OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Proposition 65

Evidence on the Carcinogenicity of Acetaminophen

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Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment
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PREFACE

Proposition 65[1] requires the publication of a list of chemicals "known to the state" to cause cancer or reproductive toxicity. The Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency maintains this list in its role as lead agency for implementing Proposition 65. The Carcinogen Identification Committee (CIC) advises and assists OEHHA, and adds chemicals to the Proposition 65 list of chemicals that cause cancer, as required by Health and Safety Code section 25249.8. The Committee serves as the state's qualified experts for determining whether a chemical has been clearly shown to cause cancer.

The Committee also provides advice and consultation regarding which chemicals should receive their review. At their meeting in October 2011, the CIC recommended that acetaminophen be placed in a 'high' priority group for future listing consideration. OEHHA selected acetaminophen for consideration for listing by the CIC, and in March 2019 OEHHA solicited from the public information relevant to the assessment of the evidence on its carcinogenicity. OEHHA reviewed and considered those submissions in preparing this document.

On December 5, 2019, the CIC is scheduled to deliberate on the carcinogenicity of acetaminophen. OEHHA developed this hazard identification document with information on the evidence of carcinogenicity of acetaminophen to assist the CIC in its deliberations. This document and the original papers discussed in the document will be provided to the CIC as part of the hazard identification materials.

OEHHA announced a public-comment period on this document upon its release. Public comments received on this document also form part of the hazard identification materials, and are provided to the CIC members prior to their formal deliberations.

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (California Health and Safety Code 25249.5 *et seq*.)

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Acronyms and Abbreviations

2-AAF	N-2-Fluorenylacetamide
3´-UTR	3´-Untranslated region
5-MT	5-Methyltryptophan
$6TG^r$	6-Thioquanine resistant
8-OHdG	8-Hydroxy-2′-deoxyguanosine (interchangeable with 8-oxodG as oxidative damage biomarkers)
8-oxodG	8-oxo-7,8-dihydro-2´-deoxyguanosine (or 8-oxodeoxyguanosine)
AC_{50}	Active Concentration 50
Acetaminophen-CYS	3-(cysteinyl) acetaminophen
Acetaminophen-GSH	3-*S*-glutathionyl-acetaminophen
ACS	American Cancer Society
AFB_1	Aflatoxin B1
AhR	Aryl hydrocarbon receptor
ALF	Acute liver failure
ALL	Acute lymphocytic leukemia
ALT	Alanine transaminase
AM_{404}	N-arachidonylphenolamine
AML	Acute myeloid leukemia
AP-1	Activator Protein-1
AR	Androgen receptor
BBN	N-butyl-N-(4-hydroxybutyl)nitrosamine
BCC	Basal cell carcinoma
BD	Basal diet
BMD	Benchmark dose
BMI	Body mass index
BUN	Blood urea nitrogen
CA	Chromosomal aberration
CAT	Catalase
CB_1	Cannabinoid receptor type 1
CCRIS	Chemical Carcinogenesis Research Information System
CD	Choline-deficient
CHO	Chinese hamster ovary
CI	Confidence interval
CIC	Carcinogen Identification Committee
CLL/SLL	Chronic lymphocytic leukemia/small lymphocytic lymphoma
C_{Max}	Peak plasma concentration

CML	Chronic myeloid leukemia
CompTox	Computational Toxicology
CPDB	Carcinogenic Potency Database
CPRD	Clinical Practice Research Datalink
CPS-II	Cancer Prevention Study II
CS	Choline-supplemented
CYP or CYP450	Cytochrome P450 enzymes
DEL	Deletion
DEN	N-nitrosodiethylamine, diethylnitrosamine
dG	Deoxyguanosine
DHPN	Dihydroxy-di-N-propylnitrosamine
DLBCL	Diffuse large B-cell lymphoma
DMAB	3,2-dimethyl-4-aminobiphenyl
DMSO	Dimethyl sulfoxide
dN-mix	Deoxyribonucleic acid mixture
DSBs	Double strand breaks
EHEN	N-ethyl-N-hydroxyethylnitrosamine
ERRα	Estrogen Related Receptor alpha
ERα	Estrogen receptor alpha
ERβ	Estrogen receptor beta
FAAH	Fatty acid amide hydrolase
GENE-TOX	Genetic Toxicology Data Bank
GGT	Gamma-Glutamyltransferase or γ-Glutamyltransferase
GHC	Group Health Cooperative
GPI	Glycosylphosphatidylinositol
GPRD	General Practice Research Database
GPs	General practitioners
GSH	Reduced glutathione
GSSG	Glutathione disulfide
GST	Glutathione *S*-transferase
GST-P	Glutathione *S*-transferase placental form (rat)
GSTP1	Glutathione *S*-transferase pi 1 (human)
H_2O_2	Hydrogen peroxide
HCC	Hepatocellular carcinomas
HD	High dose
HIC	Highest ineffective concentration
HID	Highest ineffective dose
HL	Hodgkin's lymphoma
HNF1α	Hepatocyte nuclear factor 1α
HPFS	The Health Professionals Follow-up Study

HPRT	Hypoxanthine phosphoribosyl transferase
HQ	Hydroquinone
HRP	Horseradish peroxidase
HRs	Hazard ratios
HSDB	Hazardous Substances Data Bank
HTS	High-throughput screening
i.p.	Intraperitoneal
i.v.	Intravenous
IARC	International Agency for Research on Cancer
ICD	International Classification of Diseases
IUPAC	International Union of Pure and Applied Chemistry
LD	Low dose
LEC	Lowest effective concentration
LED	Lowest effective dose
LGLL	Large granular lymphocyte leukemia
LH	Lymphohematopoietic
MC	3-Methylcholanthrene
MDA	Malondialdehyde
MEC	Multiethnic Cohort
Methyl-CCNU	1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea
mg/kg-day	Milligram per kilogram per day
miRNA	microRNA
MLL	Mixed lineage leukemia
MM	multiple myeloma
MN	Micronuclei
MNCL	Mononuclear cell leukemia
mRR	Meta-relative risk
MRP2	Multidrug resistance-associated protein 2
MSigDB	Molecular Signatures Database
NAPQI	*N*-Acetyl-*p*-benzoquinone imine
NAPSQI	*N*-Acetyl-*p*-benzosemiquinone imine
NER	Nucleotide excision repair
NFE2L2 or Nrf2	Nuclear factor erythroid 2-like 2
NHL	Non-Hodgkin's lymphoma
NHS	Nurses’ Health Study
NIH	National Institutes of Health
NK	Natural killer (cells)
NNK	4-(Methylnitrosamino)-1-(3-pryidyl)-1-butanone
N-OHAAF	N-Hydroxy-2-Fluorenylacetamide
NOK	Next of kin

NOS	Not otherwise specified
NQO	4-Nitroquinoline n-oxide
NR	Not reported
NSAIDs	Nonsteroidal anti-inflammatory drugs
NT	Not tested
NTP	National Toxicology Program
OEHHA	Office of Environmental Health Hazard Assessment
Ogg1	8-Oxoguanine DNA glycosylase
OR	Odds ratio
OS	Observational Study
OTC	Over-the-counter
OUA^r	Ouabain resistant
PAP	*p*-Aminophenol
PAP-CYS	*p*-Aminophenol cysteinyl- conjugate
PAPS	3'-Phosphoadenosine 5'-phosphosulfate
PB	Phenobarbital
PBL	Peripheral blood lymphocytes
PBTS	Peripheral blood transcriptome signatures
PCB	Polychlorinated biphenyls
PGES	Prostaglandin H synthase or prostaglandin endoperoxide synthase
PLCO	US Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial
PMA	Phorbol myristate acetate
PPAR	Peroxisome proliferator-activated receptor
ppm	Parts per million
QC	Quality control
qRT-PCR	Quantitative Reverse Transcription Polymerase Chain Reaction
RCC	Renal cell carcinoma
RNS	Reactive nitrogen species
RoC	Report on Carcinogens
RORC	RAR-related Orphan Receptor C
ROS	Reactive oxygen species
RR	Relative risk
SAMP	S-(5-acetylamino-2-hydroxyphenyl)mercaptopyruvic acid
SCC	Squamous cell carcinoma
SCE	Sister chromatid exchange
SEER	Surveillance, Epidemiology, and End Results
SES	Socioeconomic status

SHE	Syrian hamster embryo
SIRs	Standardized incidence ratios
SLRL	Sex-linked recessive lethal
SMART	Somatic mutation and recombination test
SMRs	Standardized mortality ratios
SNPs	Single nucleotide polymorphism
SOD	Superoxide dismutase
SSBs	Single strand breaks
SSVM	Sheep seminal vesicle microsomes
SULT	Sulfotransferase
$T_{1/2}$	Half-life
TCC	Transitional cell cancer
TK	Thymidine kinase
T_{max}	Time to reach maximum concentration
$TRPV_1$	Transient receptor potential vanilloid type 1
UDP	Uridine 5'-diphosphate
UDPGA	Uridine diphosphoglucuronic acid
UDS	Unscheduled DNA synthesis
UGT	Uridine 5'-diphosphate-glucuronosyltransferase or UDP-glucuronosyltransferase
UK	United Kingdom
US FDA	United States Food and Drug Administration
VDR	Vitamin D Receptor
VITAL	VITamins And Lifestyle
WHI	The Women's Health Initiative
WHI OS	Women’s Health Initiative Observational Study
WHO	World Health Organization
γ-H2AX	Phosphorylated histone 2AX (serine 139)

1. EXECUTIVE SUMMARY

Acetaminophen (N-(4-hydroxyphenyl)acetamide) is a synthetic chemical that is mainly used as an analgesic and antipyretic drug, and also has some minor industrial uses. Acetaminophen was first approved by the US Food and Drug Administration as a prescription drug in 1951, and became available without a prescription in 1955. The recommended maximum daily dose of acetaminophen is 4,000 mg.

Human exposure to acetaminophen mainly comes from the use of over-the-counter and prescription medications. *In utero* exposure and exposure via breastmilk from maternal use of this medication also occurs. There is also the potential for occupational exposure during production and use in the manufacture of other chemicals.

Epidemiological studies

A number of human epidemiological studies have investigated the relationship between acetaminophen and cancer for various cancer sites.

Kidney cancer

The association between the use of acetaminophen and kidney cancer was assessed in four cohort studies, two nested case-control studies, 12 publications from case-control studies, and two meta-analyses. Twelve case-control studies reported on renal cell carcinoma (RCC), and four assessed cancer of the renal pelvis, a rare subtype of kidney cancer.

A Danish cohort study that assessed acetaminophen use through prescription records found a non-statistically signficant increased risk of RCC, but was not able to account for important potential confounders (Friis et al. 2002). An American cohort study that collected acetaminophen use prospectively through self-report observed a statistically significant increased risk of RCC with regular and less than 10 years of use, but not with 10 or more years of use, after adjustment for body mass index (BMI), smoking, and other potential confounders (Karami et al. 2016). A cohort study from Washington state (Walter et al. 2011a) showed no significant increase in risk, and a study combining data from two large American prospective studies showed positive, but not statistically significant, increases in RCC in men and women associated with regular acetaminophen use (Cho et al. 2011).

The two most informative studies were large case-control studies nested in health databases in the US and UK. Acetaminophen prescriptions were recorded prospectively, and therefore the exposure assessment method was less subject to information bias as compared to self-report (Derby and Jick 1996; Kaye et al. 2001).

Both of these studies reported a statistically significant increasing risk of RCC with an increasing number of acetaminophen prescriptions filled. Kaye et al. (2001) adjusted for smoking and BMI.

The case-control studies generally reported small or modest but non-statistically significant increases in risk for both RCC (Cho et al. 2011; Gago-Dominguez et al. 1999; Karami et al. 2016; McCredie et al. 1988; McCredie and Stewart 1988; McCredie et al. 1995; McLaughlin et al. 1985; Rosenberg et al. 1998) and cancer of the renal pelvis (McCredie and Stewart 1988; McCredie et al. 1993; McLaughlin et al. 1985; Pommer et al. 1999). Several of the individual studies were not informative on their own because of limited statistical power due to the rarity of kidney cancer, small numbers of exposed cases and/or crude exposure assessments. The exceptions were the two largest case-control studies (Gago-Dominguez et al. 1999; Karami et al. 2016) and a large pooled analysis of case-control studies (McCredie et al. 1995), which reported roughly two-fold increased risk of RCC (either borderline significant or statistically significant) or positive dose response relationships.

Two meta-analyses also found a statistically significant increased risk between acetaminophen use and kidney cancer (Choueiri et al. 2014; Karami et al. 2016) and RCC specifically (Karami et al. 2016), although both analyses had some limitations.

Urinary bladder cancer

Three cohort studies, two nested case-control studies and six case-control studies examined the association of acetaminophen with bladder cancer. The three most informative studies assessed acetaminophen use prospectively through medical records; two reported a non-significant increased risk of bladder cancer with low to moderate use and no dose-response trend (Friis et al. 2002; Kaye et al. 2001), and the third reported a non-statistically significant increase with high use (Derby and Jick 1996). Of the two cohort studies that assessed acetaminophen use through self-report, one reported non-significant increased risks with use (Walter et al. 2011a).

The case-control studies had a mix of positive and null findings. A case-control study from Spain (Fortuny et al. 2006) observed no cancer association with acetaminophen overall, but found an increased risk of bladder cancer among acetaminophen users with a genotype (*GSTP1* Val/Val) encoding for a decrease in GSTP1 (glutathione S-transferase Pi 1) function and hence reduced capacity for glutathione conjugation with acetaminophen metabolites, such as *N*-acetyl-*p*-benzoquinone imine (NAPQI). In those with the *GSTP1* Val/Val genotype, bladder cancer risk was increased roughly two-fold in acetaminophen users and in those who used acetaminophen regularly for more than four years (though not statistically significant), suggesting that this *GST* genotype may be important to consider in studies assessing the carcinogenicity of acetaminophen. Three of the case-control studies observed no association between bladder cancer and

various metrics of acetaminophen intake (Fortuny et al. 2007; McCredie and Stewart 1988; Pommer et al. 1999), three reported non-statistically significant elevated risks of bladder cancer (Castelao et al. 2000; Fortuny et al. 2006; Piper et al. 1985), and one reported a statistically significant increase in risk of bladder cancer (Baris et al. 2013).

Urinary tract cancers

In addition to kidney and bladder cancer, there were two cohort and six case-control studies that assessed the association between acetaminophen use and cancer of other sites in the urinary tract or combined several sites of the urinary tract (three studies of renal pelvis and ureter, two studies of transitional cell cancers that included multiple sites within the urinary tract, and one study of ureter cancer).

Significant increases were not observed in the cohort studies of urinary tract cancer (Friis et al. 2002; Walter et al. 2011a). The population-based case-control study of transitional cell cancer had a statistically significant increase in risk with any acetaminophen use (Steineck et al. 1995), whereas the findings from the hospital-based case-control study were not statistically significant (Rosenberg et al. 1998). Both studies had significant limitations. The only study specifically of ureter cancer reported a statistically significant two-fold increased risk for greater than 0.1 kg of lifetime acetaminophen intake (McCredie and Stewart 1988).

Lymphohematopoietic system cancers

The association between acetaminophen use and several types of LH cancers has been studied in a number of cohort and case-control studies. For all LH cancers combined, significantly increased risks of LH cancer-related deaths were found in the one cohort study in Danish North Jutland County (Lipworth et al. 2003) but not in another in the same geographic area for incidence of LH cancer (Friis et al. 2002). A US cohort study reporting on “hematologic malignancies” found statistically significant increases in women that were high users through self-report on a questionnaire (Walter et al. 2011b).

For myeloid leukemia, statistically significant increases were reported for one cohort in men and women combined (Walter et al. 2011b) and for women but not men in one case-control study (Ross et al. 2011), with significant trends observed in terms of duration of use and tablets per week. A second case-control study reported a non-significant elevation in risk (Friedman 1982).

AML was assessed in two case-control studies: a borderline significant increase was reported in one study (Weiss et al. 2006) and a statistically significant increase in women with a dose-response trend was reported in the second study (Ross et al. 2011). CML was assessed in one case-control study that reported non-significant elevations in risk in men and women (Ross et al. 2011).

For lymphoma, a statistically significant increase was reported in one case-control study (Becker et al. 2009). A statistically significant increase in risk of two types of B-cell lymphoma was observed in a cohort study (Walter et al. 2011b), with a dose-response trend in one type. For total NHL, a non-significant elevation in risk was observed in one cohort (Friis et al. 2002). Elevations were observed in two case-control studies, which were statistically significant in women but not men in one study (Baker et al. 2005) and not significant in women the other study (Kato et al. 2002). There were increases in the risk of three cell types of NHL, but not one cell type.

Two studies reported an increased risk of multiple myeloma, which was not significant in the cohort study (Friis et al. 2002). The case-control study reported a significantly increased risk with several metrics of exposure (regular use, times per week, years of use), with a significant dose-response trend (Moysich et al. 2007).

Two studies reported on the risk in Hodgkin's lymphoma. A cohort study reported a non-significant elevation but contained only one case (Friis et al. 2002). A case-control study found significantly increased risks with significant trends by several metrics of exposure (Chang et al. 2004).

Potential confounding by smoking was possible for some of these LH cancers since it was adjusted for in only three studies (Chang et al. 2004; Moysich et al. 2007; Walter et al. 2011b).

Liver cancer

The association between acetaminophen use and liver cancer was examined in two large independent cohorts that assessed acetaminophen use through prescription records databases, one from Denmark (Friis et al. 2002; Lipworth et al. 2003) and the other from the UK (McGlynn et al. 2015; Yang et al. 2016). In the Danish cohort, elevations in mortality and incidence risk were observed that were statistically significant for mortality from liver cancer (Lipworth et al. 2003) but not incidence of liver cancer (Friis et al. 2002). This cohort could not control for potential confounders such as smoking and alcohol use. In the UK database, statistically significant elevations in the risk of liver cancer were reported, after adjusting for several covariates including smoking status, alcohol-related disorders, hepatitis B or C virus infection, and use of non-steroidal anti-inflammatory drugs (Yang et al. 2016). Dose-response analyses showed increasing risk with increasing acetaminophen prescriptions overall and when restricted to individuals without liver disease.

Other cancers

For cancers of the breast, ovary, uterine endometrium, prostate, skin, and colorectum, the association with acetaminophen use was either decreased, null, or inconsistent. The data from cohort and case-control studies from a number of other cancer sites were

too sparse to evaluate thoroughly, namely the brain, respiratory tract, gastrointestinal tract (stomach, esophagus, oral/pharyngeal cancer), pancreas, cervix, and all cancers combined.

Animal studies

Long-term carcinogenicity studies of acetaminophen have been conducted in mice and rats. Significant tumor findings were observed in three of ten studies in mice and in three of seven studies in rats. The positive findings are as follows:

Liver tumors

- In the female B6C3F1 mice exposed to acetaminophen in feed for up to 134 weeks (Amo and Matsuyama 1985), the incidence of hepatocellular adenoma or carcinoma combined was significantly increased in the high-dose group by pairwise comparison with controls, with a significant dose-related trend.
- In the male as well as the female IF mice exposed to acetaminophen in feed for up to 18 months (Flaks and Flaks 1983), the incidences of hepatocellular adenoma, and adenoma or carcinoma combined were significantly increased in the high-dose groups by pairwise comparison with controls, with significant dose-related trends. Carcinoma was also similarly increased in male mice.
- In the male as well as the female Leeds rats exposed to acetaminophen in feed for up to 18 months (Flaks et al. 1985), the incidences of hepatocellular adenomas were significantly increased in the high-dose groups by pairwise comparison with controls, with significant dose-related trends.

Pituitary gland

- In the female B6C3F1 mice exposed to acetaminophen in feed for up to 134 weeks (Amo and Matsuyama 1985), the incidence of pituitary gland adenoma was significantly increased in the high-dose group by pairwise comparison with controls, with a significant dose-related trend.

Urinary bladder

- In the male Leeds rats exposed to acetaminophen in feed for up to 18 months (Flaks et al. 1985), the incidences of rare urinary bladder papilloma and papilloma or carcinoma combined were significantly increased in the high-dose group by pairwise comparison with controls, with significant dose-related trends. In the female Leeds rats similarly exposed, the incidence of urinary bladder papilloma or carcinoma combined was significantly increased in the low-dose group by pairwise comparison with controls.

Mononuclear cell leukemia (MNCL)

- In the female F344/N rats exposed to acetaminophen in feed for up to 103 weeks (NTP 1993), the incidence of MNCL was significantly increased in the high-dose group by pairwise comparison with controls, with a significant dose-related trend.

Significant tumor findings were not observed in long-term carcinogenicity studies of acetaminophen in male and female B6C3F1 mice exposed for 103 weeks (NTP 1993), male B6C3F1 mice exposed for either 134 weeks (Amo and Matsuyama 1985) or 70 weeks (Hagiwara and Ward 1986), male and female Swiss mice exposed for 11 months (Weisburger et al. 1973), female ABC-A mice exposed for life (mean survival ≤ 40 weeks) (Wright 1967), male F344 rats exposed for 103 weeks (NTP 1993), male and female F344/DuCrj rats exposed for 104 weeks (Hiraga and Fujii 1985), or male Sprague-Dawley rats exposed for 117 weeks (Johansson 1981).

Pharmacokinetic and Mechanistic Data

Pharmacokinetics

Metabolism of acetaminophen leads to the formation of electrophilic and genotoxic metabolites, including NAPQI, *N*-acetyl-*p*-benzosemiquinone imine (NAPSQI), *p*-aminophenol (PAP), *p*-benzoquinone imine, *p*-benzoquinone, and the *N*-acetyl-*p*-aminophenoxy and *p*-aminophenoxy free radicals. Reactive oxygen species (ROS) can be formed during the metabolism of acetaminophen via either redox cycling or oxidative reactions involving various metabolic intermediates. The generation of electrophilic and genotoxic metabolites of acetaminophen can vary among individuals, due to variability in genetic factors, such as *GSTP1*, and non-genetic factors, such as nutritional status.

Genotoxicity data

Acetaminophen was not mutagenic in bacteria. Acetaminophen has tested positive for a number of other genotoxicity endpoints:

- Mutations in rodent cells *in vitro*
- DNA strand breaks in mouse liver, and in mussels *in vivo*; in human cells *in vitro*
- DNA adducts in mouse liver and kidney *in vivo*; in human granulocytes *in vitro*; in acellular systems
- DNA oxidation in mouse *in vivo*, and in rat cells *in vitro*
- Impairment of DNA repair in rat and mouse *in vivo*, and in human and rodent cells *in vitro*
- Aneuploidy in rat embryos *in vivo*
- Micronuclei (MN) formation in human lymphocytes, rat, mouse bone marrow, mouse peripheral blood and mussels *in vivo*; in human and rodent cells *in vitro*

- Chromosome aberrations (CAs) in human lymphocytes and mouse bone marrow *in vivo*; in human and rodent cells *in vitro*
- Sister chromatid exchanges (SCEs) in human lymphocytes and mouse bone marrow *in vivo*; in human and rodent cells *in vitro*

Genotoxicity of acetaminophen metabolites

There is also evidence for the genotoxicity of three metabolites of acetaminophen:

- NAPQI, which tests positive for DNA strand breaks in human and rodent cells *in vitro* and in acellular systems, and which is observed to form DNA adducts in multiple acellular systems.
- PAP, which tests positive for mutations in mice sperm *in vivo*, rodent cells *in vitro*, and in *E. coli* and *Drosophila*. PAP also forms DNA strand breaks in human and rodent cells *in vitro* and DNA adducts in human granulocytes *in vitro*. It also tests positive for chromosomal effects - MN in mouse *in vivo*; CAs in human and rodent cells *in vitro*, mouse and plants *in vivo*; SCEs in human and rodent cells *in vitro*; and intra-chromosomal recombination in yeast.
- *p*-Benzoquinone, which tests positive for mutations in *Salmonella*, and in rodent cells *in vitro*, DNA strand breaks in human and rodent cells *in vitro*, and DNA adducts in human cells and in acellular systems. Chromosomal effects include MN in human and rodent cells *in vitro*, and in mouse bone marrow and liver cells *in vivo*; CAs in mouse bone marrow *in vivo*; SCEs in human lymphocytes *in vivo*, and in rodent cells *in vitro*. *p*-Benzoquinone also inhibits topoisomerase IIα in human cells *in vitro*, and in an acellular system. It also induced effects associated with genomic instability in mouse and human cells, and decreased *Ogg1* expression in rodent cells *in vitro*.

Structure activity comparisons

The biological activity of acetaminophen was compared to five structurally related compounds: phenacetin, aniline, PAP, 2,4-diaminophenol dihydrochloride, and 3-amino-4-ethoxyacetanilide. Of these five comparison chemicals, two (phenacetin and aniline) are listed as Proposition 65 carcinogens.

Common target tumor types observed between acetaminophen and some of the comparison chemicals are urinary bladder tumors (observed for phenacetin), and pituitary tumors (observed for 3-amino-4-ethoyacetanilide). All five structurally related comparison chemicals have genotoxic activity.

Key characteristics

The key characteristics of carcinogens (IARC 2019a; Smith et al. 2016) were used to organize the data relevant to carcinogenicity from mechanistic studies of

acetaminophen. These studies provide evidence on four of the 10 key characteristics of carcinogens enumerated below.

- Is electrophilic or can be metabolically activated

  Acetaminophen can be metabolized by CYP enzymes, prostaglandin endoperoxide synthase (PGES), and other peroxidases to form electrophilic compounds. Electrophilic metabolites of acetaminophen include NAPQI, NAPSQI, *p*-benzoquinone imine, *p*-benzoquinone and the *N*-acetyl-*p*-aminophenoxy and *p*-aminophenoxy free radicals.

- Is genotoxic

  There is evidence on the genotoxicity of acetaminophen and its metabolites, NAPQI, PAP, and *p*-benzoquinone, as outlined above.

- Alters DNA repair or causes genomic instability

  There is evidence that acetaminophen alters DNA repair and causes genomic instability. The evidence includes (i) inhibition of ribonucleotide reductase (which results in impairment of nucleotide excision repair) by acetaminophen; (ii) decreased protein expression and activity of the DNA repair enzyme Ogg1 by acetaminophen and decreased gene expression of Ogg1 by *p*-benzoquinone; (iii) increased levels of γ-H2AX (indication of DNA double-strand breaks) by acetaminophen and *p*-benzoquinone; and (iv) inhibition of human topoisomerase IIα (leading to DNA double-strand breaks) by NAPQI and *p*-benzoquinone.

- Induces oxidative stress

  Evidence for induction of oxidative stress comes from *in vivo* and *in vitro* studies of acetaminophen conducted in both humans and animals, and includes observations of markers of oxidative stress in adults and children taking therapeutic doses, and in rats and mice administered doses of 150 mg/kg and 200 mg/kg, respectively. Transcriptomic studies have reported that acetaminophen can regulate genes in biological pathways related to oxidative stress, including in a study in humans exposed to therapeutic doses and a study in mice exposed to 151 mg/kg acetaminophen.

2. INTRODUCTION

2.1 Identity of Acetaminophen

Acetaminophen is a synthetic chemical that exists as a white crystalline powder. Acetaminophen consists of a benzene ring with one hydroxyl group and an acetamide group at the *para* position (Figure 1). It is a derivative of acetanilide and a member of the class of phenols. Selected chemical properties are listed in Table 1.

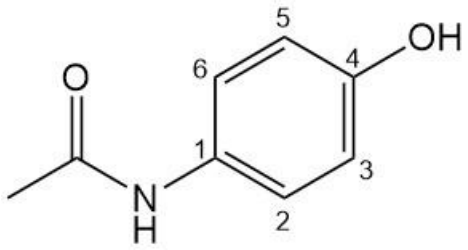


Figure 1. Structure of acetaminophen

Table 1. Selected chemical properties of acetaminophen

Name	Acetaminophen
IUPAC Systematic Name	N-(4-Hydroxyphenyl)acetamide
CAS Registry Number	103-90-2
Molecular Formula	$C_8H_9NO_2$
Molecular Weight	151.16 g/mol
Melting Point	169°C
pKa Dissociation Constant	9.50
log P (Octanol-water)	0.46
Water Solubility	0.0926 mol/L
Selected Additional Synonyms	Paracetamol; acetyl-*p*-amino-phenol; 4-acetamidophenol; N-(4-hydroxyphenyl)acetamide; *p*-hydroxyacetanilide; *p*-acetaminophenol; *p*-acetylaminophenol

(Data source: Computational Toxicology (CompTox) Chemicals Dashboard, https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID2020006#properties; accessed May 29, 2019)

2.2 Uses

Acetaminophen is mainly used as an analgesic and antipyretic drug, and also has some minor industrial uses (IARC 1999a). Current industrial uses of acetaminophen include the manufacture of azo dyes and photographic chemicals (O'Neil MJ. 2006).

Acetaminophen was first available as a US FDA (Food and Drug Administration) approved prescription drug in the US in 1951, and became available without a prescription in 1955 (IARC 1990). Currently, acetaminophen is available in more than 600 different prescription and over-the-counter (OTC) medications in the US for adults and children. In some of these medications, acetaminophen is the sole active ingredient while in many others it is present in combination with other active ingredients (US FDA 2019). Common prescription and OTC medicines containing acetaminophen include medications that treat fever, allergy, colds, flu, and various aches and pains.

2.3 Occurrence and Exposure

Because of its wide use, acetaminophen is commonly detected in septic tank effluent (Godfrey et al. 2007) and wastewater (Kolpin et al. 2002; Yang et al. 2011). Acetaminophen has also been detected in groundwater (Fram and Belitz 2011), surface water (Carmona et al. 2017; Wu et al. 2012), sea water (Brumovsky et al. 2017; Napier et al. 2018), and soil and sediment (Carmona et al. 2017). It has also been found in wildlife – *e.g.*, fish and osprey nestlings (Bean et al. 2018).

Human exposure to acetaminophen mainly comes from the use of OTC and prescription medications by the oral, intravenous injection, and rectal routes. Fifty million Americans use medicines containing acetaminophen each week (Acetaminophen Awareness Coalition 2019). In addition, *in utero* exposure and exposure via breastmilk from maternal use of this medication also occurs, since acetaminophen crosses the placenta (Nitsche et al. 2017) and is excreted in breast milk (Berlin et al. 1980; Bitzen et al. 1981). There is also the potential for occupational exposure during production and use in the manufacture of other chemicals. In 1999, it was estimated that 65,000 US workers were potentially exposed to acetaminophen (IARC 1999a).

Overdose of acetaminophen containing products accounted for over 78,000 emergency department visits in the US between 2006-2007 (Budnitz et al. 2011). It has been estimated that in the US, acetaminophen toxicity causes about 30,000 hospitalizations each year (Blieden et al. 2014). Inadvertent overdoses from medications containing acetaminophen in combination with other active ingredients accounts for nearly half of the 1,600 acetaminophen-related cases of liver failure each year in the US (McCarthy 2014). To prevent unintentional hepatotoxicity, US FDA limited the amount of acetaminophen permitted in prescription medications with multiple active ingredients to

325 milligrams (mg) acetaminophen per dosage unit (*e.g.*, tablet, capsule or other dosage unit) (US FDA 2014). OTC medications with multiple active ingredients can contain 325, 500 or 650 mg acetaminophen per dosage. The recommended maximum daily dose of acetaminophen is 4,000 mg (US FDA 2009).

3. DATA ON CARCINOGENICITY

3.1 Carcinogenicity Studies in Humans

Background

Acetaminophen was previously evaluated for its carcinogenicity in the International Agency for Research on Cancer (IARC) Monographs in 1990 and 1999. Concerns over the carcinogenicity of acetaminophen arose because it is the major metabolite of phenacetin, a recognized carcinogen that causes cancer of the renal pelvis (IARC 2012). In both of the previous IARC evaluations of acetaminophen, it was classified in Group 3 (not classifiable as to its carcinogenicity to humans) and the epidemiologic data on carcinogenicity were judged to be inadequate. In the 1999 IARC evaluation, the epidemiologic data on the carcinogenicity of acetaminophen was judged either inadequate or inconsistent by target organ. Several cohort and case-control studies examining the association of cancers at various sites with acetaminophen exposure have been published since the IARC review. Also, two overlapping meta-analyses published since the 1999 IARC evaluation included several of these case-control studies, as well as cohort studies, and reported an increased risk of kidney cancer associated with use of acetaminophen (Choueiri et al. 2014; Karami et al. 2016). This chapter reviews the full body of epidemiological evidence on acetaminophen.

There are several issues to consider when evaluating the epidemiological data that may affect the interpretation of the risk estimates presented in the individual studies, including confounding, various types of bias, reverse causation, and misclassification of exposure. In addition, it is important to understand risk factors for a particular cancer site in order to identify confounders. A brief discussion of these issues is presented below.

Confounding

Confounding is an important consideration when evaluating the results of epidemiological studies. A confounder is a variable that may distort the association between the exposure and outcome of interest. It is related to the exposure and causally related to the outcome. A variable is considered as a confounder when evaluating the relationship between an exposure (e.g., acetaminophen) and outcome (e.g., cancer) when three requirements are met:

- The variable can cause or prevent the outcome of interest.
- It is not an intermediate variable in the causal pathway between exposure and the outcome.

- It is associated with the exposure under investigation (Porta 2014; Rothman et al. 2008).

These are illustrated in this directed acyclic graph (Figure 2), in which the straight line represents a potential association and the arrows represent causal paths:

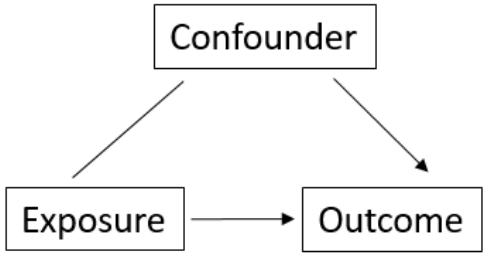


Figure 2. Directed acyclic graph (DAG), i.e., a graphical presentation of confounding

The correct identification of confounders "requires substantive knowledge about the causal network of which exposure and outcome are part (e.g., pathophysiological and clinical knowledge). Attempts to select confounders solely based on observed statistical associations may lead to bias" (Porta 2014).

Furthermore, over-adjustment can introduce bias (a systematic error) where none was present to begin with. As explained in Greenland and Rothman (2008),

> "Adjustment for variables that violate any of these criteria is sometimes called *overadjustment* and is the analytic parallel of the design error of overmatching…If a variable violates any of these criteria [referring back to the three requirements of a confounding factor described above], its use in conventional stratified analysis…can reduce the efficiency (increase the variance) of the estimation process, without reducing bias. If the variable violates the third criterion, such use can even increase bias".

Potential confounders in the association between acetaminophen and cancer, by target organ

Subsequent sections show that the most consistent results on the published epidemiologic evidence on the carcinogenicity of acetaminophen comes from reports of cancers of the:

- urinary tract
  - kidney (n=12 case-control studies, 2 nested case-control studies, 4 cohort studies),
  - urinary tract/ureter/renal pelvis (n=6 case-control studies, 2 cohort studies),

    - bladder (n=6 case-control studies, 2 nested case-control studies, 3 cohort studies) and
- lymphohematopoietic (LH) system (n=11 case-control studies, 3 cohort studies).

The discussion of confounders here focuses on these sites. For these select cancer sites, Table 2 below lists the established risk factors, as identified by IARC (2019b) and the American Cancer Society (ACS 2019d). This table may help the consideration of potential confounders when evaluating acetaminophen/cancer associations reported in studies. Of the risk factors for cancer listed in the table, several are also associated with acetaminophen exposure, thereby meeting the definition of a confounder. There is evidence that acetaminophen use is associated with smoking (Curhan et al. 2002; Ersboll et al. 2015), hypertension (Curhan et al. 2002), education/socioeconomic status (Ersboll et al. 2015; Paulose-Ram et al. 2005), race/ethnicity (Paulose-Ram et al. 2005; Yang et al. 2012), sex (Backryd 2018; Dale et al. 2015; Jawahar et al. 2012; Paulose-Ram et al. 2005; Sarganas et al. 2015), age (Backryd 2018; Paulose-Ram et al. 2005; Samuelsen et al. 2015; Sarganas et al. 2015) and body mass index (BMI)/obesity (Curhan et al. 2002; Ersboll et al. 2015).

Table 2. Risk factors and potential confounders for selected cancer sites (IARC 2019b; ACS 2019d)

Cancer site	Risk factors	Potential confounders
Kidney	Tobacco smoking, X-radiation, gamma-radiation, trichloroethylene, obesity (BMI), high blood pressure (hypertension), family history of kidney cancer, advanced kidney disease, race, a number of hereditary diseases	Tobacco smoking, BMI, hypertension, age, sex
Renal pelvis and ureter	Plants containing aristolochic acid, phenacetin, analgesic mixtures containing phenacetin, tobacco smoking	Phenacetin, tobacco smoking, age, sex
Bladder	Aluminum production, 4-aminobiphenyl, arsenic and inorganic arsenic compounds, auramine production, benzidine, chlornaphazine, cyclophosphamide, magenta production, 2-naphthylamine, painting, rubber production industry, *Schistosoma haematobium*, tobacco smoking, o-toluidine, X-radiation, gamma-radiation, ethnicity, age, gender (male), personal history of bladder/urothelial cancer, bladder birth defects, genetics/family history of bladder cancer, cyclophosphamide	Tobacco smoking, age, sex
Lympho-hemato-poietic system	Azathioprine, benzene, busulfan, 1,3-butadiene, chlorambucil, cyclophosphamide, cyclosporine, Epstein-Barr virus, etoposide with cisplatin and bleomycin, fission products (including Strontium-90), formaldehyde, *Helicobacter pylori*, hepatitis C virus, human immunodeficiency virus type 1, human T-cell lymphotropic virus type 1, Kaposi sarcoma herpes virus, lindane, melphalan, MOPP (vincristine-prednisone-nitrogen mustard-procarbazine mixture, pentachlorophenol, phosphorus-32, rubber production industry, semustine (methyl-CCNU), thiotepa, thorium-232 and its decay products, tobacco smoking, treosulfan, X-radiation, gamma-radiation, chemotherapy, Agent Orange, *Chlamydophila psittaci, Campylobacter jejuni,* genetic syndromes, ethnicity, gender (males), family history, immune system suppression, geography, body weight, diet, breast implants, organ transplants	Tobacco smoking, BMI, age, sex
Liver	Sex, race/ethnicity, chronic viral hepatitis, cirrhosis, alcohol use, tobacco use, BMI, type 2 diabetes, certain rare diseases, aflatoxins, vinyl chloride and thorium dioxide (Thorotrast), anabolic steroids, estrogen-progestogen contraceptives, plutonium, thorium-232 and its decay products	Tobacco smoking, BMI, age, sex

Protopathic Bias

Studies investigating the association between acetaminophen exposure and risk of cancer can be subject to protopathic bias, commonly referred to as reverse causation (Salas et al. 1999). It occurs when drugs are prescribed to treat symptoms that are actually early manifestations of the outcome of interest (or manifestations of precursors to the outcome of interest) (Signorello et al. 2002). Signorello et al. (2002) goes on to explain,

> "Technically, this type of bias is not true confounding, but a reversal of cause and effect, in which the outcome precedes the exposure of interest. Because analgesics are used to treat aches and pains, which are frequently the harbingers of more serious disease, epidemiologic studies relating analgesic use to disease outcomes are especially vulnerable to protopathic bias."

This type of bias can be controlled by adding a lag time, i.e., by excluding the exposure in a specified time period before the occurrence of the outcome (Arfe and Corrao 2016). A number of the epidemiological studies described below included a lag time of one or more years in their analysis.

Confounding by Indication

Several studies refer to the issue of confounding by indication, which occurs when the indication for selecting a particular treatment also affects the outcome (Kyriacou and Lewis 2016; Salas et al. 1999; Schneeweiss and Suissa 2013). In this situation, "the indication is a confounder because it correlates with the intervention and is a risk indicator for the illness" (Salas et al. 1999). It can occur when the risk of an adverse event is related to the indication for medication use; thus, those who are actually exposed are at higher or lower risk of the adverse event than those unexposed (Schneeweiss and Suissa 2013).

An example is described in a study that found positive associations between acetaminophen prescriptions and liver cancer in a large medical records database in the UK, the Clinical Practice Research Datalink (CPRD) (McGlynn et al. 2015; Yang et al. 2016) (See Section 3.1.2.3 on liver cancer). Confounding by indication was assessed as a possible explanation for the increase in risk. Patients with liver disease (e.g., cirrhosis, portal hypertension, thrombocytopenia) may be advised to avoid NSAID use due to risk of gastrointestinal bleeding and renal failure. These patients may be channeled towards receiving acetaminophen and may also be at a higher risk for liver cancer. Therefore the indication of liver disease may confound the association between acetaminophen use and liver cancer. However, results did not change materially when restricting the analyses to individuals without chronic liver disease.

The term “confounding by indication” is sometimes incorrectly used synonymously with other terms such as confounding by disease severity and protopathic bias (Joseph et al. 2014; Salas et al. 1999; Schneeweiss and Suissa 2013; Signorello et al. 2002).

Information Bias and Misclassification of Exposure

Information bias is “a flaw in measuring exposure or outcome data that results in different quality (accuracy) of information between comparison groups” (Last 2001). The misclassification of exposure or outcome measurements can be either differential (varies between study groups) or non-differential (is the same in all study groups).

Recall bias is a type of information bias, which is especially a concern in case-control studies in which cases differentially report higher drug use than controls. However, recall bias is not expected to be of concern in interpreting case-control studies of cancer and acetaminophen use because acetaminophen has not been perceived by the public to be a risk factor for cancer.

The tools used to measure acetaminophen intake fall into two main types: self-reported questionnaire or prescription database. Self-reported recall may be an issue in observational studies, and may lead to information bias and potential misclassification of exposure from inaccurate reporting. As cancer cases are not expected to differentially report higher acetaminophen exposure compared to controls (see above), any misclassification of exposure would likely occur in both cases and controls and be non-differential (unrelated to disease status), and bias risk estimates toward the null value of 1.0, signifying no association. Some case-control studies did try to validate self-reported acetaminophen use against medical records, which would alleviate some of the uncertainty in the exposure assessment.

The other method of measuring exposure is via patient prescription records. It is not a perfect measure of exposure because a prescription does not necessarily guarantee that the patient took the medication containing the chemical of interest (e.g., acetaminophen), and the patient may take additional medication obtained over-the-counter (OTC) that also contains the chemical of interest. While some studies assessed both prescription records and self-reported use of the chemical of interest, most did not account for OTC medication use that also contains the chemical of interest; this could underestimate the amount of exposure. However, the use of prescription records greatly reduces the risk of recall bias; further, non-compliance would reduce the amount of exposure and bias the risk estimates towards the null value of 1.0, signifying no association (Olsen et al. 2008). A validation study in the UK of patient prescription records for aspirin-containing prescriptions suggested that the potential impact of misclassification of aspirin use due to unrecorded OTC use was low; a similar assumption for acetaminophen can be made since it can also be obtained in the UK via a prescription or OTC (Cea Soriano et al. 2016a, b). A previous study within the UK

General Practice Research Database (GPRD) found that women were unlikely to use acetaminophen regularly or chronically without a prescription from their doctor (general practitioner) (Meier et al. 2002).

Methods

We searched several databases and identified 133 epidemiologic studies published in peer-reviewed journals on the carcinogenicity of acetaminophen for this review. Epidemiologic studies were included if they were of cohort, nested case-control, case-control, or meta-analysis study design and presented a risk estimate for the association between consumption of acetaminophen and risk of cancer (incidence or mortality), or provided enough data to calculate a crude risk estimate. We also searched reviews of the carcinogenicity of acetaminophen (e.g., Weiss 2016) for additional publications. We excluded the following types of studies:

- Those that reported on a broad category of analgesics (e.g., nonsteroidal anti-inflammatory drugs [NSAIDs], all analgesics) as these studies would not be specific enough to adequately assess the association between acetaminophen exposure and cancer risk.
- Those of cancer patients or survivors, as they were not able to investigate acetaminophen as a cause of cancer.
- Those reported only as conference abstracts since the results reported in such abstracts are considered preliminary and subject to change, as they have not been peer-reviewed for publication.

Appendix A provides a description of the details of our literature search strategy, including the search question, literature search terms by database searched, inclusion and exclusion criteria, and the literature tree.

Below we provide a review of the published epidemiological studies on the carcinogenicity of acetaminophen, by target organ site and study design. The text presents an expanded synthesis of the findings to enhance readability, whereas the details of the studies are mostly presented in the tables. Study details are highlighted in both text and table only when they are remarkable or key for interpretation. To facilitate comparison of the results across studies, the acetaminophen dose is noted if it was available from the publication. Issues concerning the dose of acetaminophen consumed in the study populations are also discussed.

3.1.1 Description of cohort studies that present results on multiple cancers

The risks of cancer associated with acetaminophen use have been investigated in a number of cohort studies. The two main types of exposure assessment methods are prescription databases and self-reported questionnaires. As discussed earlier,

prescription databases are generally considered more informative because they collect data on prescriptions prospectively and therefore are less subject to information bias. Unlike questionnaires, they do not rely on patient recall or interviews to obtain data and there is no opportunity for recall and interviewer bias (Strom 2013). They can also be linked to other electronic databases, such as medical records or mortality records. A disadvantage of this method is that data on use of over-the-counter (OTC) medications is often lacking (resulting in under-ascertainment of exposure). Furthermore, not every dispensing indicates exposure (this would result in over-ascertainment of exposure). However, a previous study within the UK GPRD prescription database found that women were unlikely to use acetaminophen regularly or chronically without a prescription (Meier et al. 2002).

Another disadvantage of relying exclusively on databases for both exposure and outcome assessment can be lack of information on potential confounding variables. On the other hand, questionnaires include any exposure to acetaminophen, but are subject to recall bias. Study participants may not accurately remember which medications they took in the years prior. In cohort studies, however, exposure misclassification is expected to be non-differential because exposure is assessed prospectively; this would bias the risk estimate toward the null. The accuracy of self-reporting can be influenced by the type of medication, drug use patterns, design of the data collection materials, and respondent characteristics (Strom 2013).

The following describes the cohorts that reported on multiple cancers, by exposure assessment method.

Cohorts with exposure assessment through prescription databases

The Danish North Jutland County cohort

Friis et al. (2002) was a cohort study conducted in North Jutland County in Denmark from 1989 to 1997 that compared cancer incidence among 39,946 individuals receiving prescriptions for acetaminophen with expected incidence based on the population. Acetaminophen exposure was ascertained through the population-based Pharmacoepidemiologic Prescription Database, a system maintained by Danish pharmacies. Cases were identified through linkage to the Danish Cancer Registry, which has recorded cancer incident cases since 1943 in Denmark. Expected numbers of first primary cancers were calculated by multiplying the number of person-years of the cohort members with the sex, 5-year age group, and 5-year calendar-year specific incidence rates of first primary cancers of all residents of North Jutland county who did not receive a prescription for acetaminophen (Friis et al. 2002). Follow-up time began one year after the first recorded prescription and ended on the date of the first primary cancer diagnosis, date of death, or December 31, 1997. The study calculated standardized incidence ratios (SIRs), or the ratio of the observed cases to the expected

number of cancers based on the incidence rates in the population; cancers investigated were all malignant neoplasms, cancers of the buccal cavity and pharynx, esophagus, stomach, colon, rectum, liver, pancreas, lung, breast, cervix uteri, corpus uteri, ovary, prostate, urinary tract, renal parenchyma (including “renal cell carcinoma” and “kidney cancer, not otherwise specified”), renal pelvis/ureter, urinary bladder, melanoma, nonmelanoma skin cancer, brain, “lymphatic and haematopoietic tissue”, non-Hodgkin’s lymphoma, Hodgkin’s disease, multiple myeloma, and leukemia (Friis et al. 2002). Two cohorts were analyzed: the total cohort comprising subjects meeting the eligibility criteria who had ever had an acetaminophen prescription and an acetaminophen-only cohort that excluded individuals who received a prescription for aspirin or NSAIDs, to disentangle the effects of acetaminophen from other analgesics.

The study found that acetaminophen use in the acetaminophen-only cohort was associated with statistically significantly increased risks of all malignant neoplasms combined, all malignant neoplasms in men only, and esophagus, lung, and nonmelanoma skin cancer. In the total cohort, acetaminophen use was associated with increased risk of cancers of the renal parenchyma (e.g., renal cell carcinoma), renal pelvis/ureter, urinary bladder, and urinary tract.

By restricting the analysis to five or more years after the first recorded acetaminophen prescription, the authors reported statistically significant elevations in the SIRs for cancers of the esophagus (SIR, 2.5; 95% CI, 1.1–5.0), lung (SIR, 1.8; 95% CI, 1.4–2.2), and renal pelvis/ureter (SIR, 3.0; 95% CI, 1.2–6.2), but not for cancer of the renal parenchyma (SIR, 1.0; 95% CI, 0.4–2.1). Liver cancer was elevated but not statistically significant (SIR, 2.0; 95% CI, 0.8–4.1). Additional analyses stratified by the number of prescriptions for some cancer sites.

The strengths of this study are that exposure was assessed through prospectively collected prescription records, captured for virtually the entire population of North Jutland county. The study also began follow-up one year after the first recorded prescription to reduce the possibility of protopathic bias. This study also had some limitations. Analyses did not adjust for any confounders besides age, sex, and calendar year. Additionally, the study did not have information on OTC acetaminophen or other analgesic use, degree of compliance with acetaminophen-containing prescriptions, or use of analgesics prior to the start of the prescription database. However, the fact that insurance in Denmark reimburses up to 75% of the cost of prescriptions decreases the chance that OTC acetaminophen is also being used, particularly in long-term users. The filling of many repeat prescriptions suggests that the medications are routinely taken until completed (Friis et al. 2002). Additionally, this prescription database has been shown to have good validity in pharmacoepidemiologic research (Friis et al. 2002; Nielsen et al. 1997; Sorensen and Larsen 1994).

Lipworth et al. (2003) evaluated cancer mortality due to specific causes in 49,890 individuals prescribed acetaminophen in this population in North Jutland county during the same time period. Mortality was evaluated through 1996. The mean follow-up time since first prescription was 3.5 years, and 31% of the cohort members died during the follow-up period. Standardized mortality ratios (SMR) were calculated as the ratio of the observed to the expected number of deaths. The expected number of deaths was calculated based on the death rates in the general population of North Jutland county, stratified by sex, five-year age groups, and one-year calendar periods. Additional analyses were stratified by latency period (i.e., time since initial prescription) and number of prescriptions. Results were presented for mortality caused by cancers of the digestive tract, respiratory tract, liver, ovary, breast, prostate, LH, and 'other and unspecified' malignant neoplasms. All sites found significantly increased risks of death with acetaminophen use in the overall cohort. SMRs decreased with longer latency periods. The authors hypothesize that a reason for elevated SMRs that decline with increasing latency is that individuals are prescribed acetaminophen for pain relief during the year or more prior to death due to illness, which is described as a common practice in Denmark. The authors refer to this as "confounding by indication" (Lipworth et al. 2003). However, as explained in the introduction of Section 3.1, the pattern of association by duration of use and the attenuated OR for a longer latency period is consistent with protopathic bias. The underlying condition for which the drug is prescribed, rather than the drug itself, results in increased mortality risk. However, this study is of limited use because the cause of death may not necessarily be an indication of cancer incidence. It is probable that many cancer cases were missed because they were not the primary cause of death. Additionally, there may be overlap with Friis et al. (2002), which assessed cancer incidence in the same population.

The Danish Diet, Cancer and Health Cohort

The prospective Danish Diet, Cancer and Health cohort study was conducted in Copenhagen and Aarhus, Denmark between 1993 and 1997 with 29,875 women and 27,177 men. Acetaminophen use was assessed through a combination of both the Danish Prescription Database and a self-reported baseline questionnaire. Information on cancer diagnoses was identified through the Danish Cancer Registry and the Danish Pathology Register. The three studies that utilized data from this cohort study did not find an association with breast cancer (Friis et al. 2008) or colorectal cancer (Friis et al. 2009). Olsen et al. (2008) found an increased risk of lung cancer with acetaminophen use.

The General Practice Research Database (GPRD)

The General Practice Research Database (GPRD), which was formerly known as the Clinical Practice Research Database (CPRD), contains information entered by general

practitioners (GPs) in the UK. This database has been used to examine cancer risks associated with acetaminophen use in several cohort and/or nested case-control studies (Garcia Rodriguez and Huerta-Alvarez 2001; Garcia Rodriguez and Gonzalez-Perez 2004a, b; Kaye et al. 2001; Ronquist et al. 2004). This database collects information on 2-3 million patients. Both acetaminophen prescriptions and cancer cases were identified through the GPRD. A previous study validating a large number of cancer cases documented a high reliability of cancer diagnoses recorded in the GPRD (Jick 1997).

The use of this database has several advantages. The UK population is covered by universal health coverage: in 1998, this database had covered a representative subset of the UK population in excess of three million persons with routinely collected computerized medical data, representing 25 million person-years of information (Garcia Rodriguez and Perez Gutthann 1998; Walley and Mantgani 1997). The GPs are the center of healthcare delivery in the UK and collect information on demographics and other variables, including smoking, weight, and height (available for >70% of the population). Therefore data are less subject to information bias as it did not rely on participants' recall. Although occasional use of non-prescription acetaminophen was not assessed, such use is considered to be infrequent. Therefore it is likely that exposure was not underestimated. A previous study within the GPRD found that women were unlikely to use acetaminophen regularly or chronically without a prescription from their GP (Meier et al. 2002). A limitation of this database is that there were no links to other databases or cancer registries to verify the diagnoses or completeness of outcome ascertainment. Acetaminophen use was associated with increased risk of kidney cancer (Kaye et al. 2001). Other studies using this cohort did not find an association between acetaminophen use and cancer. Garcia Rodriguez and Gonzalez-Perez (2004a) found a reduced risk of breast cancer. Acetaminophen use was associated with either no association (Ronquist et al. 2004) or a reduced association (Garcia Rodriguez and Gonzalez-Perez 2004b) of prostate cancer. Garcia Rodriguez and Huerta-Alvarez (2001) found no association with colorectal cancer.

Cohorts with exposure assessment through self-reported or interviewer-based questionnaires

The VITamins And Lifestyle (VITAL) cohort study

Walter et al. (2011a) assessed acetaminophen use and risk of several cancers in the VITamins And Lifestyle (VITAL) cohort in western Washington State of 62,841 men and women, with a mean age of 61.5 years. Exposure was assessed through a self-administered questionnaire at baseline that included questions on regular use of acetaminophen and other analgesics, defined as at least one day per week for at least one year over the previous 10 years ("low use" defined as less than four days per week

or less than four years; “high use” defined as four or more days per week and four or more years). After a mean follow-up of 6.5 (±1.7) years, incident cases of invasive malignancy (other than nonmelanoma skin cancer) were identified by linkage to the Surveillance, Epidemiology, and End Results (SEER) cancer registry. Participants who did not develop cancer were censored at date of withdrawal from study, emigration from the SEER region, death, or December 31, 2008.

Hazard ratios (HRs) were calculated with Cox proportional hazards models, using participants’ age as the time metric, for the associations between acetaminophen use and incident cancer malignancies. All reported results were stratified by no use, low use, and high use. HRs were calculated for total cancer incidence, gastrointestinal cancer (includes colon, rectum, pancreas, esophagus, stomach, liver, small intestine, anus and anal canal, gall bladder, biliary tract, and ‘other or ill-defined digestive organs’), colon and rectum cancer, pancreatic cancer, lung cancer, urinary tract cancer (includes kidney, renal pelvis, ureter, bladder, and ‘other or unspecified urinary organs’), kidney cancer, bladder cancer, melanoma, female cancers (includes breast, uterus, ovary, vulva, and ‘other or unspecified female genital organs’), breast cancer, uterine cancer, prostate cancer, and aggressive prostate cancer. Positive associations with acetaminophen use, although not statistically significant, were found for some cancers; however, there were no significant dose-response relationships. Although this was a large cohort, there were some limitations to this study. There was limited power to detect significant associations due to small numbers of cases in individual cancer types. Additionally, this study may have over-adjusted their multivariable regression models, and no sensitivity analyses were conducted to exclude acetaminophen use in the year prior to cancer diagnosis.

A separate publication reported the risk of hematologic malignancies in this cohort with 64,839 men and women (Walter et al. 2011b). High use of acetaminophen was associated with an increased risk of combined hematologic malignancies, myeloid neoplasms, plasma cell disorders, and “mature B-cell neoplasms other than CLL/SLL [small lymphocytic leukemia/chronic lymphocytic leukemia] or plasma cell disorders”. Users were defined as those who used the drug for at least 4 years prior to categorization to exclude the possibility of reverse causation (disease and/or symptoms that could lead to acetaminophen use). Most patients with hematologic malignancies are diagnosed within one year of symptom onset. Additionally, results were significant for acetaminophen but not for other analgesics (Walter et al. 2011b). Control for confounders in these analyses was appropriate.

The Nurses’ Health Studies (NHS and NHSII)

The Nurses’ Health Study (NHS) and Nurses’ Health Study II (NHSII) are large prospective cohort studies conducted in the US (NHS 2016). In 1976, the NHS enrolled

121,700 female registered nurses aged 30-55 years. The NHSII enrolled a similar cohort of 116,429 women aged 25-42 years in 1989. All women receive mailed questionnaires every two years to collect data on demographics, lifestyle factors, medical history, and disease outcomes. Questions regarding acetaminophen use were queried biennially in the NHS starting in 1990 and in the NHSII starting in 1989. Cancer cases were identified through self-report on the questionnaires or when a participant was reported deceased. Cancer cases were confirmed through medical records and causes of death were identified via linkage to the National Death Index. Strengths of this large prospective cohort include adequate power and detailed exposure data to evaluate timing and patterns of analgesic use. The frequently updated exposure data and prospective study design limit the potential for exposure misclassification and recall bias (Barnard et al. 2018). Additionally, many studies were able to incorporate latency periods between exposure assessment and outcome onset in order to reduce the possibility of reverse causation. A limitation of the cohort is the lack of data on medication quantity and indication for use.

Cho et al. (2011) found an increased risk of kidney cancer with acetaminophen use in this cohort. No associations with acetaminophen use were observed in these cohorts for breast (Eliassen et al. 2009; Zhang et al. 2012) and ovarian (Barnard et al. 2018; Fairfield et al. 2002; Merritt et al. 2018; Pinheiro et al. 2009) cancers, or basal cell carcinoma, squamous cell carcinoma or melanoma (Jeter et al. 2011; Jeter et al. 2012).

The Health Professionals Follow-up Study (HPFS)

In the HPFS from 1986 through 2004, acetaminophen exposure was assessed through biennial questionnaires that were administered starting in 1986 (Cho et al. 2011; Genkinger et al. 2007). Eighty-seven percent of the self-reported cases were confirmed by medical records. Those that were not confirmed by medical record were corroborated with additional information from the participant, next of kin, or death certificate. Genkinger et al. (2007) did not find an association of acetaminophen use with bladder cancer. This study was a well-conducted analysis of an established cohort; however, there was less data for acetaminophen exposure compared to aspirin and other analgesics in Genkinger et al. (2007). Cho et al. (2011) found an increased risk of kidney cancer with acetaminophen use in this cohort.

The US Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial study

The US Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial was a well-conducted prospective cohort study that included 98,807 participants, aged 55-74 years at enrollment, from 10 screening centers throughout the US (Karami et al. 2016). Enrollment was conducted between 1993 and 2001, and study participants were followed for up to 16 years. A baseline questionnaire collected medical and lifestyle data, and a supplemental questionnaire was sent in 2006–2007, which collected

information on the number of years participants took acetaminophen, aspirin, and non-aspirin NSAIDs at least once per week. The questionnaire did not distinguish between OTC and prescription forms. Cases were identified by annually mailed questionnaires, with confirmation through medical records. An increased risk of renal cell carcinoma was observed with regular use of acetaminophen (use for at least once per week prior to the supplemental questionnaire). Analyses that were restricted to participants diagnosed greater than two years after completing the supplemental questionnaire suggests that protopathic bias is unlikely. Limitations of this study include possible recall bias from self-reported questionnaires and the use of only a single questionnaire (i.e., the supplemental questionnaire) evaluating drug use.

The Multiethnic Cohort Study

Three studies examined cancer risk associated with acetaminophen use in the Multiethnic Cohort (MEC) Study. The MEC is a prospective cohort study that consists of 215,251 men and women between the ages of 45 and 75 years selected from five racial/ethnic populations (African American, Japanese American, Latino, Native Hawaiian, and white) from Los Angeles County, CA and the state of Hawaii. Exposure assessment was conducted through a baseline questionnaire, and cancer outcomes were identified through linkages to the Los Angeles County Cancer Surveillance Program, the State of California Cancer Registry, and the Hawaii Tumor Registry (all members of the Surveillance, Epidemiology, and End Results Program supported by the National Cancer Institute). The three studies that assessed acetaminophen use did not find an association with stomach cancer (Epplein et al. 2009), breast cancer (Gill et al. 2007), or ovarian cancer (Setiawan et al. 2012).

The Women's Health Initiative Observational Study

Three studies examined cancer risk associated with acetaminophen use in the Women's Health Initiative (WHI) Observational Study (OS) cohort. From 1993 to 1998, the WHI-OS enrolled 93,676 postmenopausal women (50-79 years old) at 40 sites across the US. Medication intake was collected at baseline via an interview-administered questionnaire. Medication data were validated by checking prescription records and pill bottle labels during the interview process. A second clinical visit after 3 years collected additional medication data. Participants reported medical outcomes in annual questionnaires, and cancer cases were confirmed through pathology and medical records. Three studies that used the WHI-OS did not find an association between acetaminophen use and skin cancer (Gamba et al. 2013; Wysong et al. 2014) or breast cancer (Harris et al. 2003).

Cancer Prevention Study II

Three studies examined cancer risk in association with acetaminophen use in the Cancer Prevention Study II (CPS-II). This prospective mortality study of approximately 1.2 million adults was started in 1982 by the American Cancer Society. Enrollment took place in all states in the US, as well as Puerto Rico and the District of Columbia. At baseline, participants completed a questionnaire that included questions about medications. In the early years of the CPS-II study, mortality was assessed every two years via follow-up with volunteers of the ACS. Death certificates were then obtained to ascertain cancer deaths. Later the study was linked to the National Death Index and state cancer registries to identify cancer cases. Three studies utilized this cohort to investigate associations with acetaminophen use: Thun et al. (1993) found an increased risk of rectum cancer mortality; Rodriguez et al. (1998) did not find an association with ovarian cancer mortality; and Jacobs et al. (2011) did not find an association with risk of prostate cancer incidence.

3.1.2 Human epidemiology studies by cancer site

3.1.2.1 Cancers of the urinary tract

The urinary tract consists of the kidneys, renal pelvis, ureters, bladder, and urethra. The kidney forms urine in the nephrons of the renal parenchyma, empties through the minor calyces to the major calyces to the renal pelvis, and into the narrow tubes called ureters. The ureters connect the kidneys to the bladder, which empties via the urethra. Studies that report associations for the urinary tract will thus include kidney cancer cases, as well as other cancers of the urinary tract.

Transitional cell cancer (TCC), also known as *urothelial carcinoma*, is the most common type of urinary tract cancer (>90%). It arises from cells called transitional cells that make up the lining of the renal pelvis (where the ureters meet the kidneys), ureters, bladder and urethra. Other more rare types of cancer include squamous cell carcinoma, small cell carcinoma, and adenocarcinoma (Yaxley 2016).

Several cohort and case-control studies report the International Classification of Diseases (ICD) codes, which help to identify the specific type of cancer. The ICD-9 codes for urinary tract cancers are shown in Table 3 (ICD9Data). ICD versions 8 and 10 have similar groupings.

Table 3. ICD-9 codes for cancers of the urinary tract

ICD Code	Diagnosis
188	Malignant neoplasm of bladder
189	Malignant neoplasm of kidney and other and unspecified urinary organs
189.0	Malignant neoplasm of kidney, except pelvis
189.1	Malignant neoplasm of renal pelvis
189.2	Malignant neoplasm of ureter
189.3	Malignant neoplasm of urethra
189.4	Malignant neoplasm of paraurethral glands
189.8	Malignant neoplasm of other specified sites of urinary organs
189.9	Malignant neoplasm of urinary organ, site unspecified

3.1.2.1.1 Urinary tract cancer (combined or not specified)

Several studies reported associations for the urinary tract; these studies can include kidney cancer cases, as well as other cancers of the urinary tract. The specific organs included are given as reported by the individual study, or are reported as 'not specified.'

Cohort studies

Two cohort studies examined the association between acetaminophen use and cancer of the urinary tract (Friis et al. 2002; Walter et al. 2011a) (Table 4).

Friis et al. (2002) compared cancer incidence among individuals receiving prescriptions for acetaminophen with expected incidence. Details of the study design and cohort are given in Section 3.1.1 above. This study found no association with prescriptions for acetaminophen only (excluding persons with prescriptions for aspirin or other NSAIDs) and cancers of the renal pelvis/ureter (ICD-7, 180.1/180.2)[2] or cancers of the urinary tract combined (ICD-7, 180–181), which included the renal parenchyma, pelvis/ureter, and urinary bladder. The study reported no association of acetaminophen prescription with renal pelvis/ureter when stratified by number of prescriptions; however there were only two exposed cases, which limits the ability of the study to detect an association.

Walter et al. (2011a) assessed acetaminophen use and risk of several cancers in the VITAL cohort (details are given above in Section 3.1.1). Exposure was assessed through a baseline questionnaire and the cohort was followed for a mean of 6.5 years. This study assessed acetaminophen use and risk of urinary tract cancers combined, which included cancers of the kidney, bladder, renal pelvis, ureter, or unspecified

[2] The study used the older ICD-7 codes, which are slightly different than ICD 8-10 codes.

urinary organs. There were no statistically significant associations in men and women combined or in women. High use of acetaminophen was associated with an increased risk of urinary tract cancer in men for low use (HR, 1.17; 95% CI, 0.73–1.87; 29 cases) and high use (HR, 1.15; 95% CI, 0.54–2.41; 10 cases), although neither were statistically significant.

Case-control studies

Six case-control studies were conducted on urinary tract cancers and acetaminophen use: three studies of the renal pelvis and ureter (Linet et al. 1995; Pommer et al. 1999; Ross et al. 1989), two studies of transitional cell cancers that included multiple sites within the urinary tract (Rosenberg et al. 1998; Steineck et al. 1995), and one study of ureter cancer (McCredie and Stewart 1988) (Table 4).

For cancers of the renal pelvis and ureter, all three of the case-control studies reported increased risks associated with acetaminophen use, but none were statistically significant. The highest OR in the study by Linet et al. (1995) was 1.4 (95% CI, 0.5–4.1; 9 exposed cases) for 10 or more years duration of acetaminophen use. Other categories/exposure metrics analyzed (cumulative lifetime use, regular or only use of acetaminophen) reported lower ORs.

For the two studies of transitional cell cancers of the urinary tract, both reported increased risks associated with self-reported acetaminophen use. A population-based case-control study reported an OR of 1.6 (95% CI, 1.1–2.3) with any acetaminophen use (Steineck et al. 1995). The results from a hospital-based case-control study were inconsistent (Rosenberg et al. 1998); a non-statistically significant increase in risk was reported for regular acetaminophen use that began less than one year previously (OR, 1.4; 95% CI, 0.7–2.8; 13 exposed cases). Lower risks were reported for the exposure categories representing higher acetaminophen exposure (regular use that began at least one year previously or regular use that began at least one year previously with at least five years duration).

For ureter cancer, acetaminophen use was associated with a two-fold increased risk for >0.1 and >1 kg of acetaminophen intake that was statistically significant (McCredie and Stewart 1988).

These results for urinary tract cancers, which occur in proximity to kidney cancers, are in accord with the body of literature reporting an increased risk of RCC, kidney cancer, and cancer of the renal pelvis associated with acetaminophen use.

Summary of urinary tract cancer findings

The association between acetaminophen use and cancer of the urinary tract has been assessed in two cohort studies (Friis et al. 2002; Walter et al. 2011a) and six case-control studies: three studies of the renal pelvis and ureter (Linet et al. 1995; Pommer et

al. 1999; Ross et al. 1989), two studies of transitional cell cancers that included multiple sites within the urinary tract (Rosenberg et al. 1998; Steineck et al. 1995), and one study of ureter cancer (McCredie and Stewart 1988).

Significant increases were not observed in the cohort studies of urinary tract cancer (Friis et al. 2002; Walter et al. 2011a). The population-based case-control study of transitional cell cancer had a statistically significant increase in risk with any acetaminophen use (Steineck et al. 1995), whereas the findings from the hospital-based case-control study were not statistically significant (Rosenberg et al. 1998). Both studies had significant limitations. The only study specifically of ureter cancer reported a statistically significant two-fold increased risk for greater than 0.1 kg of lifetime acetaminophen intake (McCredie and Stewart 1988).

Table 4. Cohort and case-control studies of urinary tract cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Urinary Tract – Cohort						
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	**Population:** Danish Cancer registry and prescription database. Cases excluded because of residency outside the county at the date of prescription, parent (of patient) registered as customer, error in the personal identification number, death prior to or at the date of prescription, age younger than 16 years, subjects who had a cancer diagnosis (except nonmelanoma skin cancer) prior to date of first recorded prescription, or died within the first year of follow-up N = 39,946 **Exposure assessment method:** records	**Urinary tract (all), ICD-7, 180-181: SIR, Prescription paracetamol (acetaminophen)**			Age, sex, calendar year	**Exposure information:** Exposure level: ever prescribed acetaminophen and number of prescriptions **Strengths:** Excluded person-time and cancer experience in the first year of follow-up after first acetaminophen prescription. Use of prescription database and linked to cancer registry. Almost complete capture of the population of North Jutland county. **Limitations:** Multiple comparisons performed, no adjustment for confounders such as tobacco smoking or alcohol consumption, lack of data on use of analgesic agents prior to start of prescription database and use of OTC analgesics, degree of compliance, or indications for use. **Confidence in evidence:** This study had some limitations, but is still fairly informative. The primary strength is the use of the prescription database for exposure assessment and linkage to the cancer registry. The main concern is the lack of control for confounders other than matching for age, year, and sex.
		Any	1 (0.7–1.4)	34		
		Pelvis/ureter, ICD-7, 180.1/180.2: SIR, Number of acetaminophen prescriptions				
		Any prescription	0.9 (0.1–3.2)	2		
		1	1.3 (0–7)	1		
		2-4	1.7 (0–9.7)	1		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** VITAL (VITamins And Lifestyle) study 62,841 **Exposure assessment method:** questionnaire	**Urinary tract (all): HR, 10-yr use prior to baseline in men and women combined**			Age, education, race, marital status, height, BMI, physical activity, pack-years of smoking, alcohol intake at 45 yrs, fruit and vegetable intake, red meat intake, multivitamin use, self-rated health, family history of colon, lung, and hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/chronic joint pain, migraine/chronic headaches, use of NSAIDs	**Exposure information:** Regular use defined as ≥1 d/wk for ≥1 yrs **Strengths:** Large sample size. **Limitations:** Limited power in detecting associations between acetaminophen use and risk of individual cancers or cancer subtypes. Analyses were overadjusted, reducing precision to detect a significant effect. **Confidence in evidence:** This study had some limitations, mainly that they over-adjusted for confounding and that there were small group sizes for some of the subgroup analyses. Self-reported exposure, not validated. However, this is a well-established cohort study that was overall well-conducted and is considered informative.
		No use	1	214		
		Low use (<4 d/wk or <4 yrs)	1.1 (0.76–1.59)	50		
		High use (≥4 d/wk and ≥4 yrs)	1.05 (0.6–1.83)	18		
		Trend-test *p*-value: 0.72				
		Urinary tract (all): HR, 10-yr use prior to baseline in women			Confounders controlled for in men and women combined, in addition to: family history of breast cancer, mammogram in past 2 yrs, age at menarche, age at menopause, age at first birth, years of estrogen therapy, years of combined hormone therapy, hysterectomy	
		No use	1	57		
		Low use (<4 d/wk or <4 yrs)	1.07 (0.58–1.97)	21		
		High use (≥4 d/wk and ≥4 yrs)	0.89 (0.38–2.11)	8		
		Trend-test *p*-value: 0.89				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
		Urinary tract (all): HR, 10-yr use prior to baseline in men			Confounders controlled for in men and women combined, in addition to: family history of prostate cancer, history of prostate specific antigen (PSA) testing	
		No use	1	157		
		Low use (<4 d/wk or <4 yrs)	1.17 (0.73–1.87)	29		
		High use (≥4 d/wk and ≥4 yrs)	1.15 (0.54–2.41)	10		
		Trend-test *p*-value: 0.53				
Urinary Tract (combined as specified) – Case-Control						
Ross et al. (1989) Case-Control Los Angeles **Enrollment or follow-up:** 1978-1982	**Population:** Cases: 187; Controls: 187 **Exposure assessment method:** interview	**Renal pelvis and ureter: Unadjusted OR, Analgesics containing acetaminophen**			None	**Exposure information:** Regular use defined as >30 d/yr or >30 consecutive days **Limitations:** No confidence intervals calculated.
		>30 d/yr	1.3 [(0.89–1.86)][a]	36		
		>30 consecutive days	2 [(0.74–2.47)][a]	22		
Linet et al. (1995) Case-Control USA: New Jersey, Iowa, Los Angeles **Enrollment or follow-up:** 1983-1986	**Population:** Cases: 502 (308 renal pelvis, 194 ureter); Controls: 496 **Exposure assessment method:** interview	**Renal pelvis and ureter: OR, Duration (yr) acetaminophen use**			Age, gender, geographic area, smoking	**Exposure information:** Regular use defined as ≥2 doses/week for ≥1 month **Strengths:** Cases histologically confirmed. Population-based controls.
		No regular use	1	385		
		Regular acetaminophen use	1 (0.6–1.8)	35		
		Only acetaminophen use	0.8 (0.3–2.3)	9		
		≤4 yrs	0.9 (0.4–2)	13		
		5-9 yrs	1 (0.4–2.5)	10		
		≥10 yrs	1.4 (0.5–4.1)	9		
		Renal pelvis and ureter: OR, Cumulative lifetime use (kg)				
		No regular use	1	385		
		≤1 kg	1.1 (0.5–2.1)	22		
		>1 kg	1 (0.4–2.3)	13		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Steineck et al. (1995) Case-Control Stockholm, Sweden **Enrollment or follow-up:** 1985-1987	**Population:** Cases: 325; Controls: 393 **Exposure assessment method:** questionnaire	**Squamous or transitional cell carcinoma in the urinary tract (renal pelvis, ureter, urinary bladder, urethra): OR, Acetaminophen use**			Gender, year of birth, smoking, other analgesic use	**Exposure information:** Ever use **Strengths:** Population-based controls.
		No use	1	NR		
		Use	1.6 (1.1–2.3)	119		
Rosenberg et al. (1998) Case-Control several Eastern US cities **Enrollment or follow-up:** 1976-1996	**Population:** Cases: 498 cases; Controls: 8,149 noncancer controls, 6,499 cancer controls **Exposure assessment method:** interview	**Transitional cell cancer (urinary bladder, renal pelvis, and ureter or urethra): OR, Acetaminophen use compared to noncancer controls**			Age, gender, interview year, geographic area	**Exposure information:** Regular use defined as ≥2 d/wk for ≥1 month **Strengths:** Cases confirmed by pathology reports. **Limitations:** Hospital-based controls. **Additional results:** There was no evidence of reverse causation. The authors examined regular use of acetaminophen at least two years before admission: the RR was 1.1 with noncancer controls and 0.9 with cancer controls, and the CIs included 1.0, based on 13 case users. To assess whether more intense acetaminophen use was related to the risk of transitional cell cancer, with daily use for at least a month starting at least a year before admission: the RR was 1.2 with noncancer controls and 0.9 with cancer controls, based on 11 case users.
		Never used	1	348		
		Nonregular use	1.1 (0.9–1.4)	122		
		Regular use that began ≥1 yr previously	1.1 (0.6–1.9)	15		
		<5 yrs duration, regular use ≥1 yr	1.1 (0.5–2.3)	8		
		≥5 yrs duration, regular use ≥1 yr	1.1 (0.5–2.6)	7		
		Regular use that began <1 yr previously	1.4 (0.7–2.8)	13		
Pommer et al. (1999) Case-Control West Berlin, Germany **Enrollment or follow-up:** 1990-1994	**Population:** Cases: 647 (51 renal pelvis, 25 ureter); Controls: 647 **Exposure assessment method:** interview	**Cancer of renal pelvis (ICD-9, 189.1) or ureter (ICD-9, 189.2): OR, Any paracetamol (acetaminophen) use**			Smoking, former smoking, socioeconomic status	**Exposure information:** Regular intake >1 dose/month **Strengths:** Use of population-based controls. **Limitations:** This study can't separate the effect of previous phenacetin intake (banned 1986 in west Germany) and subsequent use of acetaminophen in heavy analgesic users. Methods of control selection were not described in detail. **Confidence in evidence:** NOTE: Partial overlap with McCredie et al. 1995 which enrolled subjects 1989-1991
		No/rare analgesic use	1	31		
		Acetaminophen use	1.64 (0.21–12.49)	6		
		Cancer of renal pelvis (ICD-9, 189.1) or ureter (ICD-9, 189.2): OR, Any acetaminophen use			Smoking, former smoking, socioeconomic status, laxative intake	
		No/rare analgesic use	1	31		
		Acetaminophen use	2.25 (0.28–17.96)	6		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Ureter – Case-Control						
McCredie and Stewart (1988) Case-Control New South Wales, Australia **Enrollment or follow-up:** 1980-1982	**Population:** Cases: 55; Controls: 688 **Exposure assessment method:** questionnaire	**Ureter cancer (ICD-8 189.2): OR, Lifetime paracetamol (acetaminophen) consumption**			Sex, tobacco, phenacetin	**Exposure information:** Use considered ≥ 0.1 kg lifetime consumption **Strengths:** The use of population controls; adjustment for phenacetin use. **Limitations:** Quantitative exposure data (e.g. dose-response) was not presented. There may not have been sufficient latency between collection of exposure data (calculated up to the year of case diagnosis) and onset of disease. **Confidence in evidence:** Potential for information bias: exposure to acetaminophen assessed via self-report, lack of detail on presented the exposure assessment strategy or analysis. The OR associated with 0.1 kg of lifetime acetaminophen use was higher than that for 1 kg of use, and the confidence intervals were wide and not statistically significant.
		None	1	NR		
		≥ 1 kg acetaminophen	2 (0.8–4.5)	NR		
		≥ 0.1 kg acetaminophen	2.5 (1.1–5.9)	NR		

[a] Confidence intervals were calculated by OEHHA.

3.1.2.1.2 Kidney cancer

Kidney cancer is reported in the epidemiologic literature as kidney cancer, renal cell carcinoma (RCC) (including cancer of the renal parenchyma according to the ICD codes), transitional cell carcinoma (TCC) of the kidney, and cancer of the renal pelvis (see Table 3 for ICD codes). The American Cancer Society (ACS) estimates that approximately 73,820 new cases of kidney and renal pelvis cancer will occur in the US (44,120 in men and 29,700 in women), and about 14,770 people (9,820 men and 4,950 women) will die from the disease in 2019 (ACS 2018b). Kidney/renal pelvis cancer makes up about 4.2% of all new cancer cases in the US (NCI 2019c).

RCC (ICD-8,9 code 189.0) is the most common type of kidney cancer; it can be distinguished by its subtypes. Clear cell is the most common subtype of RCC (~70%), followed by papillary, chromophobe, other rare subtypes, and unclassified. A rarer type of kidney cancer is TCC of the renal pelvis (ICD-8,9 code 189.1), which accounts for 7% of all kidney tumors. Cancers of the renal pelvis can also be squamous cell carcinoma or adenocarcinoma, which are rarer than TCC (NCI 2019c).

Potential confounders in the association between kidney cancer (specifically RCC) and acetaminophen use are tobacco smoking and obesity/body mass index (ACS 2017; Curhan et al. 2002; IARC 2019b). Additionally, phenacetin is an important potential confounder when assessing cancer of the renal pelvis (See Table 2).

Phenacetin is an analgesic that causes cancer of the renal pelvis and ureter, although its role in RCC is less clear (IARC 2012). OTC sales have been banned in most countries, and phenacetin was withdrawn from many analgesic compounds worldwide long before the legal bans, which occurred in 1977 in Australia, 1985 in Denmark, 1986 in Germany, and 1987 in Belgium. Phenacetin was withdrawn from the market in 1978 in Canada, 1980 in the UK and 1983 in the USA (IARC 2012). The latency period between phenacetin exposure and renal pelvis cancer appears to be at least 10 years (McCredie et al. 1993).

In studies of cancer of the renal pelvis, we reported the risk estimates associated with phenacetin intake, when available. Failure to detect an increased risk could indicate methodologic deficiencies in a study, such as the exposure assessment strategy or an insufficient latency time between exposure and ascertainment of cancer outcome. This is identified in the study descriptions below.

Details of studies are presented in the tables and are described briefly below.

Cohort studies

Four cohort studies reported on the association between acetaminophen and cancer of the kidney (Cho et al. 2011; Friis et al. 2002; Karami et al. 2016; Walter et al. 2011a) (Table 5).

The study design and limitations of Friis et al. (2002) are discussed above in Section 3.1.1. The study did not find an association between any prescription of acetaminophen and cancer of the "renal parenchyma", which the study specified as "including 'renal cell carcinoma' (ICD-7, 180.3) and 'kidney cancer, not otherwise specified' (ICD-7, 180.0)" (SIR, 1.0; 95% CI, 0.4–2.1; 7 exposed cases). When stratified by number of prescriptions during the study period, the risk was non-significantly increased in individuals with 10 or more prescriptions (SIR, 2.5; 95% CI, 0.7–6.4; 4 exposed cases), and there was a dose response trend with number of increasing prescriptions (*p* value for trend: 0.04). The small number of exposed cases limits the ability of the study to detect an association.

Cho et al. (2011) used data from two large prospective studies, the NHS and the HPFS, to examine acetaminophen use and risk of RCC. Exposure was assessed through biennial questionnaires. This study found positive, but not statistically significant, increases in RCC in men (RR, 1.47; 95% CI, 0.84–2.56), women (RR, 1.26; 95% CI, 0.84–1.88), and men and women combined (RR, 1.32; 95% CI, 0.96–1.84) who reported regular use of acetaminophen at the time of the first interview in the 1990 NHS and 1986 HPFS cohorts. Analyses of duration of acetaminophen use did not demonstrate increases in RCC. However, statistical power was limited since many subgroup analyses were comprised of few cases per group. The authors also conducted subgroup analyses with only clear cell carcinoma, the major histologic subtype of RCC, and found similar results to those for all histologic types of RCC.

Walter et al. (2011a) is discussed above in Section 3.1.1. After a mean follow-up of 6.5 years, this study did not find an increase in kidney cancer in men and women combined for either low (30 cases) or high (11 cases) users of acetaminophen. The histologic subtype of kidney cancer was not specified. The latency period may have been too short to detect an association.

The PLCO Screening Trial was conducted in centers throughout the US, and collected information on acetaminophen use via a supplemental questionnaire (Karami et al. 2016). A statistically significant increase RCC was observed with regular use of acetaminophen (use for at least once per week for three months or longer, at least two years prior to the interview) (HR, 1.68; 95% CI, 1.19–2.39). Analyses that were restricted to participants diagnosed greater than two years after completing the supplementary questionnaire suggests that protopathic bias is unlikely. In subgroup analyses by duration of use, there was an increase in participants with less than 10

years of regular use (HR, 2.09; 95% CI, 1.39–3.14). There was no increase in long-term use (10 or more years) (HR, 1.08; 95% CI, 0.55–2.10); however, statistical power was limited in this group given there were only 10 cases. The trend test *p*-value for duration of use was 0.06. Results did not differ by clear cell histologic subtype.

Nested case-control studies

Two case-control studies nested in health databases, one in the US (Derby and Jick 1996) and one in the UK (Kaye et al. 2001), reported increasing risk of RCC with an increasing number of acetaminophen prescriptions filled (Table 5). Both studies adjusted for smoking and BMI and recorded acetaminophen prescriptions prospectively in the database.

Derby and Jick (1996) conducted a case-control study within the Group Health Cooperative (GHC) Database of Puget Sound, WA USA that enrolled 222 incident cases of renal cancer (ICD-8, 189) diagnosed during the period 1980-1991 and 885 controls matched on sex, age within 1 year, and duration of GHC membership. The GHC was a consumer-owned cooperative health maintenance organization (founded in 1945) that was acquired by Kaiser Permanente in 2015. The GHC maintained its own tumor registry and routinely followed up with the SEER registry. The GHC plan, with roughly 380,000 members in 1994, provided virtually complete prepaid medical coverage for drugs, outpatient care and services in GHC-maintained hospitals, where most of its members sought hospital care. Information captured during GHC members' clinical visits, which included height, weight, occupation and smoking status were available from computer files and clinical records.

Information on all OTC and prescription drugs, including acetaminophen, were obtained from GHC pharmacy files for all cases and controls. GHC pharmacies were free, until 1986 when a small co-payment was required. A previous validation study from the GHC reported 92% agreement in the information obtained from pharmacy files and interviews (Jick 1997). Therefore, the GHC records were a good proxy for acetaminophen use. Being exposed to acetaminophen was defined as having a prescription for acetaminophen or any combination drug that contained acetaminophen. The acetaminophen dose for each category of prescription filled was estimated from the average acetaminophen prescription amount most frequently filled at GHC during the study period, and was as follows: none, 1, 2-9 (<0.2 kg), 10-19 (0.2-0.4 kg), 20-39 (0.5-0.9 kg), and ≥40 (≥1 kg) prescriptions. To account for protopathic bias, exposure to acetaminophen use in the year before diagnosis was not considered in the analysis, as analgesics may have been prescribed to relieve pain symptoms caused by the onset of disease.

Compared to non-users, heavy acetaminophen users (40 or more filled prescriptions per lifetime) experienced a significantly elevated risk of kidney cancer (OR, 2.6; 95% CI,

1.1-6.0; 9 exposed cases) and there was a positive dose-response relationship (p-value for trend = 0.01). These results were from an unadjusted analysis and could be biased due to potential confounding by tobacco smoking and BMI. When adjusted for smoking, BMI, and history of urinary tract infections, the increased risk persisted among heavy acetaminophen users, although the confidence intervals were wide and not statistically significant (OR, 4.5; 95% CI, 0.7–29.9; three exposed cases).

To explore the possibility of protopathic bias, an analysis was conducted by the timing of onset of acetaminophen use as well as the indication for use. Heavy users first took acetaminophen five to 15 years before the case index date, which makes it unlikely that acetaminophen was being used to treat early symptoms of renal cancer. The indications for use (arthritis, lumbar disc, trigeminal neuralgia, and bone disease) of the heavily exposed cases were unrelated to the symptoms of, nor risk factors for, renal cancer.

This study also reported results on bladder cancer (see Section 3.1.2.1.3). The strengths of this study are that it was a large study within a well-defined cohort that prospectively collected information on several variables. The exposure assessment was prospective and likely to be accurate and virtually complete, although it is possible that the records of prescriptions filled or purchase of OTC analgesics may not fully reflect the amount of acetaminophen taken.

Kaye et al. (2001) replicated the results of the American GHC study within the UK GPRD. Details, strengths, and limitations of this database are described above. Acetaminophen exposure was ascertained from routinely collected prescription records and analyzed for the period one to five years before diagnosis. There was a 2-fold increase in the risk of renal cancer (OR, 2.3; 95% CI, 1.0–5.3; 12 exposed cases) associated with 20 or more prescriptions of acetaminophen, after adjusting for smoking, BMI, history of hypertension, and diuretic use. A dose-response relationship of increasing OR with an increasing number of prescriptions was also observed in a linear spline model as well as a categorical data analysis, although a formal test for trend was not conducted. The indication of acetaminophen use was also assessed; none were early symptoms of kidney disease. This study also reported a slightly increased risk of renal pelvis/transitional cell cancer that was not statistically significant (OR, 1.2; 95% CI, 0.4–3.1; 20 exposed cases) and did not adjust for any confounders.

This study had several strengths. The study was large and well suited to study a rare outcome such as kidney cancer; it was population-based, acetaminophen exposure and several key variables were recorded prospectively and routinely into a large database, and it was able to adjust for several important confounders.

Case-control studies

There have been 12 published reports from case-control studies on the association between acetaminophen use and kidney cancer (Chow et al. 1994; Gago-Dominguez et al. 1999; Karami et al. 2016; Kreiger et al. 1993; McCredie et al. 1988; McCredie and Stewart 1988; McCredie et al. 1993; McCredie et al. 1995; McLaughlin et al. 1985; Mellemgaard et al. 1994; Pommer et al. 1999; Rosenberg et al. 1998) (Table 5), including a large pooled analysis of case-control studies of RCC conducted internationally (McCredie et al. 1995) (Table 6). Two case-control studies assessed acetaminophen use through records collected prospectively; they were conducted within a well-defined cohort (Derby and Jick 1996; Kaye et al. 2001) and are reviewed as nested case-control studies above. In the remainder of the case-control studies, cases were verified for diagnosis and acetaminophen exposure was assessed through self-report. Three studies used prompts to aid recall (Gago-Dominguez et al. 1999; McCredie et al. 1995; McLaughlin et al. 1985) and only one study attempted to verify data from self-report (Gago-Dominguez et al. 1999). Population-based controls were enrolled in all but one study, which enrolled hospital-based controls (Rosenberg et al. 1998). Although there may be errors in the data due to self-report that could result in misclassification of exposure, this is expected to be non-differential as acetaminophen has not previously been linked to renal pelvis cancer. There were 10 studies of RCC (Chow et al. 1994; Gago-Dominguez et al. 1999; Karami et al. 2016; Kreiger et al. 1993; McCredie et al. 1988; McCredie et al. 1993; McCredie et al. 1995; McLaughlin et al. 1985; Mellemgaard et al. 1994; Rosenberg et al. 1998), three studies of cancer of the renal pelvis (McCredie et al. 1993; McLaughlin et al. 1985; Pommer et al. 1999), one study of kidney cancer not further specified (McCredie and Stewart 1988) and one study that assessed transitional cell carcinoma, which included kidney cancer (Rosenberg et al. 1998).

The largest case-control analysis, which pooled data from 1732 RCC cases and 2309 controls, was conducted by McCredie et al. (1995). This study pooled data from three previously published studies conducted in Australia (McCredie et al. 1993), Denmark (Mellemgaard et al. 1994), and Minnesota, USA (Chow et al. 1994) as well as additional data from Germany and Sweden. Most of these individual studies are not presented in detail because the results have been superseded by the larger, pooled analysis, with the exception of McCredie et al. (1993), which additionally presented results for cancer of the renal pelvis. Select characteristics of the studies included in this analysis are presented in Table 6.

In McCredie et al. (1995), cases with a verified diagnosis of RCC during the period 1989-1991 were identified through population-based registries in four study areas (Sydney, Australia; Denmark; Uppsala, Sweden; and Minnesota, USA) and through hospitals/pathologists in Germany. Controls were frequency matched by age and

gender and selected from the same study bases as the cases using various methods. Controls were identified through registers covering the entire population (Denmark and Uppsala), electoral rolls (Sydney), residential lists (Germany) or Health Care Financing Administration lists (Minnesota, ages 65-79 years) or random digit dialing (Minnesota, ages 20-64 years).

Although acetaminophen use was assessed through self-report, the exposure assessment strategy was detailed. During an interview using a standardized questionnaire, participants were asked if they had ever taken analgesics (by brand name) 20 or more times in their lives before 1987. An extensive checklist of country-specific brand names was available. For "regular" acetaminophen use (i.e., at least twice a week for one month or longer), further information was sought on age at starting and stopping, and the amount, frequency and duration of consumption. A drug matrix was created, using information from drug compendia and pharmaceutical companies for the amount in milligrams of active ingredient per tablet or sachet of each brand of analgesic and any changes in its composition over time. The matrix was used to calculate each subject's lifetime total consumption of analgesic (e.g. grams of acetaminophen).

Acetaminophen use was generally not associated with RCC in men, but there was a non-statistically significant increased risk in women (OR for ≥0.1 kg acetaminophen, 1.3; 95% CI: 0.9-2.0), with the highest OR observed in the category of highest use (OR >5.0 kg acetaminophen, 2.5; 95% CI, 1.0-6.2; p-value for trend not presented). Phenacetin was not associated with RCC in this study. Limitations of this study were that the reference category included non-regular analgesic users and exposure information was heterogeneous despite significant efforts to harmonize the questionnaires across centers. This study included subjects from five centers in four countries with varying patterns of analgesic use. Country-specific brand names of analgesics were compiled, but different criteria were used to construct the drug lists across the various centers.

McLaughlin et al. (1985) conducted the first case-control study published on the association between acetaminophen and RCC (ICD-8, 189.0) and renal pelvis cancer (ICD-8, 189.1) in an area of Minnesota where incidence and mortality from kidney cancer in the US were unusually high (McLaughlin et al. 1985). Analyses were adjusted for age and cigarette smoking. For RCC, there were some positive associations with acetaminophen use in women but the confidence intervals were wide and not statistically significant. The results were less consistent for men: a non-statistically significant increased risk of RCC was noted in men with ≤36 months regular use (OR, 3.1; 95% CI, 0.9–11.8), but not with more than 36 months of regular use (OR, 0.7; 95% CI, 0.1–3.4). However, It should be noted that this study also reported that phenacetin use was associated with RCC risk in both men and women, with a trend in increasing

risk with increasing exposure, and the highest ORs observed in the highest category of phenacetin use (regular use >36 months). For cancer of the renal pelvis, positive associations, although not statistically significant, were observed for use of acetaminophen-containing analgesics in men and women, although the confidence intervals were too wide to permit a conclusion. This study further attempted to analyze the independent effects of acetaminophen and phenacetin, but the results were not informative because there were too few exposed cases. No conclusion could be reached on the association between acetaminophen use and kidney cancer from this study because:

1) The associations with acetaminophen use were inconsistent. (However, phenacetin use was associated with increased risks of RCC and renal pelvis cancers.)

2) There were few cases that used acetaminophen containing products, and even fewer that used acetaminophen only; thus, there was limited power to detect an effect in this study. Strengths were the detailed exposure assessment, including questions on frequency and duration of analgesic use, and the use of prompts to aid recall. A follow-up case-control study in this area was conducted (Chow et al. 1994) and included in the pooled analysis (McCredie et al. 1995).

A series of case-control studies from Australia were published shortly after the McLaughlin et al. (1985) study, with some overlap of the populations (McCredie et al. 1988; McCredie and Stewart 1988; McCredie et al. 1993; McCredie et al. 1995). These studies reported elevated ORs, not statistically significant, for the association between acetaminophen use and kidney cancer.

Acetaminophen use was associated with a non-statistically significant elevated risk of RCC [reported as cancer of the renal parenchyma (ICD-8 189.0)] in a case-control study using cases reported to the New South Wales Central Cancer Registry between July 1977 and February 1982 (McCredie et al. 1988) (OR, 1.2; 95% CI, 0.8–1.8).

Acetaminophen use was not significantly associated with an increased risk of cancer of the renal pelvis (ICD-8 189.1), nor was there a trend in increasing risk with increasing use (>0.1 kg acetaminophen: OR, 1.24 (95% CI, 0.6–2.3); >1 kg acetaminophen: OR, 0.80 (95% CI, 0.4–1.7)) in a case-control study (McCredie and Stewart 1988) that included cases diagnosed between August 1980 and February 1982 from the New South Wales Central Cancer Registry. ORs for acetaminophen use were adjusted for exposure to phenacetin and tobacco smoking. Kidney disease controls may have been included in these analyses (it is not clearly stated in the paper). Although these controls were selected for conditions unrelated to analgesic use, they may have been similar to cases with respect to risk factors for kidney disease. This could lead to an underestimation of the odds ratio.

A non-significant increased risk associated with acetaminophen use and renal pelvis cancer (OR, 3.27; 95% CI, 0.25–43.02) (ICD-9, 189.1) was reported in a case-control study conducted in Berlin, Germany (Pommer et al. 1999). The methods of control selection were not described in detail in this study and the confidence intervals were very wide, limiting interpretation of these findings. A case-control study of RCC (ICD-9, 189.0) from Ontario, Canada (Kreiger et al. 1993) also found no association with acetaminophen use in women (OR, 0.9; 95% CI, 0.5–2.0) or men (OR, 0.8; 95% CI, 0.3–1.7).

There was also a slight elevation in the risk of RCC associated with acetaminophen use in two other studies, one of which was statistically significant. In a hospital-based case-control study from several eastern US cities, regular acetaminophen use that began less than one year before diagnosis had an OR of 1.8 (95% CI, 0.9–3.5 using non-cancer controls); although there were too few exposed cases and no clear pattern to assess whether there was a trend in increasing risk with increasing use (Rosenberg et al. 1998). In this study, there was concern over whether the control group was appropriate for this study, as they had similar acetaminophen use as cases (approximately 30%). In a large case-control study from Los Angeles, regular acetaminophen use was associated with a statistically significant increased risk of RCC (OR, 1.7; 95% CI, 1.3–2.1) with a significant dose-response relationship (p-value for linear trend not reported) (Gago-Dominguez et al. 1999). Although acetaminophen use was self-reported, a validation study in this population found concordance between self-report and physician records, minimizing concerns over information bias.

Karami et al. (2016) investigated the association between RCC and acetaminophen use in three analyses: 1) a large population-based case-control study (US Kidney Cancer Study), 2) a post-trial observational cohort study (PLCO; discussed above), and 3) a meta-analysis including these data and other publications (discussed below). Among case-control participants of the US Kidney Cancer Study, RCC risk was statistically significantly associated with OTC acetaminophen use (OR, 1.35; 95% CI, 1.01–1.83). There was a positive trend with increasing duration (p-value for trend = 0.01), with a two-fold risk for 10 or more years of use (OR, 2.01; 95% CI, 1.30–3.12). No association with prescription acetaminophen use was detected.

Meta-analyses

There have been two meta-analyses reporting on the association between RCC and acetaminophen use (Figures 3 and 4) (Choueiri et al. 2014; Karami et al. 2016). Both meta-analyses reported an increased risk between acetaminophen use and kidney cancer (meta-relative risk [mRR] for Choueiri et al. (2014), 1.28; 95% CI, 1.15-1.44; mRR for Karami et al. (2016), 1.25; 95% CI, 1.10-1.41; I^2 = 37.73% for case-control and cohort studies), although both studies were limited by either the studies they included or the analyses that were conducted (Figures 3 and 4) (I-squared is a measure of

inconsistency or heterogeneity between studies[3]). Karami et al. (2016) did not include the study by McCredie et al. (1988) on RCC. Choueiri et al. (2014) double counted subjects: it included the McCredie et al. (1995) international pooled analysis, as well as the individual studies that overlapped with the pooled analysis: McCredie et al. (1993), Chow et al. (1994), and Mellemgaard et al. (1994). The only sensitivity analysis conducted by Karami et al. (2016) was restricting the analysis to RCC.

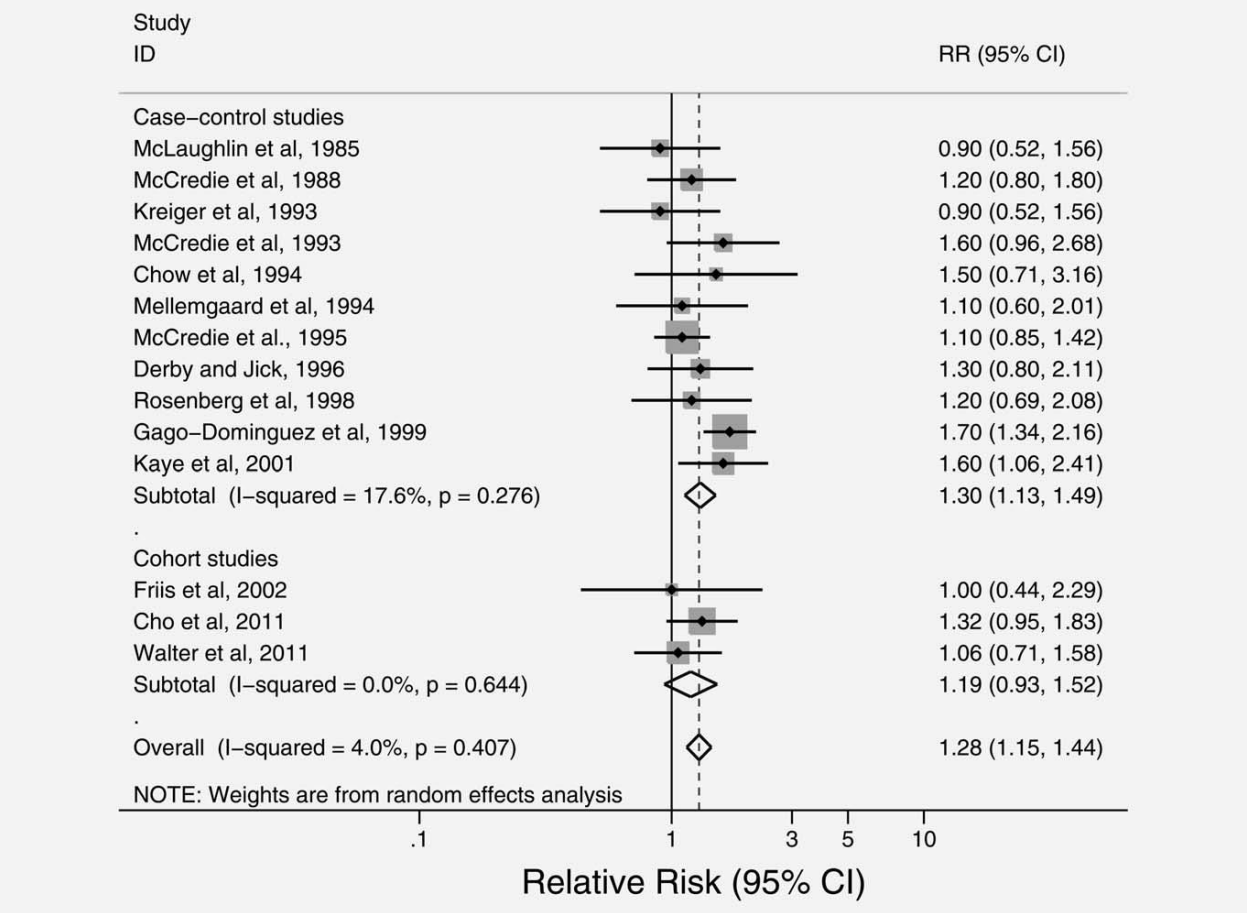


Figure 3. Forest plot from the meta-analysis on acetaminophen use and kidney cancer by Choueiri et al. (2014)

[3] The I^2 statistic describes the percentage of variation across studies that is due to heterogeneity instead of chance and can be directly compared between meta-analyses with different numbers of studies and different types of outcome data (Higgins et al. 2003).

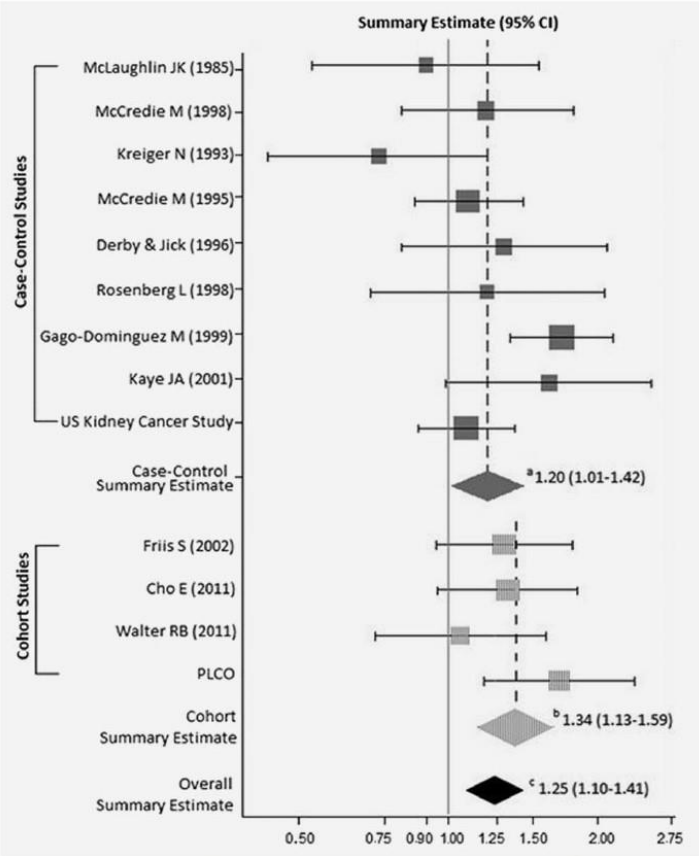


Figure 4. Forest plot from the meta-analysis on acetaminophen use and kidney cancer by Karami et al. (2016)

Summary of kidney cancer findings

Several epidemiological studies have assessed the hypothesis of whether acetaminophen, a metabolite of the analgesic phenacetin, causes cancer of the kidney. Phenacetin has been identified as a cause of cancer of the renal pelvis and ureter, although its role in RCC is less clear (IARC 2012). Kidney cancer is fairly uncommon; RCC is the most common subtype, while cancer of the renal pelvis is rare.

The association between the use of the analgesic acetaminophen and kidney cancer was assessed in four cohort studies (Cho et al. 2011; Friis et al. 2002; Karami et al. 2016; Walter et al. 2011a), two nested case-control studies (Derby and Jick 1996; Kaye et al. 2001), and 12 publications from case-control studies (Chow et al. 1994; Gago-Dominguez et al. 1999; Karami et al. 2016; Kreiger et al. 1993; McCredie et al. 1988; McCredie and Stewart 1988; McCredie et al. 1993; McCredie et al. 1995; McLaughlin et al. 1985; Mellemgaard et al. 1994; Pommer et al. 1999; Rosenberg et al. 1998). Twelve studies reported on RCC (Cho et al. 2011; Chow et al. 1994; Gago-Dominguez et al. 1999; Karami et al. 2016; Kreiger et al. 1993; McCredie et al. 1988; McCredie et al. 1993; McCredie et al. 1995; McLaughlin et al. 1985; Mellemgaard et al. 1994; Rosenberg et al. 1998), and only four assessed cancer of the renal pelvis (Kaye et al.

2001; McCredie et al. 1993; McLaughlin et al. 1985; Pommer et al. 1999), reflecting the rarity of this subtype of kidney cancer.

Tobacco smoking and BMI are important confounders in the association between kidney cancer (particularly RCC) and acetaminophen use. Phenacetin is an analgesic that was largely discontinued in the 1970s and 1980s as it was found to cause cancer of the renal pelvis and ureter (IARC 2012); it is therefore an important factor to adjust for in analyses of cancer of the renal pelvis in studies that assessed exposure during the years that phenacetin was available.

A Danish cohort study that assessed acetaminophen use through prescription records found a non-statistically signficant increased risk of RCC (Friis et al. 2002). However, this study presented SIRs that were calculated using the general population from the same Danish region as the cases as a reference, and thus may differ from cases in the profile of important potential confounders, such as tobacco smoking or BMI, for which this study was unable to account. An American cohort study that collected acetaminophen use prospectively through self-report observed a statistically significant increased risk of RCC with regular and less than 10 years of use, but not with 10 or more years of use, after adjustment for BMI, smoking, and other potential confounders (Karami et al. 2016). A cohort study from Washington state (Walter et al. 2011a) showed no significant increase in risk, and a study combining data from two large American prospective studies showed positive, but not statistically significant, increases in RCC in men and women associated with regular acetaminophen use (Cho et al. 2011).

The two most informative studies were large case-control studies nested in health databases in the US and UK. Acetaminophen prescriptions were recorded prospectively, and therefore the exposure assessment method was less subject to information bias as compared to self-report (Derby and Jick 1996; Kaye et al. 2001). Both of these studies reported a statistically significant increasing risk of RCC with an increasing number of acetaminophen prescriptions filled. Kaye et al. (2001) adjusted for smoking and BMI.

The case-control studies generally reported small or modest but non-statistically significant increases in risk for both RCC (Cho et al. 2011; Gago-Dominguez et al. 1999; Karami et al. 2016; McCredie et al. 1988; McCredie and Stewart 1988; McCredie et al. 1995; McLaughlin et al. 1985; Rosenberg et al. 1998) and cancer of the renal pelvis (McCredie and Stewart 1988; McCredie et al. 1993; McLaughlin et al. 1985; Pommer et al. 1999). Several of the individual studies were not informative on their own because of limited statistical power due to the rarity of kidney cancer, small numbers of exposed cases and/or crude exposure assessments. The exceptions were the two largest case-control studies (Gago-Dominguez et al. 1999; Karami et al. 2016) and a large pooled analysis of case-control studies (McCredie et al. 1995), which reported

roughly two-fold increased risk of RCC (either borderline significant or statistically significant) or positive dose response relationships.

Two meta-analyses also found a statistically significant increased risk between acetaminophen use and kidney cancer (Choueiri et al. 2014; Karami et al. 2016) and RCC specifically (Karami et al. 2016), although both analyses had their limitations.

The studies were conducted in different geographic regions, with different designs, and different time periods, which can minimize the possibility of chance. Most studies accounted for protopathic bias by excluding the year before case diagnosis from the analysis. Population-based controls were enrolled in all but one case-control study (Rosenberg et al. 1998), which helps to minimize the possibility of selection bias. Cases were generally verified for diagnosis, minimizing concerns of outcome misclassification. Acetaminophen exposure was assessed through self-report in the (non-nested) case-control studies and although errors in recall may result in misclassification of exposure, this is expected to be non-differential (as acetaminophen has not previously been linked to renal pelvis cancer) and bias the risk estimates towards the null. The most informative studies adjusted for the important confounders, tobacco smoking and BMI. Several studies additionally assessed confounding by indication by stratifying on the indications for acetaminophen use.

All of the four cohort studies that reported on the association between acetaminophen and cancer of the kidney found an increased risk, which was statistically significant in Karami et al. (2016). All but the Danish cohort adjusted for tobacco smoking and BMI. Several studies (Derby and Jick 1996; Gago-Dominguez et al. 1999; Karami et al. 2016; Kaye et al. 2001) also reported dose-response relationships, strengthening the credibility of these findings. The most informative studies prospectively collected data on acetaminophen use in pharmaceutical records databases, ensuring that exposure occurred before cancer onset (Derby and Jick 1996; Friis et al. 2002; Kaye et al. 2001).

Table 5. Cohort and case-control studies of kidney cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Kidney (renal cell carcinoma, RCC) – Cohort						
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	**Population:** Danish Cancer registry and prescription database. Cases excluded because of residency outside the county at the date of prescription, parent (of patient) registered as customer, error in the personal identification number, death prior to or at the date of prescription, age younger than 16 years, subjects who had a cancer diagnosis (except nonmelanoma skin cancer) prior to date of first recorded prescription, or died within the first year of follow-up N = 39,946 **Exposure assessment method:** records	**ICD-7, 180.0/180.3[a]: SIR, Number of acetaminophen prescriptions**			Age, sex, calendar year	**Exposure information:** Exposure level: ever prescribed acetaminophen and number of prescriptions **Strengths:** Excluded person-time and cancer experience in the first year of follow-up after first acetaminophen prescription. Use of prescription database and linked to cancer registry. Almost complete capture of the population of North Jutland county. **Limitations:** Multiple comparisons performed, no adjustment for confounders such as tobacco smoking or alcohol consumption, lack of data on use of analgesic agents prior to start of prescription database and use of OTC analgesics, degree of compliance, or indications for use. **Confidence in evidence:** This study had some limitations, but is still fairly informative. The primary strength is the use of the prescription database for exposure assessment and linkage to the cancer registry. The main concern is the lack of control for confounders other than matching for age, year, and sex.
		Any prescription	1 (0.4–2.1)	7		
		2-4	0.6 (0–3.2)	1		
		5-9	1.8 (0.2–6.4)	2		
		≥10	2.5 (0.7–6.4)	4		
		Trend-test *p*-value: 0.04				
Cho et al. (2011) Cohort US **Enrollment or follow-up:** 1990-2006 (NHS), 1986-2006 (HPFS); 16 (women), 20 (men) yrs follow-up time	**Population:** Nurses' Health Study and Health Professionals Follow-up Study N=77,525 women; 49,403 men **Exposure assessment method:** questionnaire	**RR, Baseline medication use in women & men pooled**			Age in months, calendar year, smoking status, BMI, history of hypertension, physical activity, fruit intake, vegetable intake, alcohol intake	**Exposure information:** Exposure level: Regular use (≥2 times/wk) at baseline **Strengths:** Information on use of analgesics ascertained multiple times during follow-up. High follow-up rates (97% in NHS; 91% in HPFS). **Limitations:** Confounding by indication (eg, patients with RCC started to take analgesics before diagnosis to treat the symptoms) may have been an issue for these widely used drugs. Because phenacetin was available in the US up to the mid-1980s, results with
		No regular use	1	289		
		Regular use	1.32 (0.96–1.84)	44		
		RR, Cumulative duration of regular use in women & men				
		No regular use	1	231		
		>0 to <4 y	0.94 (0.69–1.3)	50		
		4 to <10 y	1.03 (0.61–1.74)	16		
		≥10 y	1.05 (0.65–1.69)	21		
		Trend-test *p*-value: 0.90				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
		RR, Baseline medication use in women			Smoking status, BMI, history of hypertension, physical activity, fruit, vegetable intake, alcohol, age in months, calendar year, parity	follow-up started in 1986 and 1990 might have been confounded by past use of phenacetin. Small number of cases in some subgroup analyses. **Confidence in evidence:** Low concerns about most potential biases. The NHS and HPFS are well-established cohorts; multiple follow-up questionnaires were administered. The main concern is the use of self-reported data. Overall, this is an informative study.
		No regular use	1	123		
		Regular use	1.26 (0.84–1.88)	30		
		RR, Cumulative duration of regular use in women				
		No regular use	1	93		
		>0 to <4 y	0.93 (0.62–1.41)	31		
		4 to <10 y	0.86 (0.44–1.68)	10		
		≥10 y	1.12 (0.65–1.93)	17		
		Trend-test *p*-value: 0.87				
		RR, Baseline medication use in men			Age in months, calendar year, smoking status, BMI, history of hypertension, physical activity, fruit, vegetable intake, alcohol	
		No regular use	1	166		
		Regular use	1.47 (0.84–2.56)	14		
		RR, Cumulative duration of regular use in men				
		No regular use	1	138		
		>0 to <4 y	0.97 (0.59–1.57)	19		
		4 to <10 y	1.37 (0.59–3.17)	6		
		≥10 y	0.83 (0.3–2.29)	4		
		Trend-test *p*-value: 0.98				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** VITAL (VITamins And Lifestyle) study 62,841 **Exposure assessment method:** questionnaire	**HR, 10-yr use prior to baseline in men and women combined**			Age, education, race, marital status, height, BMI, physical activity, pack-years of smoking, alcohol intake, fruit, vegetable, red meat intake, multivitamin use, self-rated health, family history of colon, lung, hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/ chronic joint pain, migraine/ chronic headaches, NSAIDs	**Exposure information:** Regular use defined as ≥1 d/wk for ≥1 yrs **Strengths:** Large sample size. **Limitations:** Limited power in detecting associations between acetaminophen use and risk of individual cancers or cancer subtypes. Analyses were overadjusted, reducing precision to detect a significant effect. **Confidence in evidence:** This study had some limitations, mainly that they over-adjusted for confounding, there were small group sizes for some of the subgroup analyses, and the follow-up time was fairly short (6.5 yrs). Self-reported exposure, not validated. However, this is a well-established cohort study that was overall well-conducted and is considered informative.
		No use	1	120		
		Low use (<4 d/wk or <4 yrs)	1.11 (0.69–1.79)	30		
		High use (≥4 d/wk and ≥4 yrs)	0.96 (0.46–1.98)	11		
		Trend-test *p*-value: 0.91				
Karami et al. (2016) Cohort US **Enrollment or follow-up:** 1993-2009; up to 16 yrs follow-up time	**Population:** Participants in the US Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO), aged 55-74 yr N=98,807 **Exposure assessment method:** questionnaire	**ICD-O-2, 649: HR, Regular use**			Sex, BMI, education, race, smoking status, hypertension, study center	**Exposure information:** Regular use defined as ≥once per week **Strengths:** Large cohort size. Conducted sensitivity analyses. **Limitations:** Small group size in some subgroups of long-term users. **Confidence in evidence:** The main limitation was that exposure was assessed through self-reported questionnaire. Otherwise it is a well-conducted study and can be considered informative overall.
		No	1	73		
		Yes	1.68 (1.19–2.39)	59		
		<10 yr	2.09 (1.39–3.14)	36		
		≥10 yrs	1.08 (0.55–2.1)	10		
		Trend-test *p*-value: 0.06				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Kidney (RCC) – Nested Case-Control						
Derby and Jick (1996) Nested Case-Control Puget Sound, WA USA **Enrollment or follow-up:** 1980-1991	**Population:** Group Health Cooperative (GHC) Cases: 222; Controls: 885 **Exposure assessment method:** records	**ICD-8, 189; Malignant neoplasm of other and unspecified urinary organs: Unadjusted OR, Acetaminophen - number prescriptions filled (Lifetime consumption)**			None	**Exposure information:** NR **Strengths:** Exposure information routinely collected prospectively in pharmacy records of both prescription and OTC use. For each subject, the number of prescriptions filled is known. Population-based within a well-defined cohort. **Limitations:** Information on confounding factors was complete for less than half of the participants. **Additional results:** The risk patterns within strata of age and sex were similar to those in the unstratified population. Results of the crude analyses were similar to that in the total population. The risk patterns within strata of age and sex were similar to those in the unstratified population. **Confidence in evidence:** Well-designed study, strong exposure assessment, data collected prospectively from records with 92% correlation with data obtained from interview. Data on duration of acetaminophen use were available, but not analyzed to reflect cumulative exposure. Information on confounding was available for only three cases in the highest exposure category.
		None	1	84		
		User at entry (<40 prescriptions & prescription filled in the year of GHC entry)	1.3 (0.8–2.1)	28		
		1	0.9 (0.6–1.5)	26		
		2-9 (<0.2 kg)	1.3 (0.9–1.9)	61		
		10-19 (0.2-0.4 kg)	1.3 (0.6–2.8)	9		
		20-39 (0.5-0.9 kg)	1.9 (0.7–5.6)	5		
		≥40 (≥1 kg)	2.6 (1.1–6)	9		
		Trend-test *p*-value: 0.01				
		ICD-8, 189; Malignant neoplasm of other and unspecified urinary organs: Adjusted OR, Acetaminophen - number prescriptions filled (Lifetime consumption)			Smoking, BMI, history of urinary tract infection	
		None	1	84		
		≥40	4.5 (0.7–29.9)	3		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Kaye et al. (2001) Nested Case-Control UK **Enrollment or follow-up:** 1995–1998	**Population:** Cases: 109; Controls: 434 **Exposure assessment method:** records	**OR, Acetaminophen - number prescriptions filled**			BMI, history of hypertension, diuretic use, smoking, age, sex, general practice, duration of prescription in the database, index date	**Exposure information:** NR **Strengths:** Population based design, acetaminophen prescriptions and demographic variables were prospectively and routinely collected, dose-response analyses conducted through two modelling strategies (p-value for trend not reported). **Limitations:** OTC use was not collected, although a previous study showed that women in the GPRD were unlikely to take acetaminophen without a prescription. **Confidence in evidence:** Prospectively collected data, population-based design, adequate control for potential confounders, thorough analysis to rule out confounding by indication. Exposure assessed by medical records.
		None	1	55		
		1-5	1.4 (0.9–2.4)	31		
		6-19	2.1 (0.9–4.6)	11		
		≥20	2.3 (1–5.3)	12		
Kidney (RCC) – Case-Control						
McCredie et al. (1995) Case-Control International (Sydney, Australia; Denmark; Uppsala, Sweden; Minnesota, USA; Berlin, Heidelberg, Germany) **Enrollment or follow-up:** 1989-1991	**Population:** Cases: 1732; Controls: 2309 **Exposure assessment method:** interview	**OR, Men and women pooled - lifetime intake before 1987**			Center, age, BMI, pack years tobacco, gender	**Exposure information:** Reference group included those who had never taken analgesics, those who had never been regular takers, and regular takers with a lifetime total of < 0.1 kg of any analgesic. Intake of acetaminophen self-reported. **Strengths:** Large sample size, dose-response analysis, highly exposure category. **Limitations:** Reference category included persons potentially exposed to acetaminophen at a level of two 500 mg acetaminophen tablets 2X/week for 1 year. Sensitivity analyses excluding these exposed persons from the reference group were not conducted. The exposure data were heterogeneous: subjects were included from 5 centers in4 countries with varying patterns of analgesic use. Different criteria were used to construct the drug lists across the various centers. No visual aid was offered to the respondents during the interviews to assist in recall and there was no
		Reference group	1	1313		
		<0.1 kg	0.7 (0.5–1.2)	34		
		≥0.1 kg	1.1 (0.9–1.5)	119		
		0.1-1.0 kg	1.1 (0.8–1.6)	68		
		1.1-5.0 kg	0.9 (0.6–1.5)	31		
		>5.0 kg	1.9 (0.9–3.9)	20		
		Trend-test *p*-value: 0.2				
		OR, Women - lifetime intake before 1987			Center, age, BMI, pack years tobacco	
		Reference group	1	474		
		<0.1 kg	1 (0.5–1.9)	19		
		≥0.1 kg	1.3 (0.9–2)	64		
		0.1-1.0 kg	1.3 (0.8–2.1)	32		
		1.1-5.0 kg	1.1 (0.6–-2.0)	18		
		>5.0 kg	2.5 (1–6.2)	14		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
		OR, Men - lifetime intake before 1987			Center, age, BMI, pack years tobacco	attempt to validate self-reported usage of prescription medications. **Confidence in evidence:** This study overlapped partially with previously published studies (McCredie et al. 1993, Chow et al. 1994, Mellemgaard et al.1994, Pommer et al. 1999). No concerns for selection bias, although non-differential exposure misclassification due to self-report was possible. Reference group included users for reasons described in the paper and sensitivity analysis including reference group of nonusers was not reported.
		Reference group	1	839		
		<0.1 kg	0.6 (0.3–1.1)	15		
		≥0.1 kg	1 (0.7–1.4)	55		
		0.1-1.0 kg	1.1 (0.7–1.7)	36		
		1.1-5.0 kg	0.8 (0.4–1.6)	13		
		>5.0 kg	1.1 (0.3–4.0)	6		
McLaughlin et al. (1985) Case-Control Minnesota, USA (Minneapolis-St Paul 7-county metropolitan area) **Enrollment or follow-up:** 1974-1979	**Population:** Cases: 495 RCC (313 men, 182 women); Controls: 697 (428 men, 269 women) **Exposure assessment method:** interview	**RCC (ICD-8, 189.0): OR, Women - acetaminophen-containing analgesics**			Age, cigarette smoking	**Exposure information:** NR **Strengths:** Population-based. High response rates for cases and controls. Information sought on frequency and duration of analgesic use. **Limitations:** Few subjects took acetaminophen only (4 cases, 7 controls). Small number of renal pelvis cancers. **Confidence in evidence:** This study alone does not permit a conclusion on the association between acetaminophen use and kidney cancer. There was limited power to detect an effect of acetaminophen; few cases used acetaminophen containing products, and even fewer used acetaminophen only. The confidence intervals were wide. In exposure-responses analyses, there was no clear trend for acetaminophen containing products (even after control for phenacetin use) for RCC or renal pelvis cancer in men, whereas, as expected, increasing phenacetin use was associated with increased risks of RCC and renal pelvis cancers. No major concerns about selection bias, outcome misclassification, differential exposure misclassification/recall bias, or confounding,
		Never	1	122		
		Ever	1.2 (0.8–1.9)	60		
		Irregular use	1.4 (0.8–2.2)	53		
		Regular use, ≤36mo	0.4 (0.1–2.8)	2		
		Regular use, >36mo	1.2 (0.3–4.6)	5		
		RCC (ICD-8, 189.0): OR, Men - acetaminophen-containing analgesics				
		Never	1	230		
		Ever	0.7 (0.5–1)	83		
		Irregular use	0.7 (0.5–1)	70		
		Regular use, ≤36mo	3.1 (0.9–11.8)	10		
		Regular use, >36mo	0.7 (0.1–3.4)	3		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
McCredie et al. (1988) Case-Control New South Wales, Australia **Enrollment or follow-up:** 1977-1982	**Population:** Cases: 360; Controls: 985 **Exposure assessment method:** questionnaire	**RCC (ICD-8, 189.0): OR, Any paracetamol (acetaminophen) use in men and women combined**			Age, sex	**Exposure information:** Users considered those who took ≥0.1 kg **Strengths:** Population-based, histologically verified diagnoses. **Limitations:** Dose-response analyses not presented for acetaminophen even though quantitative data were collected. Potential confounding by tobacco smoking. **Confidence in evidence:** Information bias/non-differential exposure misclassification is possible due to self-report of analgesic use. Potential confounding due to lack of adjustment for tobacco smoking.
		≥0.1 kg	1.2 (0.8–1.8)	55		
Kreiger et al. (1993) Case-Control Ontario, Canada **Enrollment or follow-up:** 1986-1987	**Population:** Cases: 518 (312 men, 202 women); Controls: 1381 (664 men, 705 women) **Exposure assessment method:** questionnaire	**RCC (ICD-9, 189.0): OR, Women - acetaminophen use**			Age, active cigarette smoking status, combined Quetelet index (BMI)	**Exposure information:** Exposed defined as use ≥ every other day for ≥ 1 month prior to 1980 **Strengths:** Population-based. **Limitations:** Few acetaminophen users, dose-response analyses were not conducted. **Confidence in evidence:** Quantitative data were not reported and number of exposed cases may have been too low to detect an effect. Study design and analyses were adequate.
		No phenacetin or acetaminophen	1	166		
		Acetaminophen only	0.9 (0.5–2)	10		
		Any acetaminophen	0.6 (0.4–1.6)	10		
		RCC (ICD-9, 189.0): OR, Men - acetaminophen use				
		No phenacetin or acetaminophen	1	265		
		Acetaminophen only	0.8 (0.3–1.7)	8		
		Any acetaminophen	0.9 (0.4–1.8)	11		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Rosenberg et al. (1998) Case-Control several Eastern US cities **Enrollment or follow-up:** 1976-1996	**Population:** Cases: 383; Controls: 8149 noncancer controls, 6499 cancer controls **Exposure assessment method:** interview	**OR, Acetaminophen use**			Age, gender, interview year, geographic area	**Exposure information:** Regular use defined as ≥2 days/wk for ≥1 month **Strengths:** Thorough analyses were conducted to rule out confounding by indication. **Limitations:** Hospital controls have higher prevalence of acetaminophen use than the general population, roughly equal to the prevalence in cases (~30%) (risk estimates not reported here). **Confidence in evidence:** Exposure was self-reported and the assessment strategy was not described in detail, the hospital controls had a higher exposure to acetaminophen than the general population and roughly the same as cases, ~30%.
		Never used	1	254		
		Nonregular use (<2 days/week for a month)	1.1 (0.9–1.4)	103		
		Regular use that began <1 yr previously	1.8 (0.9–3.5)	11		
		Regular use that began ≥1 yr previously	1.2 (0.7–2.1)	14		
		Regular use ≥1 yr, <5 yrs duration,	1.3 (0.6–2.7)	8		
		Regular use ≥1 yr, ≥5 yrs duration	1.1 (0.5–2.6)	6		
Gago-Dominguez et al. (1999) Case-Control Los Angeles, USA **Enrollment or follow-up:** 1986-1994	**Population:** Cases: 1276; Controls: 1204 **Exposure assessment method:** interview	**OR, Acetaminophen use**			Education, BMI, history of hypertension, cigarettes smoked per day, current smoking status, regular use amphetamines	**Exposure information:** Regular use defined as ≥2 times/week for ≥1 month **Strengths:** Attempted to validate self-reported usage of prescription analgesics with physician records. No difference in concordance between physician records and self-reports between the case and the control groups. Visual aids were used to assist self-reported recall. Interviewer bias was minimized because virtually all matched case–control pairs were interviewed by the same interviewer. Phenacetin use was adjusted for. **Confidence in evidence:** Exposure was self-reported but validation of self-report found concordance between physician records and self-report.
		No regular/ irregular use of analgesics	1	616		
		Regular use	1.7 (1.3–2.1)	300		
		Exclusive acetaminophen use	1.6 (1.1–2.4)	NR		
		OR, Acetaminophen maximum weekly dose (g)				
		<2	1.3 (0.9–1.9)	67		
		2 – <4	1.7 (1.1–2.6)	63		
		4 – <8	1.8 (1.2–2.6)	83		
		≥8	2.1 (1.3–3.3)	79		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Karami et al. (2016) Case-Control US Kidney Cancer Study: Detroit, MI and Chicago, IL **Enrollment or follow-up:** 2002-2007	**Population:** US Kidney Cancer Study Cases: 1217; Controls: 1235 **Exposure assessment method:** interview	**ICD-O-3, 64.9: OR, OTC acetaminophen use and duration**			Sex, race, hypertension, BMI, smoking status, age, education, study center, family history of cancer and dialysis treatment	**Exposure information:** Regular use defined a ≥1 time/wk for ≥3 months, ≥2 years prior to interview **Strengths:** Population-based design, inclusion of only histologically confirmed RCC cancers **Limitations:** Not possible to assess intensity or consistency of use, and exposure misclassification in defining regular use may have biased results towards the null. **Confidence in evidence:** Plausible that those who take OTC acetaminophen consume more than those who are prescribed - therefore higher OR for OTC than prescription. Combining both OTC and Rx could dilute these results.
		No	1	1081		
		Ever	1.35 (1.01–1.83)	133		
		<1 yr	1.08 (0.44–2.64)	12		
		1 - <5 yr	0.79 (0.41–1.53)	24		
		5 - <10 yr	1.37 (0.71–2.65)	27		
		≥10 yr	2.01 (1.3–3.12)	63		
		Trend-test *p*-value: 0.01				
Kidney (urinary pelvis/UUT) – Nested Case-Control						
Kaye et al. (2001) Nested Case-Control UK **Enrollment or follow-up:** 1995–1998	**Population:** Cases: 20; Controls: 434 **Exposure assessment method:** records	**Renal pelvis/transitional cell cancer: Unadjusted OR, Acetaminophen use**			None	**Exposure information:** NR **Strengths:** Population based design, acetaminophen prescriptions and demographic variables were prospectively and routinely collected, dose-response analyses conducted through two modelling strategies (p-value for trend not reported). **Limitations:** OTC use was not collected, although a previous study showed that women in the GPRD were unlikely to take acetaminophen without a prescription. **Confidence in evidence:** Prospectively collected data, population-based design, adequate control for potential confounders, thorough analysis to rule out confounding by indication. Exposure assessed by medical records.
		1 - 5 years before the index date	1.2 (0.4–3.1)	20		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Kidney (urinary pelvis/UUT) – Case-Control						
McLaughlin et al. (1985) Case-Control Minnesota, USA (Minneapolis-St Paul 7-county metropolitan area) **Enrollment or follow-up:** 1974-1979	**Population:** Cases: 74 renal pelvis cancer (50 men, 24 women); Controls: 697 (428 men, 269 women) **Exposure assessment method:** interview	**Renal pelvis (ICD-8, 189.1): OR, Women - acetaminophen-containing analgesics**			Age, cigarette smoking	**Exposure information:** NR **Strengths:** Population-based. High response rates for cases and controls. Information sought on frequency and duration of analgesic use. **Limitations:** Few subjects took acetaminophen only (4 cases, 7 controls). Small number of renal pelvis cancers. **Confidence in evidence:** This study alone does not permit a conclusion on the association between acetaminophen use and kidney cancer. There was limited power to detect an effect of acetaminophen; few cases used acetaminophen containing products, and even fewer used acetaminophen only. The confidence intervals were wide. In exposure-responses analyses, there was no clear trend for acetaminophen containing products (even after control for phenacetin use) for RCC or renal pelvis cancer in men, whereas, as expected, increasing phenacetin use was associated with increased risks of RCC and renal pelvis cancers. No major concerns about selection bias, outcome misclassification, differential exposure misclassification/recall bias, or confounding,
		Never	1	13		
		Ever	2.2 (0.8–5.8)	11		
		Irregular use	1.8 (0.6–5.4)	7		
		Regular use ≤ 36 mo	2.4 (0.1–35.6)	1		
		Regular use ≥ 36 mo	5.8 (0.8–40)	3		
		Trend-test *p*-value: <0.05				
		Renal pelvis (ICD-8, 189.1): OR, Women - long-term use of acetaminophen-containing analgesics				
		No phenacetin or acetaminophen	1	9		
		No phenacetin, > 36 mo acetaminophen	10.4 (0.2–768.3)	1		
		Renal pelvis (ICD-8, 189.1): OR, Men - acetaminophen-containing analgesics				
		Never	1	33		
		Ever	1.2 (0.6–2.5)	17		
		Irregular use	1 (0.5–2.2)	13		
		Regular use ≤ 36 mo	4.7 (0.5–43.7)	2		
		Regular use ≥ 36 mo	2.5 (0.3–17.7)	2		
		Renal pelvis (ICD-8, 189.1): OR, Men - long-term use of acetaminophen-containing analgesics				
		No phenacetin or acetaminophen	1	23		
		No phenacetin, > 36 mo acetaminophen	-	0		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
McCredie and Stewart (1988) Case-Control New South Wales, Australia **Enrollment or follow-up:** 1980-1982	**Population:** Cases: 73; Controls: 688 **Exposure assessment method:** questionnaire	**Renal pelvis (ICD 8, 189.1): OR, Lifetime acetaminophen consumption**			Sex, tobacco, phenacetin	**Exposure information:** Use considered ≥ 0.1 kg lifetime consumption **Strengths:** Population-based; adjustment for phenacetin use. **Limitations:** Exposure assessment strategy was not described in detail. Quantitative exposure data (e.g. dose-response) was not presented. There may not have been sufficient latency between collection of exposure data (calculated up to the year of case diagnosis) and onset of disease **Confidence in evidence:** Potential for information bias: exposure to acetaminophen assessed via self-report, lack of detail on presented the exposure assessment strategy or analysis. The OR associated with 0.1 kg of lifetime acetaminophen use was higher than that for 1 kg of use, and the confidence intervals were wide and not statistically significant.
		None	1	NR		
		≥ 1 kg acetaminophen	0.8 (0.4–1.7)	NR		
		≥ 0.1 kg acetaminophen	1.24 (0.6–2.3)	NR		
McCredie et al. (1993) Case-Control New South Wales, Australia **Enrollment or follow-up:** 1989-1990	**Population:** Cases: 147; Controls: 523 **Exposure assessment method:** questionnaire	**Renal pelvis (ICD-9, 189.1): OR, Paracetamol (acetaminophen) only**			Age, sex, interview method, cigarette smoking, educational level	**Exposure information:** Ever exposure defined as ≥ 20 times in their lifetime **Strengths:** The diagnoses were confirmed through histology or other methods. A single interviewer obtained data. Frequency and duration of analgesic use were queried. **Limitations:** Exposure-response analyses were not conducted. **Confidence in evidence:** Detailed exposure and outcome assessment and analyses.
		Acetaminophen only	1.3 (0.6–2.7)	21		
		Renal pelvis (ICD-9, 189.1): OR, Amount of acetaminophen in any form			Age, sex, interview method, cigarette smoking, educational level, phenacetin/ aspirin compounds	
		Non-consumers	1	106		
		Acetaminophen in any form	1.3 (0.7–2.4)	40		
		≤ 0.48 kg	0.9 (0.2–3)	5		
		0.49 - 1.36 kg	0.9 (0.3–2.5)	10		
		≥ 1.37 kg	2 (0.9–4.4)	26		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Pommer et al. (1999) Case-Control West Berlin, Germany **Enrollment or follow-up:** 1990-1994	**Population:** Cases: 51 renal pelvis; Controls: 647 **Exposure assessment method:** interview	**Renal pelvis cancer (ICD-9, 189.1): OR, acetaminophen use**			Smoking, former smoking, socioeconomic status	**Exposure information:** Intake defined as ≥1 dose/month **Strengths:** Use of population-based controls. **Limitations:** This study can't separate the effect of previous phenacetin intake (banned 1986 in west Germany) and subsequent use of acetaminophen in heavy analgesic users. Methods of control selection were not described in detail.
		No/rare analgesic intake	1	20		
		Acetaminophen use	4.76 (0.38–59.37)	6		
		Renal pelvis cancer (ICD-9, 189.1): OR, acetaminophen use			Smoking, former smoking, socioeconomic status, laxative intake	
		No/rare analgesic intake	1	20		
		Acetaminophen use	3.27 (0.25–43.02)	6		

[a] Cases included both “renal cell carcinoma” (ICD-7, 180.3) and “kidney cancer, not otherwise specified” (ICD-7, 180.0)

Table 6. Case-control studies of kidney cancer and acetaminophen use included in pooled study by McCredie et al. (1995)

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
McCredie et al. (1993) Case-Control New South Wales, Australia **Enrollment or follow-up:** 1989-1990	**Population:** Cases: 503 RCC, 149 renal pelvis cancer; Controls: 523 **Exposure assessment method:** interview	**ICD-9, 189.0 (RCC): RR, acetaminophen use**			Age, sex, interview method, cigarette smoking, obesity, phenacetin/ aspirin compounds	**Exposure information:** Regular use defined as ≥20 times during lifetime **Strengths:** The diagnoses were confirmed through histology or other methods. A single interviewer obtained data. Frequency and duration of analgesic use were queried. **Limitations:** Exposure-response analyses were not conducted. **Confidence in evidence:** Detailed exposure and outcome assessment and analyses.
		Non-consumer (<20 times in lifetime)	1	411		
		Acetaminophen only	1.5 (0.9–2.4)	55		
		Acetaminophen in any form	1.5 (1–2.3)	73		
		≤0.48 kg	1.9 (1–3.6)	26		
		0.49-1.36 kg	0.9 (0.4–1.9)	16		
		≥1.37 kg	1.7 (0.9–3.3)	31		
		ICD-9, 189.0 (RCC): RR, Use in subjects who never took phenacetin/aspirin compounds			Age, sex, interview method, cigarette smoking, obesity	
		Non-consumer (<20 times in lifetime)	1	411		
		Ever regularly	1.6 (1–2.8)	38		
		<0.36 kg	1.3 (0.6–3.2)	12		
		0.36-1.94 kg	1.6 (0.7–3.8)	14		
		>1.94 kg	2.1 (0.8–5.2)	12		
		ICD-9, 189.0 (RCC): RR, Duration of use in subjects who never took phenacetin/aspirin compounds			Age, sex, interview method, cigarette smoking, obesity	
		Non-consumer (<20 times in lifetime)	1	411		
		<1 yr acetaminophen use	0.9 (0.3–3.3)	5		
		1-7 yr acetaminophen use	1.5 (0.7–3.2)	18		
		>7 yr acetaminophen use	2.3 (1–5.4)	15		
		ICD-9, 189.0 (RCC): RR, Years first started use in subjects who never took phenacetin/ aspirin			Age, sex, interview method, cigarette smoking, obesity	
		Non-consumer (<20x in lifetime)	1	411		
		1982-1987	1.4 (0.6–3.7)	11		
		1972-1983	1.3 (0.6–3)	14		
		Before 1972	2.3 (0.9–5.6)	13		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Chow et al. (1994) Case-Control Minnesota **Enrollment or follow-up:** 1988-1990	**Population:** Cases: 690; Controls: 707 **Exposure assessment method:** interview	**RCC (ICD-9, 189.0): OR Women - Lifetime cumulative intake (kg)**			Age, smoking, BMI	**Exposure information:** Regular use defined as ≥2 times/wk for ≥1 month before 1987 **Strengths:** Exposure assessment was detailed and quantitative. Information sought on frequency and duration of analgesic use. Pharmaceutical records were used to ascertain content of acetaminophen from self-reported medications used regularly by study subjects. Lifetime cumulative exposure (in kg) was sought for acetaminophen use; annual exposure to acetaminophen was estimated from the subject's dose and the ingredient amount in the drug reported during the year of exposure. Response rates were high (>80%) for cases and population-based controls. **Limitations:** There was limited power to detect an association between acetaminophen use and RCC (8 male and 7 female cases reported acetaminophen use), especially since the analyses were stratified by sex. **Additional results:** P-value for trend was not reported. Further adjustment for other factors associated with RCC in the present study, ie., protein intake and a history of kidney diseases, did not alter the findings with lifetime consumption of analgesics. Examination by age started, usual amount and duration of use of aspirin-containing and aspirin-free medications also did not produce consistent associations. Moreover, RCC was not associated with analgesic use among next of kin (NOK) cases. **Confidence in evidence:** There was limited power to detect an effect due to few acetaminophen exposed cases, even though the study was conducted in an area with high incidence of kidney cancer. There were no significant concerns with the assessment of exposure or outcome, the analysis, nor the selection of study participants.
		Non-user	1	101		
		Acetaminophen only	2.1 (0.6–6.9)	7		
		<0.1	1.1 (0.2–5.7)	3		
		0.1-1	1 (0.3–2.8)	7		
		1.1-5	0.9 (0.4–2.1)	10		
		>5	0.9 (0.2–4.6)	3		
		RCC (ICD-9, 189.0): OR, Men - Lifetime cumulative intake (kg)				
		Non-user	1	195		
		Acetaminophen only	1.2 (0.5–3.2)	8		
		<0.1	1.2 (0.4–3.6)	6		
		0.1-1	0.7 (0.3–1.9)	7		
		1.1-5	0.7 (0.2–1.8)	6		
		>5	0.4 (0–4.2)	1		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Mellemgaard et al. (1994) Case-Control Denmark **Enrollment or follow-up:** 1989-1991	**Population:** Cases: 368 (226 men, 142 women); Controls: 396 (237 men and 159 women) **Exposure assessment method:** interview	**OR, Women-Paracetamol (acetaminophen) use**			Age, BMI, history of hypertension, SES	**Exposure information:** Use defined as ≥2 times/wk for ≥1 month **Strengths:** Little potential for selection bias as cases and controls were identified from the entire Danish population. **Confidence in evidence:** Complete overlap with McCredie et al. 1995.
		Never used analgesics	1	105		
		Ever	1 (0.4–2.5)	11		
		<1000g lifetime use	2 (0.6–6.5)	8		
		>1000g lifetime use	0.5 (0.1–1.8)	3		
		OR, Men-Acetaminophen use			Age, smoking history of hypertension, SES	
		Never used analgesics	1	181		
		Ever	1.1 (0.5–3)	11		
		<1000g lifetime use	1.4 (0.5–4.3)	16		
		>1000g lifetime use	0.9 (0.2–4)	7		

3.1.2.1.3 Bladder cancer

Several studies examined the association of acetaminophen use and risk of bladder cancer. Bladder cancer accounts for approximately 4.6% of all new cancer cases in the US (SEER 2019). About 80,470 new cases of bladder cancer will occur in the US (61,700 in men and 18,770 in women), and about 17,670 people (12,870 men and 4,800 women) will die from the disease (ACS 2019a). Risk factors for bladder cancer include smoking; occupational exposure to aromatic amines, polycyclic aromatic hydrocarbons, and chlorinated hydrocarbons; *Schistosoma haematobium* and other chronic bladder infections; and genetic predisposition (Burger et al. 2013; Pelucchi et al. 2006; WCRF/AICR 2018). The main potential confounder to account for in the association between bladder cancer and acetaminophen use is tobacco smoking. Details of studies are presented in Table 7 and are described below.

Cohort studies

Three cohort studies examined the association of acetaminophen with bladder cancer (Friis et al. 2002; Genkinger et al. 2007; Walter et al. 2011a) (Table 7).

Friis et al. (2002) did not find an association with any prescription for acetaminophen only and cancer of the urinary bladder (ICD-7, 181) overall. It found a non-statistically significant increased risk for two-four prescriptions (SIR, 1.8; 95% CI, 0.9 – 3.2; 11 exposed cases), but did not find an association in other groups stratified by number of prescriptions. However, there was limited power to detect an effect, since a number of subgroups were small. Other details and limitations of this study are discussed above in Section 3.1.1.

Genkinger et al. (2007) assessed acetaminophen and bladder cancer risk in a cohort of men from the HPFS. The study did not find an association between regular acetaminophen use and bladder cancer from 1986 to 2002 using 1986 analgesic information (RR, 0.96; 95% CI, 0.67–1.39). There was also no association from 1988 to 2002 for 1986 and 1988 analgesic information (RR, 0.90; 95% CI, 0.49–1.65). The study reports that findings were similar when participants were stratified by smoking status, age, pack years of cigarette smoking, geographic region, and fluid intake (data not shown in publication). Study details can be found above in Section 3.1.1.

Walter et al. (2011a) found that use of acetaminophen was associated with an increased risk of bladder cancer in men and women combined, but the risk estimate was not statistically significant (HR, 1.39; 95% CI, 0.74–2.60 and HR, 1.50; 95% CI, 0.57–3.89 for low and high use, respectively). There were 19 and 6 cases in the low and high use groups, respectively; thus, the statistical power to detect an effect was limited. Study details can be found above in Section 3.1.1.

Nested case-control studies

Two case-control studies nested in health databases reported increased risks of bladder cancer with acetaminophen use (Derby and Jick 1996; Kaye et al. 2001) (Table 7). Derby and Jick (1996) enrolled 504 incident cases of bladder cancer (ICD-8, 188) and 2009 controls matched on sex, age within 1 year, and duration of GHC membership. The study reported an OR of 1.3 (95% CI, 0.6–2.8; 9 exposed cases) for those who filled 40 or more acetaminophen prescriptions per lifetime. When stratified by age, the OR associated with filling 40 or more acetaminophen prescriptions was 3.3 (95% CI, 0.9–12.6; 4 exposed cases) in those younger than 65 years, but there was no association in those 65 years or older (OR, 0.9; 95% CI, 0.3–2.4; 5 exposed cases). Kaye et al. (2001) identified 189 bladder cancer cases and 744 controls matched on age, sex, general practice, year of first prescription, and index date within the GPRD in the UK. The study found a non-statistically significant increased risk of bladder cancer in those who filled 6-19 prescriptions (OR, 1.3; 95% CI, 0.8–2.2; 26 exposed cases) after adjusting for smoking and BMI, but not in those filling 1-5 or greater than 20 prescriptions.

Case-control studies

The evidence was inconsistent for the association between acetaminophen use and risk of bladder cancer in the six case-control studies published on this topic (Table 7). Three of these studies observed no association between bladder cancer and various metrics of acetaminophen intake (Fortuny et al. 2007; McCredie and Stewart 1988; Pommer et al. 1999), whereas three studies reported non-statistically elevated risks of bladder cancer (Castelao et al. 2000; Fortuny et al. 2006; Piper et al. 1985). Baris et al. (2013) reported a statistically significant increase in risk with regular use.

Of the four case-control studies reporting elevations in bladder cancer, three controlled for tobacco smoking in the analyses (Baris et al. 2013; Castelao et al. 2000; Fortuny et al. 2006), which is a risk factor for bladder cancer and may also be associated with acetaminophen use. Castelao et al. (2000) reported an OR of 1.43 (95% CI, 0.87–2.35; 90 exposed cases) in the highest category of cumulative acetaminophen intake (>886g), after adjusting for education, smoking, years employed as hairdresser/barber, NSAID use, and phenacetin use. Piper et al. (1985) reported an OR of 1.5 (95% CI, 0.4–7.2; 6 exposed cases) for use of acetaminophen only. In a case-control study from Spain, regular use of acetaminophen was not associated with bladder cancer overall (OR, 0.8; 95% CI, 0.4–1.3); however, there was a significant interaction of acetaminophen use with the glutathione S-transferase Pi 1 (*GSTP1*) I105V genotype (p = 0.008 for interaction) (Fortuny et al. 2006). Among the individuals with homozygous decrease-of-function alleles (*GSTP1* Val/Val), the use of acetaminophen was non-statistically significantly associated with increased risk of bladder cancer compared to non-users of the same genotype (OR, 1.8; 95% CI, 0.9–3.6). Subjects who were homozygous for

this mutant allele and used acetaminophen regularly for more than four years had an increased risk of bladder cancer (OR, 2.5; 95% CI, 0.4–15.6). Baris et al. (2013) reported an increase in risk with regular use (OR, 1.3; 95% CI, 1.1–1.7). Acetaminophen use was not statistically significantly associated with any category of duration of use and there was no dose-response trend.

Meta-analyses

In a meta-analysis of two cohort and eight case-control studies, acetaminophen use was not associated with bladder cancer (meta-RR, 1.01; 95% CI, 0.88–1.17) (Zhang et al. 2013).

Summary of bladder cancer findings

Three cohort studies, two nested case-control studies and six case-control studies examined the association of acetaminophen with bladder cancer. The three most informative studies assessed acetaminophen use prospectively through medical records; two reported a non-significant increased risk of bladder cancer with low to moderate use and no dose-response trend (Friis et al. 2002; Kaye et al. 2001), and the third reported a non-statistically significant increase with high use (Derby and Jick 1996). Of the two cohort studies that assessed acetaminophen use through self-report, one reported non-significant increases with use (Walter et al. 2011a).

The case-control studies had a mix of positive and null findings. A case-control study from Spain (Fortuny et al. 2006) observed no cancer association with acetaminophen overall, but found an increased risk of bladder cancer among acetaminophen users with a genotype (*GSTP1* Val/Val) encoding for a decrease in GSTP1 function and hence reduced capacity for glutathione conjugation with acetaminophen metabolites, such as *N*-acetyl-*p*-benzoquinone imine (NAPQI). In those with the *GSTP1* Val/Val genotype, bladder cancer risk was increased roughly two-fold in acetaminophen users and in those who used acetaminophen regularly for more than four years (though not statistically significant), suggesting that this *GST* genotype may be important to consider in studies assessing the carcinogenicity of acetaminophen. Three of the case-control studies observed no association between bladder cancer and various metrics of acetaminophen intake (Fortuny et al. 2007; McCredie and Stewart 1988; Pommer et al. 1999), three reported non-statistically significant elevated risks of bladder cancer (Castelao et al. 2000; Fortuny et al. 2006; Piper et al. 1985), and one reported a statistically significant increase in risk of bladder cancer (Baris et al. 2013).

Table 7. Cohort and case-control studies of bladder cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Bladder – Cohort						
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	**Population:** Danish Cancer registry and prescription database. Cases excluded because of residency outside the county at the date of prescription, parent (of patient) registered as customer, error in the personal identification number, death prior to or at the date of prescription, age younger than 16 years, subjects who had a cancer diagnosis (except nonmelanoma skin cancer) prior to date of first recorded prescription, or died within the first year of follow-up; N = 39,946 **Exposure assessment method:** records	**ICD-7, 181: SIR, Number of acetaminophen prescriptions**			Age, sex, calendar year	**Exposure information:** Exposure level: ever prescribed acetaminophen and number of prescriptions **Strengths:** Excluded person-time and cancer experience in the first year of follow-up after first acetaminophen prescription. Use of prescription database and linked to cancer registry. Almost complete capture of the population of North Jutland county. **Limitations:** Multiple comparisons performed, no adjustment for confounders such as tobacco smoking or alcohol consumption, lack of data on use of analgesic agents prior to start of prescription database and use of OTC analgesics, degree of compliance, or indications for use. **Confidence in evidence:** This study had some limitations, but is still fairly informative. The primary strength is the use of the prescription database for exposure assessment and linkage to the cancer registry. The main concern is the lack of control for confounders other than matching for age, year, and sex.
		Any prescription	1 (0.7–1.5)	25		
		1	0.9 (0.4–1.8)	8		
		2-4	1.8 (0.9–3.2)	11		
		5-9	1 (0.3–2.5)	4		
		≥10	0.4 (0–1.3)	2		
Genkinger et al. (2007) Cohort US **Enrollment or follow-up:** 1986-2004	**Population:** Men in the Health Professionals Follow-Up Study N=49,448 **Exposure assessment method:** questionnaire	**RR, Regular use in 1986**			Age, period, pack-years of smoking, current smoking status, geographic area, fluid intake	**Exposure information:** Exposure levels: no use, regular use (≥1 time/week for ≥3 months for ≥2 yrs prior to interview) **Strengths:** Exposure reassessed every two years, long follow-up time (18 years). **Limitations:** Small number of acetaminophen-exposed cases. **Confidence in evidence:** Study was well-conducted but there was not as much data for acetaminophen exposure compared to aspirin and other analgesics, which limited the ability of the study to detect an association between bladder cancer and acetaminophen.
		Nonusers	1	575		
		Regular use	0.96 (0.67–1.39)	32		
		RR, Regular use in 1986 and 1988				
		Nonusers	1	406		
		Regular use	0.9 (0.49–1.65)	12		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** VITAL (VITamins And Lifestyle) study 62,841 **Exposure assessment method:** questionnaire	**HR, 10-yr use prior to baseline in men and women combined**			Age, education, race, marital status, height, BMI, physical activity, smoking, alcohol intake, fruit, vegetable, red meat intake, multivitamin use, self-rated health, family history of colon, lung, hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/ chronic joint pain, migraine/ chronic headaches, use of NSAIDs	**Exposure information:** Regular use defined as ≥1 d/wk for ≥1 yrs **Strengths:** Large sample size. **Limitations:** Limited power in detecting associations between acetaminophen use and risk of individual cancers or cancer subtypes. Analyses were overadjusted, reducing precision to detect a significant effect. **Confidence in evidence:** This study had some limitations, mainly that they over-adjusted for confounding and that there were small group sizes for some of the subgroup analyses. Self-reported exposure, not validated. However, this is a well-established cohort study that was overall well-conducted and is considered informative.
		No use	1	76		
		Low use (<4 d/wk or <4 yrs)	1.39 (0.74–2.6)	19		
		High use (≥4 d/wk and ≥4 yrs)	1.5 (0.57–3.89)	6		
		Trend-test *p*-value: 0.24				
Bladder – Nested Case-Control						
Derby and Jick (1996) Nested Case-Control Puget Sound, WA USA **Enrollment or follow-up:** 1980-1991	**Population:** Group Health Cooperative (GHC) Cases: 504; Controls: 2009 **Exposure assessment method:** records	**(ICD 8, 188): Unadjusted RR, Acetaminophen – no. prescriptions filled (lifetime consumption)**			None	**Exposure information:** NR **Strengths:** Exposure information routinely collected prospectively in pharmacy records of both prescription and OTC use. For each subject, the number of prescriptions filled is known. Population-based within a well-defined cohort. **Limitations:** Information on confounding factors was complete for less than half of the participants. **Confidence in evidence:** Well-designed study, strong exposure assessment, data collected prospectively from records with 92% correlation with data obtained from interview. Data on duration of acetaminophen use were available, but not analyzed to reflect cumulative exposure.
		None	1	205		
		User at entry (<40 prescriptions & filled in the year of GHC entry	1.4 (1–1.9)	76		
		1	1 (0.8–1.4)	63		
		2-9 (<0.2 kg)	1 (0.8–1.3)	125		
		10-19 (0.2-0.4 kg)	1.1 (0.6–2)	16		
		20-39 (0.5-0.9 kg)	1.1 (0.6–2.3)	10		
		≥40 (≥1 kg)	1.3 (0.6–2.8)	9		
		(ICD 8, 188): Unadjusted RR, <65 years old, Acetaminophen – no. prescriptions filled (lifetime consumption)			None	
		None	1	78		
		≥40	3.3 (0.9–12.6)	4		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
		(ICD 8, 188): Unadjusted RR, ≥65 years old, Acetaminophen – no. prescriptions filled (lifetime consumption)			None	
		None	1	127		
		≥40	0.9 (0.3–2.4)	5		
Kaye et al. (2001) Nested Case-Control UK **Enrollment or follow-up:** 1995–1998	**Population:** Cases: 189; Controls; 744 **Exposure assessment method:** records	**OR, Acetaminophen - number prescriptions filled**			BMI, smoking, age, sex, general practice, duration of prescription in the database, index date	**Exposure information:** NR **Strengths:** Population based design, acetaminophen prescriptions and demographic variables were prospectively and routinely collected, dose-response analyses conducted through two modelling strategies (p-value for trend not reported). **Limitations:** OTC use was not collected, although a previous study showed that women in the GPRD were unlikely to take acetaminophen without a prescription. **Confidence in evidence:** Prospectively collected data, population-based design, adequate control for potential confounders, thorough analysis to rule out confounding by indication. Exposure assessed by medical records.
		None	1	102		
		1-5	0.8 (0.5–1.2)	48		
		6-19	1.3 (0.8–2.2)	26		
		≥20	0.7 (0.4–1.4)	13		
Bladder – Case-Control						
Piper et al. (1985) Case-Control NY, US **Enrollment or follow-up:** 1975-1980	**Population:** Cases: 173; Controls: 173 **Exposure assessment method:** interview	**Unadjusted OR, Acetaminophen use**			None	**Exposure information:** ≥30 d/yr **Limitations:** conditional logistic regression analysis was not appropriate given that pairs were matched on a wide age range (+/- 10 yrs)
		No regular use	1	NR		
		Acetaminophen only	1.5 (0.4–7.2)	6		
		Phenacetin or acetaminophen or both	3.8 (1.4–13)	19		
McCredie and Stewart (1988) Case-Control New South Wales, Australia **Enrollment or follow-up:** 1980-1982	**Population:** Cases: 162 women; Controls: 381 women **Exposure assessment method:** questionnaire	**(ICD 8, 188): OR, Lifetime consumption**			Sex, tobacco, phenacetin	**Exposure information:** Use considered ≥ 0.1 kg lifetime consumption **Strengths:** The use of population controls; adjustment for phenacetin use. **Limitations:** Quantitative exposure data (e.g. dose-response) was not presented. There may not have been sufficient latency between collection of exposure data (calculated up to the year of case diagnosis) and onset of disease
		None	1	NR		
		≥ 1 kg acetaminophen	0.7 (0.4–1.3)	NR		
		≥ 0.1 kg acetaminophen	0.7 (0.4–1.3)	NR		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Pommer et al. (1999) Case-Control West Berlin, Germany **Enrollment or follow-up:** 1990-1994	**Population:** Cases: 571; Controls: 647 **Exposure assessment method:** interview	**OR, Intake ≥1 kg acetaminophen**			Smoking, former smoking, socioeconomic status	**Exposure information:** Intake defined as >1 dose/month **Strengths:** Use of population-based controls. **Limitations:** This study can't separate the effect of previous phenacetin intake (banned 1986 in west Germany) and subsequent use of acetaminophen in heavy analgesic users. Methods of control selection were not described in detail. **Confidence in evidence:** NOTE: Partial overlap with McCredie1995 which enrolled subjects 1989-1991
		No analgesic use	1	286		
		Acetaminophen use	0.77 (0.31–1.9)	11		
		OR, Intake ≥1 kg acetaminophen			Smoking, former smoking, socioeconomic status, laxative intake	
		No analgesic use	1	286		
		Acetaminophen use	0.83 (0.33–2.07)	11		
Castelao et al. (2000) Case-Control Los Angeles, CA USA **Enrollment or follow-up:** 1987-1996	**Population:** Cases: 1514; Controls: 1514 **Exposure assessment method:** interview	**OR, Exclusive users - Acetaminophen - Cumulative lifetime exposure (g)**			Education, smoking, years employed as hairdresser/ barber	**Exposure information:** Regular use defined as ≥2 times/wk for ≥1 month **Strengths:** Population-based controls.
		Regular use	1.03 (0.67–1.58)	60		
		< 114	1.07 (0.56–2.04)	27		
		114–885	0.82 (0.39–1.72)	17		
		≥886	1.93 (0.73–5.11)	15		
		OR, Any users - Acetaminophen - Cumulative lifetime exposure (g)			Education, smoking, years employed as hairdresser/ barber, NSAID use, phenacetin use	
		Regular use	0.85 (0.6–1.19)	224		
		< 114	0.75 (0.47–1.2)	62		
		114–885	0.68 (0.43–1.09)	57		
		≥886	1.43 (0.87–2.35)	90		
Fortuny et al. (2006) Case-Control Spain (Barcelona, Valles/Bages, Alacant, Tenerife, Asturias) **Enrollment or follow-up:** 1997-2000	**Population:** Cases: 958; Controls: 1029 **Exposure assessment method:** interview	**OR, Paracetamol (acetaminophen) use**			Age, gender, region, smoking status, use of other NSAID/ analgesics	**Exposure information:** Regular use defined as ≥2 times/wk for ≥1 month **Strengths:** high participation rates, large sample size, high quality exposure and genotype information, and detailed assessment of drug composition and dose. Thorough sensitivity analyses were conducted to rule out that hospital controls would have higher drug consumption than the general population. **Limitations:** Limited statistical power particularly for stratified analyses, and possible selection bias
		Nonusers (<20 times lifelong)	1	664		
		Ever users	0.8 (0.6–1)	243		
		Non-regular (<20 times lifelong, 2 times/wk for 1 month	0.8 (0.6–1.1)	204		
		Regular (2 times/wk for ≥1 month)	0.8 (0.4–1.3)	39		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
		OR, Acetaminophen - Duration of use (y)				
		Never users	1	664		
		≤2.5	0.6 (0.3–1.5)	9		
		2.6-6.08	1 (0.4–2.3)	14		
		>6.09	1 (0.4–2.7)	11		
		Trend-test *p*-value: 0.13				
		OR, Acetaminophen - Cumulative dose (g)				
		Never users	1	664		
		1-709	0.7 (0.3–1.7)	10		
		710-1,386	0.7 (0.3–2.1)	8		
		>1,387	1.1 (0.5–2.5)	14		
		Trend-test *p*-value: 0.13				
		OR, Use of acetaminophen and GSTP1 I105V polymorphisms				
		Homozygous for wild-type (Ile/Ile)	0.5 (0.4–0.8)	NR		
		Heterozygous (Ile/Val)	1 (0.7–1.4)	NR		
		Homozygous for variant (Val/Val)	1.8 (0.9–3.6)	NR		
		Homozygous for variant (Val/Val), >4 yrs of use	2.5 (0.4–15.6)	NR		
Fortuny et al. (2007) Case-Control New Hampshire, USA **Enrollment or follow-up:** 1998-2001	**Population:** Cases: 376; Controls: 463 **Exposure assessment method:** interview	**OR, Exclusive acetaminophen use, duration (yrs)**			Age, sex, smoking	**Exposure information:** Regular use defined as ≥4 times/wk for ≥1 month **Strengths:** Population controls. Verified cases by histopathological review.
		Never use	1	211		
		Ever use	0.8 (0.5–1.6)	21		
		≤4.5 yrs	0.8 (0.3–2)	8		
		4.5-16 yrs	0.9 (0.4–2.5)	9		
		>16 yrs	0.8 (0.2–3.1)	4		
		Trend-test *p*-value: 0.672				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
		Invasive: OR, acetaminophen use, stratified by tumor invasion, tumor grade, TP53 IHC intensity			Age, sex, smoking, use of NSAIDs and analgesics	
		Noninvasive	0.6 (0.3–1.1)	NR		
		Invasive	1.1 (0.5–2.2)	NR		
		Noninvasive, low grade	0.5 (0.2–0.9)	NR		
		Noninvasive, high grade	2 (0.6–6.4)	NR		
		TP53 IHC intensity <3	0.4 (0.2–0.8)	NR		
		TP53 IHC intensity ≥3	1.7 (0.8–3.5)	NR		
Baris et al. (2013) Case-Control New England, US **Enrollment or follow-up:** 2001-2004	**Population:** Cases: 1,171; Controls: 1,418 **Exposure assessment method:** interview	**OR, Acetaminophen use**			Age, gender, race, Hispanic status, state, smoking status	**Exposure information:** Regular use defined as ≥2 times/wk for ≥1 month **Strengths:** Cases histologically confirmed. Population-based controls.
		Never use	1	314		
		Regular use	1.3 (1.1–1.7)	293		
		<5 yrs	1.5 (1.1–2)	142		
		5–9 yrs	1.1 (0.7–1.8)	48		
		10-19 yrs	1.1 (0.7–1.7)	42		
		≥20 yrs	1.1 (0.6–2)	27		
		Trend-test *p*-value: 0.966				

3.1.2.2 Lymphohematopoietic cancers

The association between acetaminophen and cancer of the lymphohematopoietic (LH) system has been assessed in three cohort studies (Friis et al. 2002; Lipworth et al. 2003; Walter et al. 2011b) and 10 case-control studies (Baker et al. 2005; Becker et al. 2009; Chang et al. 2004; Couto et al. 2015; Friedman 1982; Kato et al. 2002; Moysich et al. 2007; Ognjanovic et al. 2011; Ross et al. 2011; Weiss et al. 2006). One study that assessed acetaminophen with several outcomes among a cohort of acute myeloid leukemia (AML) patients was excluded because it did not investigate acetaminophen as a cause of AML (Finn et al. 2015). Some of the studies assessed multiple cancer sites, as shown in Table 8 below.

Table 8. Types of LH cancer investigated in each study

Reference	Combined LH cancers	Leukemia, NOS	Myeloid leukemia, NOS	AML	CML	ALL	Childhood leukemia	Lymphoma, NOS	NHL	MM	HL
Cohort											
Friis et al. (2002)	✓	✓							✓	✓	✓
Lipworth et al. (2003)	✓										
Walter et al. (2011b)	✓		✓						✓[a]		
Case-control											
Baker et al. (2005)									✓		
Becker et al. (2009)								✓			
Chang et al. (2004)											✓
Couto et al. (2015)							✓				
Friedman (1982)		✓	✓								
Kato et al. (2002)									✓		
Moysich et al. (2007)										✓	
Ognjanovic et al. (2011)							✓				
Ross et al. (2011)			✓	✓	✓						
Weiss et al. (2006)		✓		✓		✓					

LH, lymphohematopoietic; NOS, not otherwise specified; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; ALL, acute lymphocytic leukemia; NHL, non-Hodgkin lymphoma; MM, multiple myeloma; HL, Hodgkin's lymphoma

[a] Walter et al. (2011b) specifically examined CLL/SLL, a type of NHL

LH cancers are a heterogeneous group of cancers that can be divided into the main types of lymphoid neoplasms, and myeloid neoplasms and acute leukemias. According to the WHO Classification of Hematologic Malignancies 2016, myeloid neoplasms and acute leukemias can be divided into the following classifications: myeloproliferative neoplasms; myeloid/lymphoid neoplasms with eosinophilia and rearrangement of PDGFRA, PDGFRB, or FGFR1, or with PCM1-JAK2; myelodysplastic/myeloproliferative neoplasms; myelodysplastic syndromes; AML and related neoplasms; blastic plasmacytoid dendritic cell neoplasm; acute leukemias of ambiguous lineage; B-lymphoblastic leukemia/lymphoma; and T-lymphoblastic leukemia/lymphoma (Arber et al. 2016). Lymphoid neoplasms can be classified as mature B-cell neoplasms, mature T and NK neoplasms, Hodgkin lymphoma, post-transplant lymphoproliferative disorders, and histiocytic and dendritic cell neoplasms (Swerdlow et al. 2016).

Of the potential risk factors for LH cancers, tobacco smoking and BMI are considered potential confounders in the association with acetaminophen. Details of studies are presented in Tables 9-12 and are described below.

Cohort studies

Three cohort studies examined risk of lymphohematopoietic cancers with acetaminophen use.

Friis et al. (2002) found that any acetaminophen prescription was associated with a non-statistically significantly increased risk of total LH cancers (ICD-7, 200-205) (SIR, 1.2; 95% CI, 0.8–1.6) (Table 9), non-Hodgkin lymphoma (ICD-7, 200, 202) (SIR, 1.2; 95% CI, 0.7–2.0) (Table 12), and multiple myeloma (ICD-7, 203) (SIR, 1.6; 95% CI, 0.6–3.2) (Table 12) compared to expected rates in the population of North Jutland county in Denmark from 1989-1997. Any acetaminophen prescription was not associated with risk of leukemia (ICD-7, 204) (SIR, 0.9; 95% CI, 0.5–1.6) (Table 11). The study reported the risk associated with Hodgkin lymphoma, but found only one case that was exposed to acetaminophen (Table 12). Limitations of the study were lack of control for important covariates and limited power to detect significant associations (details are discussed in Section 3.1.1).

In the same Danish population, Lipworth et al. (2003) found a two-fold significantly increased risk of mortality from LH cancers with any acetaminophen prescription in men and women combined. The SMRs were attenuated with longer follow-up times, but remained significant with a five-year or longer latency period (Table 9). The results of this study should be interpreted with caution because not all LH cancers are highly fatal and therefore ascertainment of cases may not be accurately reflected on death certificates. See Section 3.1.1 for study details.

The details of Walter et al. (2011b) are described above in Section 3.1.1. Exposure was assessed through a self-administered questionnaire at baseline. This study found a statistically significant increased risk of LH cancers with high use of acetaminophen (HR, 1.84; 95% CI, 1.35–2.50, *p* trend: 0.004) (Table 9). In order to assess the possibility of reverse causation, incident cases that occurred within two years of baseline were excluded from analyses, and the risk remained significantly increased with high use (HR, 1.50; 95% CI, 1.04–2.18). When stratified by sex, risk was higher in women (HR, 2.15; 95% CI, 1.41–3.28; *p* trend: 0.001) than men (HR, 1.55; 95% CI, 0.97–2.50; *p* trend: 0.549) for total LH cancers. High use of acetaminophen was also associated with an increased risk of myeloid neoplasms (HR, 2.26; 95% CI, 1.24–4.12; *p* trend, 0.10) (Table 11), plasma cell disorders (HR, 2.42; 95% CI, 1.08–5.41; *p* trend, 0.007), and "mature B-cell neoplasms other than CLL/SLL or plasma cell disorders" (HR, 1.81; 95% CI, 1.12–2.93; *p* trend, 0.055) (Table 12) in men and women combined. Acetaminophen use was not associated with risk of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL).

Case-control studies

Leukemia (childhood)

In the two case-control studies of infant leukemia, one population-based from the US and Canada (Ognjanovic et al. 2011) and another hospital-based from Brazil (Couto et al. 2015), maternal acetaminophen use was not associated with AML, ALL, or acute leukemia with MLL rearrangements (Table 10). Acetaminophen exposure was self-reported through interview.

Leukemia (adult)

Three case-control studies assessed acetaminophen use in relation to adult leukemia and its subtypes (Friedman 1982; Ross et al. 2011; Weiss et al. 2006) (Table 11).

Friedman (1982) conducted a case-control study of leukemia and its subtypes nested within members of the Kaiser Permanente health plan of Northern California. Acetaminophen use was obtained through review of physicians' records, which were collected prospectively. Analyses using hospital controls were attenuated compared to the results using member controls. In the analyses using member controls, acetaminophen use was positively associated with all leukemias combined and myeloid leukemia, but the confidence intervals were wide and the results were not statistically significant. No associations were seen when using hospital controls.

In a hospital-based case-control study in Buffalo, NY (Weiss et al. 2006), acetaminophen use at least once per week was significantly associated with leukemia (OR, 1.75; 95% CI, 1.06–2.88; 39 exposed cases); non-statistically significant positive associations were also observed for AML and ALL. For leukemia overall, risk increases

were also observed for the metrics of frequency (times per week), years of use and cumulative use but the risk estimates and tests for trend were not statistically significant.

In a population-based case-control study in Minnesota (Ross et al. 2011), statistically significant increased risks of myeloid leukemia was observed in women, but not men, who regularly used acetaminophen (≥weekly for ≥1 yr) (OR, 1.60; 95% CI, 1.04–2.47). In women, significant dose-response associations (p-value for trend < 0.05) were observed with increasing years of use and tablets per week taken. Positive associations with AML and chronic myeloid leukemia (CML) were also observed in women but not in men (most were not statistically significant). Increasing number of tablets per week was associated with statistically significant increasing risk of AML (*p* trend = 0.03) and suggestive for CML (*p* trend = 0.10) in women.

Lymphoma, Non-Hodgkin Lymphoma (NHL), NOS and its subtypes

The only case-control study of lymphoma, NOS reported a statistically signficant OR of 2.29 (95% CI, 1.49–3.51) associated with acetaminophen use (Becker et al. 2009) (Table 12). There have been two case-control studies of NHL and acetaminophen use published to date (Baker et al. 2005; Kato et al. 2002). Kato et al. (2002) reported on total NHL in women while Baker et al. (2005) reported on NHL and its cellular subtypes: NHL (diffuse large B-cell lymphoma, DLBCL), NHL (follicular), NHL (small lymphocytic leukemia/chronic lymphocytic leukemia, SLL/CLL), NHL (T-cell lymphoma) in men and women. In both studies, consistently women who had ever used acetaminophen had odds ratios above one (some of which were statistically significant), while there was no association observed in men for NHL (DLBCL), NHL (follicular) and NHL (SLL/CLL). Kato et al. (2002) reported increased risks of NHL that were not statistically significant associated with three or more years of acetaminophen use. For NHL (all types combined), Baker et al. (2005) reported some statistically significant increased risks associated with acetaminophen use in women, but not in men. When the exposure metrics were categorized into no, moderate, high use or duration, statistically significant increased risks in women were noted in the lower category of use compared to no use, but not in the high use category (Table 12). Women had a higher prevalence of higher acetaminophen use than men.

Multiple Myeloma (MM)

Multiple myeloma is a mature B cell neoplasm (Swerdlow et al. 2016). The only case-control study of multiple myeloma (Moysich et al. 2007) reported a roughly three-fold increased risk that was statistically significant with regular use of acetaminophen compared to those who did not use regularly (OR, 2.95; 95% CI, 1.72–5.08) (Table 12). All of the risk estimates were statistically significant in all of the categories of acetaminophen use analyzed, regardless of exposure metric. The ORs were highest in

the highest categories of use: >7 times/wk (4.36; 95% CI, 1.7–11.23) and >10 yrs (3.26; 95% CI, 1.52–7.02). This study adjusted for tobacco smoking.

Hodgkin Lymphoma (HL)

Only one case-control study reported on the association between acetaminophen intake and Hodgkin lymphoma (HL) (Chang et al. 2004); this study stratified by a number of variables to determine which factors are associated with acetaminophen intake (i.e., age, education, income, ethnicity, gender, religion, and state of residence) (Table 12). The statistically significant OR for regular acetaminophen use was 1.72 (95% CI, 1.29–2.31) compared to non-regular use. This association persisted regardless of gender and cigarette smoking.

Summary of lymphohematopoietic cancer findings

The association between acetaminophen use and several types of LH cancers has been studied in a number of cohort and case-control studies. For all LH cancers combined, significantly increased risks of LH cancer-related deaths were found in the one cohort study in Danish North Jutland County (Lipworth et al. 2003) but not in another in the same geographic area for incidence of LH cancer (Friis et al. 2002). A US cohort study reporting on “hematologic malignancies” found statistically significant increases in women that were high users through self-report on a questionnaire (Walter et al. 2011b).

For myeloid leukemia, statistically significant increases were reported for one cohort in men and women combined (Walter et al. 2011b) and for women but not men in one case-control study (Ross et al. 2011), with significant trends observed in terms of duration of use and tablets per week. A second case-control study reported a non-significant elevation in risk (Friedman 1982).

AML was assessed in two case-control studies: a borderline significant increase was reported in one study (Weiss et al. 2006) and a statistically significant increase in women with a dose-response trend was reported in the second study (Ross et al. 2011). CML was assessed in one case-control study that reported non-significant elevations in risk in men and women (Ross et al. 2011).

For lymphoma, a statistically significant increase was reported in one case-control study (Becker et al. 2009). A statistically significant increase in risk of two types of B-cell lymphoma was observed in a cohort study (Walter et al. 2011b), with a dose-response trend in one type. For total NHL, a non-significant elevation in risk was observed in one cohort (Friis et al. 2002). Elevations were observed in two case-control studies, which were statistically significant in women but not men in one study (Baker et al. 2005) and not significant in women the other study (Kato et al. 2002). There were increases in the risk of three cell types of NHL, but not one cell type.

Two studies reported an increased risk of multiple myeloma, which was not significant in the cohort study (Friis et al. 2002). The case-control study reported a significantly increased risk with several metrics of exposure (regular use, times per week, years of use), with a significant dose-response trend (Moysich et al. 2007).

Two studies reported on the risk in Hodgkin's lymphoma. A cohort study reported a non-significant elevation but contained only one case (Friis et al. 2002). A case-control study found significantly increased risks with significant trends by several metrics of exposure (Chang et al. 2004).

Potential confounding by smoking was possible for some of these LH cancers since it was adjusted for in only three studies (Chang et al. 2004; Moysich et al. 2007; Walter et al. 2011b).

Table 9. Cohort studies of lymphohematopoietic cancers combined and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	**Population:** Danish Cancer registry/prescription database. Cases excluded because of residency outside county at date of prescription, parent (of patient) registered as customer, error in personal identification number, death prior to/at date of prescription, age <16 yrs, subjects who had a cancer diagnosis (except nonmelanoma skin cancer) prior to date of first recorded prescription, or died within the first year of follow-up; N = 39,946 **Exposure assessment method:** records	**ICD-7, 200-205: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year	**Exposure information:** Exposure level: ever prescribed acetaminophen and number of prescriptions **Strengths:** Excluded person-time and cancer experience in the first year of follow-up after first acetaminophen prescription. Use of prescription database and linked to cancer registry. Almost complete capture of the population of North Jutland county. **Limitations:** Multiple comparisons performed, no adjustment for confounders such as tobacco smoking or alcohol consumption, lack of data on use of analgesic agents prior to start of prescription database and use of OTC analgesics, degree of compliance, or indications for use. **Confidence in evidence:** This study had some limitations, but is still fairly informative. The primary strength is the use of the prescription database for exposure assessment and linkage to the cancer registry. The main concern is the lack of control for confounders other than matching for age, year, and sex.
		Prescription	1.2 (0.8–1.6)	33		
Lipworth et al. (2003) Cohort Denmark **Enrollment or follow-up:** 1989-1996	**Population:** Individuals who filled prescriptions identified through the Pharmacoepidemiological Prescription Database N = 17,760 men; 32,130 women **Exposure assessment method:** records	**Mortality: SMR, Prescribed paracetamol (acetaminophen) (1989-1996)**			Age, sex, calendar year	**Strengths:** Conducted latency analyses. **Limitations:** The main limitation is that cause of death would miss cancer incidences. Also, there was no information on indication for use, likely protopathic bias, short follow-up period, limited control for confounding. **Confidence in evidence:** This study is not considered informative because cause of death likely does not reflect cancer incidence. Additionally, this study uses the same population as Friis et al. 2002 and it is possible there was some double-counting of cases.
		Total	2.3 (2–2.6)	286		
		Men	2.8 (2.4–3.3)	140		
		Women	2 (1.6–2.3)	146		
		Mortality: SMR, Prescribed acetaminophen, stratified by latency period			Age, sex, calendar year	
		<1 yr	4.1 (3.4–4.8)	135		
		1-2 yrs	1.9 (1.5–2.3)	94		
		3-4 yrs	1.2 (0.8–1.7)	33		
		≥5 yrs	1.8 (1.1–2.6)	24		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
		Mortality: SMR, Number of prescriptions			Age, sex, calendar year	
		1	2.9 (2.4–3.4)	120		
		2-4	1.9 (1.4–2.4)	65		
		5-9	2.4 (1.8–3.2)	51		
		≥10	1.8 (1.4–2.4)	50		
Walter et al. (2011b) Cohort US **Enrollment or follow-up:** 2000-2008	**Population:** Men and women in the Vitamins and Lifestyle (VITAL) study N=64,839 **Exposure assessment method:** questionnaire	**"Hematologic malignancies": HR, 10-yr use prior to baseline in men and women combined**			Age, sex, race/ethnicity, education, smoking, self-rated health, history of rheumatoid arthritis, history of nonrheumatoid arthritis or chronic neck/back/joint pain, history of migraines or frequent headaches, history of fatigue/lack of energy, family history of leukemia/ lymphoma	**Exposure information:** Exposure level: “no use,” “low use” (<4 d/wk or <4 yrs), and “high use” (≥4 d/wk and ≥4 yrs). **Strengths:** Large cohort study **Limitations:** No information on dosage, possible reverse causation **Confidence in evidence:** Some limitations, but overall considered informative.
		Nonuser	1	405		
		Low use (<4 d/wk or <4 yrs)	1.16 (0.92–1.47)	96		
		High use (≥4 d/wk and ≥4 yrs)	1.84 (1.35–2.5)	52		
		Trend-test *p*-value: 0.004				
		"Hematologic malignancies": HR, 10-yr use prior to baseline in men and women combined, excluding cases occurring within 2 yrs of baseline				
		Nonuser	1	NR		
		High use (≥4 d/wk and ≥4 yrs)	1.5 (1.04–2.18)	NR		
		"Hematologic malignancies": HR, 10-yr use prior to baseline in men				
		Nonuser	1	265		
		Low use (<4 d/wk or <4 yrs)	1.12 (0.81–1.54)	48		
		High use (≥4 d/wk and ≥4 yrs)	1.55 (0.97–2.5)	19		
		Trend-test *p*-value: 0.549				
		"Hematologic malignancies": HR, 10-yr use prior to baseline in women				
		Nonuser	1	140		
		Low use (<4 d/wk or <4 yrs)	1.22 (0.87–1.73)	48		
		High use (≥4 d/wk and ≥4 yrs)	2.15 (1.41–3.28)	33		
		Trend-test *p*-value: 0.001				

Table 10. Case-control studies of childhood leukemia and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Ognjanovic et al. (2011) Case-Control US and Canada **Enrollment or follow-up:** 1996-2006	**Population:** Cases: 441; Controls: 323 **Exposure assessment method:** interview	**ALL (Acute lymphoblastic leukemia): OR Regular use before knowledge of pregnancy**			Maternal age, race, alcohol consumption during pregnancy, household income	**Exposure information:** Regular use defined as ≥5 and ≥10 times before and after knowledge of pregnancy, respectively **Strengths:** Population-based controls. **Additional results:** Also evaluated any use of acetaminophen; results were similar to regular use.
		No regular use	1	215		
		Regular use (≥5 times)	1.11 (0.7–1.78)	46		
		ALL (Acute lymphoblastic leukemia): OR Regular use after knowledge of pregnancy				
		No regular use	1	191		
		Regular use	1.27 (0.85–1.9)	70		
		AML (Acute myeloid leukemia): OR Regular use before knowledge of pregnancy				
		No regular use	1	146		
		Regular use (≥5 times)	0.83 (0.45–1.52)	22		
		AML (Acute myeloid leukemia): OR Regular use after knowledge of pregnancy				
		No regular use	1	137		
		Regular use	0.77 (0.46–1.3)	31		
Couto et al. (2015) Case-Control Brazil **Enrollment or follow-up:** 1999-2007	**Population:** Cases: 231; Controls: 411 **Exposure assessment method:** interview	**ALL (Acute lymphocytic leukemia): OR Maternal exposure during pregnancy, children <2 yrs**			Child skin color, maternal age, maternal education, birth weight, pesticide exposure, hormone intake during pregnancy	**Exposure information:** Use not defined **Limitations:** Hospital-based controls.
		No reported exposure	1	74		
		Reported exposure	0.56 (0.28–1.1)	17		
		ALL (Acute lymphocytic leukemia): OR Maternal exposure during pregnancy, children 0-11 mo				
		No reported exposure	1	25		
		Reported exposure	0.69 (0.27–1.74)	10		
		ALL (Acute lymphocytic leukemia): OR, Maternal exposure during pregnancy, children 12-23 mo				
		No reported exposure	1	49		
		Reported exposure	0.41 (0.15–1.15)	7		
		AML (Acute myeloid leukemia): OR, Maternal exposure during pregnancy, children <2 yrs				
		No reported exposure	1	23		
		Reported exposure	0.48 (0.15–1.48)	6		
		Acute leukemia with MLL rearrangements: OR, Maternal exposure during pregnancy, children <1 yr				
		No reported exposure	1	10		
		Reported exposure	1.55 (0.49–4.94)	6		

Table 11. Cohort and case-control studies of adult leukemia and its subtypes and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Leukemia (combined) – Cohort						
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Friis et al. (2002) above in Table 9*.	**ICD-7, 204: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year	*See Friis et al. (2002) above in Table 9.*
		Prescription	0.9 (0.5–1.6)	11		
Leukemia (combined) – Case-Control						
Friedman (1982) Case-Control CA, USA **Enrollment or follow-up:** 1971-1976	**Population:** Cases: 409; Controls: 409 hospital; 409 Kaiser member **Exposure assessment method:** records	**ICD 8, Codes 200, 204, 205: RR, Use of acetaminophen, hospital controls**			Year of admission, sex, year of birth, duration of membership	**Exposure information:** Any prescription **Strengths:** Exposure assessed through prescription records. **Limitations:** Hospital-based and member-based controls.
		Any use	0.44 (0.2–1)	8		
		ICD 8, Codes 200, 204, 205: RR, Use of acetaminophen, member controls				
		Any use	1.13 (0.43–2.91)	9		
Weiss et al. (2006) Case-Control Buffalo NY **Enrollment or follow-up:** 1981-1998	**Population:** Cases: 169; Controls: 676 **Exposure assessment method:** questionnaire	**OR Frequency of use**			Year of survey, age, sex	**Exposure information:** Regular use defined as ≥ once per week for ≥ 6 months **Strengths:** Cases confirmed through a tumor registry and diagnostic index. **Limitations:** Hospital controls.
		Never	1	95		
		Ever	1.53 (1.03–2.26)	74		
		<1 time/wk	1.74 (1.06–2.86)	34		
		≥1 time/wk	1.75 (1.06–2.88)	39		
		Trend-test *p*-value: 0.23				
		OR Duration of use				
		Never	1	95		
		≤10 yrs	1.42 (0.85–2.39)	30		
		>10 yrs	1.44 (0.83–2.52)	27		
		Trend-test *p*-value: 0.22				
		OR Cumulative use				
		Never	1	95		
		Mod. use (≤2 tablet-yrs)	1.86 (1.11–3.12)	33		
		High use (>2 tablet-yrs)	1.3 (0.73–2.34)	23		
		Trend-test *p*-value: 0.30				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Leukemia (ALL (Acute lymphoblastic/lymphocytic leukemia)) – Case-Control						
Weiss et al. (2006) Case-Control Buffalo NY **Enrollment or follow-up:** 1981-1998	**Population:** Cases: 169; Controls: 676 **Exposure assessment method:** questionnaire	**OR Acetaminophen use**			Year of survey, age, sex	**Exposure information:** Regular use defined as ≥ once per week for ≥ 6 months **Strengths:** Cases confirmed through a tumor registry and diagnostic index. **Limitations:** Hospital controls.
		Never	1	18		
		Ever	1.73 (0.79–3.78)	18		
Leukemia (Myeloid) – Cohort						
Walter et al. (2011b) Cohort US **Enrollment or follow-up:** 2000-2008	**Population:** Men and women in the Vitamins and Lifestyle (VITAL) study N=64,839 **Exposure assessment method:** questionnaire	**HR, 10-yr use prior to baseline in men and women combined**			Age, sex, race/ethnicity, education, smoking, self-reported health, history of rheumatoid arthritis, history of nonrheumatoid arthritis or chronic neck/back/joint pain, history of migraines or frequent headaches, history of fatigue/lack of energy, family history of leukemia/lymphoma	**Exposure information:** Exposure level: “no use,” “low use” (<4 d/wk or <4 yrs), and “high use” (≥4 d/wk and ≥4 yrs). **Strengths:** Large cohort study **Limitations:** No information on dosage, possible reverse causation **Confidence in evidence:** Some limitations, but overall considered informative.
		Nonuser	1	88		
		Low use (<4 d/wk or <4 yrs)	1.55 (0.98–2.43)	28		
		High use (≥4 d/wk and ≥4 yrs)	2.26 (1.24–4.12)	14		
		Trend-test *p*-value: 0.10				
		Restricted to myelodysplastic syndrome or AML: HR, 10-yr use prior to baseline in men and women combined				
		Nonuser	1	NR		
		High use (≥4 d/wk and ≥4 yrs)	2.3 (1.12–4.73)	NR		
Leukemia (Myeloid) – Case-Control						
Friedman (1982) Case-Control CA, USA **Enrollment or follow-up:** 1971-1976	**Population:** Cases: 409; Controls: 409 hospital; 409 Kaiser member **Exposure assessment method:** records	**ICD 8, 205: RR, Use of acetaminophen, hospital controls**			Year of admission, sex, year of birth, duration of membership	**Exposure information:** Any prescription **Strengths:** Exposure assessed through prescription records. **Limitations:** Hospital-based and member-based controls.
		Any use	0.67 (0.19–2.34)	4		
		ICD 8, 205: RR, Use of acetaminophen, member controls				
		Any use	1.67 (0.4–6.87)	5		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Ross et al. (2011) Case-Control Minnesota **Enrollment or follow-up:** 2005-2009	**Population:** Cases: 670 (278 women, 392 men); Controls: 701 (358 women, 343 men) **Exposure assessment method:** questionnaire	**OR Duration of use in women**			Age, BMI, other analgesic use	**Exposure information:** Regular use defined as ≥1 time/wk for ≥1 yr **Strengths:** Population-based controls. Cases reviewed by pathologists.
		Nonuser	1	209		
		1-5 yrs	1.7 (0.87–3.29)	21		
		6-10 yrs	1.24 (0.63–2.44)	19		
		>10 yrs	1.96 (1–3.84)	23		
		Trend-test *p*-value: 0.04				
		OR Duration of use in men				
		Nonuser	1	341		
		1-5 yrs	1.66 (0.77–3.59)	20		
		6-10 yrs	1.44 (0.6–3.47)	14		
		>10 yrs	0.55 (0.25–1.21)	11		
		Trend-test *p*-value: 0.32				
		OR Tablets per week in women				
		Nonuser	1	209		
		User	1.6 (1.04–2.47)	63		
		<7	1.06 (0.59–1.89)	24		
		≥7	2.37 (1.34–4.18)	39		
		Trend-test *p*-value: 0.003				
		OR Tablets per week in men				
		Nonuser	1	341		
		User	1.09 (0.67–1.77)	45		
		<7	1.08 (0.56–2.07)	22		
		≥7	1.09 (0.57–2.12)	23		
		Trend-test *p*-value: 0.77				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Leukemia (AML (Acute myeloid leukemia)) – Case-Control						
Weiss et al. (2006) Case-Control Buffalo NY **Enrollment or follow-up:** 1981-1998	**Population:** Cases: 169; Controls: 676 **Exposure assessment method:** questionnaire	**OR Acetaminophen use**			Year of survey, age, sex	**Exposure information:** Regular use defined as ≥ once per week for ≥ 6 months **Strengths:** Cases confirmed through a tumor registry and diagnostic index. **Limitations:** Hospital controls.
		Never	1	77		
		Ever	1.5 (0.98–2.3)	56		
Ross et al. (2011) Case-Control Minnesota **Enrollment or follow-up:** 2005-2009	**Population:** Cases: 670 (278 women, 392 men); Controls: 701 (358 women, 343 men) **Exposure assessment method:** questionnaire	**OR Tablets per week in women**			Age, BMI, other analgesic use	**Exposure information:** Regular use defined as ≥1 time/wk for ≥1 yr **Strengths:** Population-based controls. Cases reviewed by pathologists.
		Nonuser	1	133		
		User	1.46 (0.87–2.44)	35		
		<7	0.98 (0.49–1.96)	14		
		≥7	2.16 (1.11–4.23)	21		
		Trend-test *p*-value: 0.03				
		OR, Tablets per week in men				
		Nonuser	1	217		
		User	1.06 (0.61–1.83)	28		
		<7	1.29 (0.64–2.61)	17		
		≥7	0.82 (0.37–1.84)	11		
		Trend-test *p*-value: 0.44				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Leukemia (CML (Chronic myeloid leukemia)) – Case-Control						
Ross et al. (2011) Case-Control Minnesota **Enrollment or follow-up:** 2005-2009	**Population:** Cases: 670 (278 women, 392 men); Controls: 701 (358 women, 343 men) **Exposure assessment method:** questionnaire	**OR Tablets per week in women**			Age, BMI, other analgesic use	**Exposure information:** Regular use defined as ≥1 time/wk for ≥1 yr **Strengths:** Population-based controls. Cases reviewed by pathologists.
		Nonuser	1	59		
		User	1.24 (0.64–2.42)	17		
		<7	0.66 (0.24–1.86)	5		
		≥7	1.98 (0.88–4.45)	12		
		Trend-test *p*-value: 0.10				
		OR Tablets per week in men				
		Nonuser	1	94		
		User	1.07 (0.51–2.23)	12		
		<7	0.54 (0.15–1.92)	3		
		≥7	1.64 (0.67–4.05)	9		
		Trend-test *p*-value: 0.36				

Table 12. Cohort and case-control studies of lymphoma and its subtypes and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Lymphoma (type not specified) – Case-Control						
Becker et al. (2009) Case-Control Europe **Enrollment or follow-up:** 1998-2004	**Population:** Cases: 2362; Controls: 2458 **Exposure assessment method:** questionnaire	**OR, Acetaminophen use**			Center, age, sex	**Exposure information:** Exposure levels: any use **Strengths:** Large dataset **Limitations:** Possible selection bias as the controls were selected from hospital-based studies. Self-reported medical history. Non-specific lymphoma and no pathological review. Limited analysis of analgesics.
		No use	1	374		
		Use	2.29 (1.49–3.51)	NR		
Total NHL (Non-Hodgkin's lymphoma) (all subtypes combined) – Cohort						
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Friis et al. (2002) above in Table 9.*	**ICD-7, 200, 202: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year	*See Friis et al. (2002) above in Table 9.*
		Prescription	1.2 (0.7–2)	14		
Total NHL (Non-Hodgkin's lymphoma) (all subtypes combined) – Case-Control						
Baker et al. (2005) Case-Control Buffalo, NY **Enrollment or follow-up:** 1982-1998	**Population:** Hospital-based controls Cases: 625; Controls: 2512 **Exposure assessment method:** questionnaire	**OR, Men - regular acetaminophen use**			Age, race, year of survey	**Exposure information:** Regular use defined as ≥once/wk for 6 consecutive months **Strengths:** Conducted pathology review of the diagnosis. **Limitations:** Hospital-based controls- possible selection bias as the controls were selected from the hospital getting treatment for non-neoplastic conditions. Controls were using analgesics and this may dilute the acetaminophen use between cases and controls. It may not represent unbiased measure of association.
		No	1	290		
		Yes	0.75 (0.48–1.17)	29		
		OR, Men - frequency of acetaminophen use				
		1-6 times/wk	0.62 (0.37–1.03)	21		
		≥7 times/wk	1.54 (0.65–3.67)	8		
		OR, Men - duration of acetaminophen use				
		1-10 yrs	0.63 (0.34–1.15)	14		
		>10 yrs	0.92 (0.5–1.7)	15		
		OR, Men - cumulative acetaminophen use				
		Moderate use (≤10 tablet-yrs)	0.72 (0.44–1.16)	25		
		High use (>10 tablet-yrs)	1 (0.32–3.15)	4		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
		OR, Women - regular use				
		No	1	186		
		Yes	1.71 (1.18–2.5)	64		
		OR, Women - frequency of acetaminophen use				
		1-6 times/wk	1.8 (1.2–2.7)	53		
		≥7 times/wk	1.38 (0.65–2.93)	11		
		OR, Women - duration of acetaminophen use				
		1-10 yrs	1.78 (1.15–2.76)	42		
		>10 yrs	1.59 (0.9–2.81)	22		
		OR, Women - cumulative acetaminophen use				
		Moderate use (≤10 tablet-yrs)	1.95 (1.3–2.92)	56		
		High use (>10 tablet-yrs)	0.95 (0.42–2.17)	8		
Kato et al. (2002) Case-Control New York **Enrollment or follow-up:** 1995-1998	**Population:** Women, aged 20-79 yrs Cases: 376; Controls: 463 **Exposure assessment method:** interview	**OR, Acetaminophen use**			Age at index date, family history of hematologic cancer, college education, BMI 10 yrs before interview, surrogate status, year of interview	**Exposure information:** Regular use defined as ≥ 1 time/month for ≥ 6 months **Strengths:** Population-based controls. Diagnosis confirmed through medical records and pathology slides. **Limitations:** Exposure self-reported.
		No use	1	NR		
		≤3 yrs	0.98 (0.42–2.32)	15		
		3.01-10 yrs	1.26 (0.51–3.09)	15		
		>10 yrs	1.39 (0.45–4.26)	13		
		Trend-test *p*-value: 0.10				
NHL (B-cell lymphoma) – Cohort						
Walter et al. (2011b) Cohort US **Enrollment or follow-up:** 2000-2008	**Population:** Men and women in the Vitamins and Lifestyle (VITAL) study N=64,839 **Exposure assessment method:** questionnaire	**Plasma cell disorders: HR, 10-yr use prior to baseline in men and women combined**			*See Walter et. (2011b) above in Table 9.*	**Exposure information:** Exposure level: “no use,” “low use” (<4 d/wk or <4 yrs), and “high use” (≥4 d/wk and ≥4 yrs). **Strengths:** Large cohort study **Limitations:** No information on dosage, possible reverse causation
		Nonuser	1	44		
		Low use (<4 d/wk or <4 yrs)	1.63 (0.88–3.04)	14		
		High use (≥4 d/wk and ≥4 yrs)	2.42 (1.08–5.41)	7		
		Trend-test *p*-value: 0.007				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
		Mature B-cell neoplasms other than CLL/SLL or plasma cell disorders: HR, 10-yr use prior to baseline in men and women combined				**Confidence in evidence:** Some limitations, but overall considered informative.
		Nonuser	1	169		
		Low use (<4 d/wk or <4 yrs)	0.84 (0.56–1.26)	30		
		High use (≥4 d/wk and ≥4 yrs)	1.81 (1.12–2.93)	23		
		Trend-test *p*-value: 0.055				
NHL (DLBCL (Diffuse large B-cell lymphoma)) – Case-Control						
Baker et al. (2005) Case-Control Buffalo, NY **Enrollment or follow-up:** 1982-1998	**Population:** Hospital-based controls Cases: 625; Controls: 2512 **Exposure assessment method:** questionnaire	**OR, Men - regular use**			Age, race, year of survey	*See Baker et al. (2005) above.*
		No	1	290		
		Yes	0.68 (0.3–1.53)	84		
		OR, Women - regular use				
		No	1	186		
		Yes	2.51 (1.23–5.14)	50		
NHL (Follicular B cell lymphoma) – Case-Control						
Baker et al. (2005) Case-Control Buffalo, NY **Enrollment or follow-up:** 1982-1998	**Population:** Hospital-based controls Cases: 625; Controls: 2512 **Exposure assessment method:** questionnaire	**OR, Men - regular use**			Age, race, year of survey	*See Baker et al. (2005) above.*
		No	1	290		
		Yes	0.77 (0.37–1.59)	107		
		OR, Women - regular use				
		No	1	186		
		Yes	1.6 (0.9–2.86)	94		
NHL (SLL/CLL) – Cohort						
Walter et al. (2011b) Cohort US **Enrollment or follow-up:** 2000-2008	**Population:** Men and women in the Vitamins and Lifestyle (VITAL) study N=64,839 **Exposure assessment method:** questionnaire	**HR, 10-yr use prior to baseline in men and women combined**			*See Walter et. (2011b) above in Table 9.*	**Exposure information:** Exposure level: "no use," "low use" (<4 d/wk or <4 yrs), and "high use" (≥4 d/wk and ≥4 yrs). **Strengths:** Large cohort study **Limitations:** No information on dosage, possible reverse causation **Confidence in evidence:** Some limitations, but overall considered informative.
		Nonuser	1	70		
		Low use (<4 d/wk or <4 yrs)	0.93 (0.5–1.73)	13		
		High use (≥4 d/wk and ≥4 yrs)	0.84 (0.31–2.28)	4		
		Trend-test *p*-value: 0.261				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
NHL (SLL) – Case-Control						
Baker et al. (2005) Case-Control Buffalo, NY **Enrollment or follow-up:** 1982-1998	**Population:** Hospital-based controls Cases: 625; Controls: 2512 **Exposure assessment method:** questionnaire	**OR, Men - regular use**			Age, race, year of survey	*See Baker et al. (2005) above.*
		No	1	290		
		Yes	0.23 (0.03–1.77)	36		
		OR, Women - regular use				
		No	1	186		
		Yes	2.41 (1.08–5.41)	39		
NHL (T-cell lymphoma) – Case-Control						
Baker et al. (2005) Case-Control Buffalo, NY **Enrollment or follow-up:** 1982-1998	**Population:** Hospital-based controls Cases: 625; Controls: 2512 **Exposure assessment method:** questionnaire	**OR, Men - regular use**			Age, race, year of survey	*See Baker et al. (2005) above.*
		No	1	290		
		Yes	0.73 (0.16–3.24)	32		
		OR, Women - regular use				
		No	1	186		
		Yes	0.54 (0.11–2.76)	17		
MM (Multiple myeloma) – Cohort						
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Friis et al. (2002) above in Table 9.*	**ICD-7, 203: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year	*See Friis et al. (2002) above in Table 9.*
		Prescription	1.6 (0.6–3.2)	7		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
MM (Multiple myeloma) – Case-Control						
Moysich et al. (2007) Case-Control Buffalo, NY **Enrollment or follow-up:** 1982-1998	**Population:** Cases: 117; Controls: 483 **Exposure assessment method:** questionnaire	**OR, Acetaminophen use**			Age, smoking status, year of questionnaire completion	**Exposure information:** Regular use defined as ≥1/wk for ≥6 months **Limitations:** Hospital controls. Exposure self-reported.
		No regular use	1	82		
		Regular use	2.95 (1.72–5.08)	30		
		1-6 times/wk	2.57 (1.39–4.76)	21		
		>7 times/wk	4.36 (1.7–11.23)	9		
		Trend-test *p*-value: <0.001				
		OR, Duration of use				
		No regular use	1	82		
		1-10 yrs	2.75 (1.41–5.38)	16		
		>10 yrs	3.26 (1.52–7.02)	14		
		Trend-test *p*-value: <0.001				
HL (Hodgkin's lymphoma) – Cohort						
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Friis et al. (2002) above in Table 9.*	**ICD-7, 201: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year	*See Friis et al. (2002) above in Table 9.*
		Prescription	1.4 (0–8)	1		
HL (Hodgkin's lymphoma) – Case-Control						
Chang et al. (2004) Case-Control Boston, MA and CT **Enrollment or follow-up:** 1997-2000	**Population:** Cases: 565; Controls: 679 **Exposure assessment method:** questionnaire	**OR, Acetaminophen use**			Age, sex, state of residence, smoking history, use of other analgesics	**Exposure information:** Exposure level: regular use (≥2 times/wk) **Limitations:** Possible selection bias because controls were often of higher educational level and SES.
		Non-regular use	1	NR		
		Regular use	1.72 (1.29–2.31)	NR		
		OR, Acetaminophen use				
		Never	1	NR		
		Occasional	1.88 (1.4–2.53)	NR		
		Regular	2.17 (1.58–2.98)	NR		
		Trend-test *p*-value: 0.001				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
		OR, Regular use, stratified by age				
		No regular use	1	NR		
		Regular use, 15-54 y	1.75 (1.27–2.41)	NR		
		Regular use, 55-79 y	1.55 (0.74–3.21)	NR		
		OR, Regular use, stratified by sex				
		No regular use	1	NR		
		Regular use, men	1.74 (1.13–2.68)	NR		
		Regular use, women	1.7 (1.14–2.53)	NR		
		OR, Regular use, stratified by histologic subtype				
		No regular use	1	NR		
		Regular use, nodular sclerosis	1.63 (1.16–2.29)	NR		
		Regular use, mixed cellularity	1.42 (0.72–2.8)	NR		
		OR, Regular use, stratified by tumor EBV genome status				
		No regular use	1	NR		
		Regular use, positive	1.81 (1.06–3.08)	NR		
		Regular use, negative	1.58 (1.11–2.24)	NR		

3.1.2.3 Liver cancer

In the US, liver cancer (liver and intrahepatic bile duct cancer) was the fifth and eighth leading cause of cancer death in men and women, respectively, accounting for approximately 41,000 cancer cases and 29,000 deaths in 2017 (Islami et al. 2017). Risk factors for liver cancer that are also associated with acetaminophen use are tobacco smoking, obesity and alcoholic beverages (ACS 2019b; IARC 2019b).

The association between acetaminophen use and liver cancer has been assessed in two independent cohorts; there were no case-control studies (Table 13). Both cohorts noted positive associations.

In a Danish study that compared cancer incidence in an acetaminophen exposed cohort to the general population, Friis et al. (2002) did not find a signficant association of acetaminophen prescription with liver cancer (SIR, 1.8; 95% CI, 0.7–3.6; 7 exposed cases) (see Section 3.1.1 for study details). However, a mortality analysis of this cohort (Lipworth et al. 2003) found a statistically significant increased risk of liver cancer mortality in men (SMR, 2.6; 95% CI, 1.8–3.8) who were prescribed acetaminophen. Mortality from liver cancer in women was not statistically significant (SMR, 1.6; 95% CI, 0.9–2.6). The risk was similar after a latency period of 5 or more years in men and women combined (SMR, 2.6; 95% CI, 1.1–5.2; 8 cases). However, this study did not control for confounders such as smoking and alcohol use. This study was limited by the use of death certificates to ascertain outcome, which may not reflect cancer incidence, particularly for low fatality diseases.

Two publications from a nested case-control study of liver cancer conducted within a large medical records database in the UK, the Clinical Practice Research Datalink (CPRD) (previously the GPRD), reported some positive associations between acetaminophen prescriptions and liver cancer (McGlynn et al. 2015; Yang et al. 2016). CPRD is a large, automated population-based medical record database that contains prospectively collected information both on prescribed medications and clinical diagnoses for approximately 8.5% of the population of the UK. Cases and controls were drawn from the database from 1988 to 2011 who were 10-90 years old. Controls were selected from individuals who were in the database at the case's index date and had no cancer diagnosis prior to that date, and were matched based on age, sex, general practice, and number of prior years in the CPRD.

McGlynn et al. (2015) had the primary objective of assessing statin use; acetaminophen use was modelled only as a covariate, and the study reported a univariate OR of 1.52 (95% CI 1.31–1.75). Yang et al. (2016) further explored these findings in a more detailed analysis of acetaminophen use. Acetaminophen use was associated with an increased risk of liver cancer that was, in general, more pronounced in heavy and long-

term users. Dose-response trends were observed in some analyses. The authors presented several sensitivity analyses. For example, they stratified by the presence of liver disease to assess confounding by indication (OR, 1.24; 95% CI, 1.05–1.47) and also omitted acetaminophen use two years prior to diagnosis from the analysis to assess the possibility of protopathic bias (OR, 1.20; 95% CI, 1.02–1.42). Analyses were adjusted for several covariates including BMI, smoking status, alcohol-related disorders, hepatitis B or C virus infection, diabetes, rare metabolic disorders, use of NSAIDs, antidiabetic medications, and statins. In general, the adjusted ORs were attenuated from the crude ORs. In the tables in this report, only the most informative analyses are presented.

Increasing risk was observed with increasing acetaminophen prescriptions overall and when restricted to individuals without liver disease (p-value for trend < 0.01). This trend was also present in individuals without liver disease with the highest intensity of acetaminophen use (time between first and last prescriptions >5 years).

Confounding by indication was assessed as a possible explanation for the increase in risk. The authors state that patients at highest liver cancer risk (e.g., cirrhosis, portal hypertension with thrombocytopenia) may be advised to avoid NSAID use due to risk of gastrointestinal bleeding and renal failure and suggest these patients may be channeled towards receiving acetaminophen. Therefore these indications may confound the association between acetaminophen use and liver cancer (Yang et al. 2016). However, results did not change materially when restricting the analyses to individuals without chronic liver disease, supporting that confounding by indication was not an explanation for these positive findings.

Protopathic bias, or reverse causation, was examined as a potential explanation for the risk increases. Because acetaminophen may be used to treat pain in early stages of liver cancer before diagnosis, one might expect the association to be attenuated when changing the index date from one year to two years prior to the date of diagnosis. However, the statistically significant results were essentially unchanged in this sensitivity analysis (OR 1.20, 95% CI 1.02–1.42 for ever-use of acetaminophen 2 years prior to diagnosis vs. OR 1.18, 95% CI 1.00–1.39 for ever-use of acetaminophen 1 year prior to diagnosis), arguing against protopathic bias as an explanation. Nevertheless, potential reverse causation could not be ruled out as it is unknown whether these persons had pre-clinical liver cancer.

This study had several strengths. Several validation studies have been conducted within the CPRD, increasing the reliability of the data. It is population-based and is representative of the population in terms of age, sex, most diseases, and prescriptions written; thus minimizing the potential for selection bias and improving validity and generalizability of the findings (Strom 2013). Prescription records to assess

acetaminophen use were prospectively collected, minimizing the potential for information bias and exposure misclassification (see beginning of Section 3.1). Additionally, these analyses adjusted for alcohol-related disorders, accounting for potential confounding of alcohol use.

Summary of liver cancer findings

The association between acetaminophen use and liver cancer was examined in two large independent cohorts that assessed acetaminophen use through prescription records databases, one from Denmark (Friis et al. 2002; Lipworth et al. 2003) and the other from the UK (McGlynn et al. 2015; Yang et al. 2016). In the Danish cohort, elevations in mortality and incidence risk were observed that were statistically significant for mortality from liver cancer (Lipworth et al. 2003) but not incidence of liver cancer (Friis et al. 2002). This cohort could not control for potential confounders such as smoking and alcohol use. In the UK database, statistically significant elevations in the risk of liver cancer were reported, after adjusting for several covariates including smoking status, alcohol-related disorders, hepatitis B or C virus infection, and use of NSAIDs (Yang et al. 2016). Dose-response analyses showed increasing risk with increasing acetaminophen prescriptions overall and when restricted to individuals without liver disease.

Table 13. Cohort and nested case-control studies of liver cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Cohort						
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	**Population:** Danish Cancer registry and prescription database. Cases excluded because of residency outside the county at the date of prescription, parent (of patient) registered as customer, error in the personal identification number, death prior to or at the date of prescription, age younger than 16 years, subjects who had a cancer diagnosis (except nonmelanoma skin cancer) prior to date of first recorded prescription, or died within the first year of follow-up N = 39,946 **Exposure assessment method:** records	**ICD-7, 155: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year	**Exposure information:** Exposure level: ever prescribed acetaminophen and number of prescriptions **Strengths:** Excluded person-time and cancer experience in the first year of follow-up after first acetaminophen prescription. Use of prescription database and linked to cancer registry. Almost complete capture of the population of North Jutland county. **Limitations:** Multiple comparisons performed, no adjustment for confounders such as tobacco smoking or alcohol consumption, lack of data on use of analgesic agents prior to start of prescription database and use of OTC analgesics, degree of compliance, or indications for use. **Confidence in evidence:** This study had some limitations, but is still fairly informative. The primary strength is the use of the prescription database for exposure assessment and linkage to the cancer registry. The main concern is the lack of control for confounders other than matching for age, year, and sex.
		Prescription	1.8 (0.7–3.6)	7		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Lipworth et al. (2003) Cohort Denmark **Enrollment or follow-up:** 1989-1996	**Population:** Individuals who filled prescriptions identified through the Pharmacoepidemiological Prescription Database N = 17,760 men; 32,130 women **Exposure assessment method:** records	**Mortality: SMR, Prescribed acetaminophen (1989-1996)**			Age, sex, calendar year	**Exposure information:** NR **Strengths:** Conducted latency analyses. **Limitations:** The main limitation is that cause of death would miss cancer incidences. Also, there was no information on indication for use, likely protopathic bias, short follow-up period, limited control for confounding. **Confidence in evidence:** This study is not considered informative because cause of death likely does not reflect cancer incidence. Additionally, this study uses the same population as Friis et al. 2002 and it is possible there was some double-counting of cases.
		All	2.2 (1.6–2.9)	48		
		Men	2.6 (1.8–3.8)	31		
		Women	1.6 (0.9–2.6)	17		
		Mortality: SMR, Prescribed acetaminophen, stratified by latency period				
		<1 yr	3.8 (2.3–5.9)	19		
		1-2 yrs	1.7 (0.97–2.9)	15		
		3-4 yrs	1.1 (0.4–2.4)	6		
		≥5 yrs	2.6 (1.1–5.2)	8		
		Mortality: SMR, Number of prescriptions				
		1	2.7 (1.6–4.1)	20		
		2-4	2.1 (1.1–3.6)	13		
		5-9	1.9 (0.8–3.9)	7		
		≥10	1.6 (0.7–3.1)	8		
Nested Case-Control						
McGlynn et al. (2015) Nested Case-Control UK **Enrollment or follow-up:** 1988-2011	**Population:** A nested case-control study was conducted within the Clinical Practice Research Datalink (CPRD) of the United Kingdom (UK). Cases: 1195; Controls: 4640 **Exposure assessment method:** records	**Univariate OR, Paracetamol (acetaminophen) use**			None	**Exposure information:** Exposure levels: any use **Limitations:** Limited assessment of acetaminophen use and liver cancer. Possible that some secondary liver cancers were included.
		No use	1	404		
		Acetaminophen use	1.52 (1.31–1.75)	791		

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
Yang et al. (2016) Nested Case-Control United Kingdom **Enrollment or follow-up:** 1988 to 2011	**Population:** In addition to the full case-control match, we completed an additional match based on the presence of chronic liver disease. For the 170 cases with a history of chronic liver disease, 680 controls selected among individuals with liver disease in the CPRD were matched to these cases at a four-to-one ratio using the same matching factors as in the primary match. Similarly, the remaining 1025 cases without liver disease were matched to 4100 controls without chronic liver disease. This approach allows sufficient sample size for stratified analyses by chronic liver disease. Cases: 1195; Controls: 4640 **Exposure assessment method:** records	**OR, Excluding exposure 2 years prior to case diagnosis. Any paracetamol (acetaminophen) use (no. of prescriptions)**			BMI, smoking status, alcohol-related disorders, hepatitis B or C virus infection, diabetes, rare metabolic disorders, use of NSAIDs, antidiabetic medications, and statins	**Exposure information:** Ever use defined as ≥2 prescriptions **Strengths:** Conducted several sensitivity tests to rule out confounding by indication and protopathic bias. Analyses were adjusted for alcohol-related disorders, collected prospectively, to rule out confounding by alcohol use. Reliability of the data were ensured through several validation studies within CPRD. The study was population-based, representative of the general population, thus minimizing the potential for selection bias, and improving the validity and generalizability of the findings. **Limitations:** None. **Confidence in evidence:** No concerns of selection or information bias or confounding.
		0 – 1	1	620		
		≥ 2	1.2 (1.02–1.42)	575		
		2-9	1.14 (0.94–1.37)	271		
		10-19	1.14 (0.84–1.53)	86		
		20-39	1.47 (1.09–1.98)	94		
		≥40	1.3 (0.98–1.73)	124		
		Trend-test *p*-value: 0.07				
		Individuals without liver disease: OR, acetaminophen use (no. of prescriptions)				
		0-1	1	501		
		≥2	1.24 (1.05–1.47)	524		
		2-9	1.09 (0.89–1.33)	224		
		10-19	1.54 (1.14–2.08)	91		
		20-39	1.21 (0.88–1.66)	75		
		≥40	1.61 (1.22–2.12)	134		
		Trend-test *p*-value: <0.01				
		Individuals without liver disease: OR, 2-5 years between first and last prescription (no. of prescriptions)				
		0 - 1	1	501		
		≥2	1.15 (0.89–1.49)	114		
		2-9	1.15 (0.83–1.61)	58		
		10-19	1.7 (1.02–2.83)	29		
		20-39	0.87 (0.5–1.54)	18		
		≥ 40	0.9 (0.39–2.11)	9		
		Trend-test *p*-value: 0.99				

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled	Comments, strengths, and weaknesses
		Individuals without liver disease: OR, >5 years between first and last prescription (number of prescriptions)				
		0 - 1	1	501		
		≥2	1.35 (1.09–1.67)	307		
		2-9	1.06 (0.78–1.44)	79		
		10-19	1.4 (0.95–2.05)	49		
		20-39	1.35 (0.93–1.97)	54		
		≥40	1.71 (1.28–2.29)	125		
		Trend-test *p*-value: <0.01				

3.1.2.4 Hormone-related cancers

Breast cancer

In the US, it is estimated that breast cancer will be responsible for approximately 268,600 new diagnoses of invasive cancer cases, 62,930 new cases of carcinoma in situ, and 41,760 deaths in women in 2019 (ACS 2019c; Torre et al. 2017). Risk factors for breast cancer that are also associated with acetaminophen use are obesity and alcoholic beverages. Female hormone use, menopausal status, and reproductive factors are important risk factors for breast cancer, although their association with acetaminophen exposure is unknown (ACS 2019b; IARC 2019b).

Cohort studies

Eighteen cohort studies evaluated the use of acetaminophen and risk of breast cancer in women (Bosco et al. 2011; Clarke et al. 2017; Eliassen et al. 2009; Friis et al. 2002; Friis et al. 2008; Gallicchio et al. 2006; Gallicchio et al. 2007; Garcia Rodriguez and Gonzalez-Perez 2004a; Gill et al. 2007; Harris et al. 1999; Harris et al. 2003; Kehm et al. 2019; Kwan et al. 2007; Lipworth et al. 2003; Marshall et al. 2005; Rahme et al. 2005; Walter et al. 2011a; Zhang et al. 2012) (Appendix Table B1). Lipworth et al. (2003) included two male breast cancer cases. Most studies found either no association or decreases, mostly non-statistically significant, in the risk of breast cancer, (Bosco et al. 2011; Clarke et al. 2017; Friis et al. 2002; Friis et al. 2008; Garcia Rodriguez and Gonzalez-Perez 2004a; Gill et al. 2007; Harris et al. 1999; Harris et al. 2003; Kehm et al. 2019; Walter et al. 2011a), localized and non-localized breast cancer (Marshall et al. 2005), invasive breast cancer (Gallicchio et al. 2007; Marshall et al. 2005), breast carcinoma (Gallicchio et al. 2006), or breast cancer recurrence (Kwan et al. 2007). Eliassen et al. (2009) found no association of acetaminophen use with invasive breast cancer in premenopausal women, and Zhang et al. (2012) and Rahme et al. (2005) found no or decreased association of breast cancer in postmenopausal women. Most studies adjusted for several risk factors for breast cancer, but the covariates used differed by study. More than half of the studies assessed dose-response and only one study found a statistically inverse association with longer duration of acetaminophen use (p-value for trend = 0.03) (Bosco et al. 2011).

The only study that found statistically significant positive associations was a Danish mortality study (Lipworth et al. 2003). However, this study was limited by the use of death certificates to ascertain outcome, which may not reflect cancer incidence, particularly for low fatality disease.

Case-control studies

The five case-control studies of breast cancer were consistent in finding no association with acetaminophen use (Ashok et al. 2011; Brasky et al. 2010; Harris et al. 2006; Meier et al. 2002; Terry et al. 2004) or a small decrease in risk with higher use (Meier et al. 2002) (Appendix Table B2). Three of these studies adjusted for BMI (Harris et al. 2006; Meier et al. 2002; Terry et al. 2004) and one study additionally adjusted for alcohol use (Harris et al. 2006). Of the two studies that assessed dose-response, neither observed an association (Brasky et al. 2010; Meier et al. 2002).

Meta-analyses

A meta-analysis that included unpublished data found a reduced risk of breast cancer with acetaminophen use (meta-OR for case control studies, 0.85, 95% CI 0.76–0.95; meta-RR for cohort studies 0.95, 95% CI, 0.88-1.01; meta-RR for estrogen positive tumors, 0.92; 95% CI, 0.85-1.00) (de Pedro et al. 2015).

Summary of breast cancer findings

Overall, acetaminophen use was associated with either no association or a mostly non-significant reduced risk of breast cancer in women in 18 cohort and five case-control studies, even after adjustment for important potential confounders such as BMI and alcohol use in several of these studies. The one exception was the cohort study of Lipworth et al. (2003) that used death certificates to assess outcome. Many of these studies assessed dose-response and found no association. These null/negative findings were consistent over different time periods, geographic locations, and study design, minimizing the possibility of chance as an explanation.

Ovarian cancer

Ovarian cancer is rare, representing 1.3% of all new cancer cases in the U.S. In 2019, it is estimated that there will be 22,530 new cases and 13,980 deaths in the US from ovarian cancer (NCI 2019a). Ovaries are composed of three main types of cells; each cell type can develop into a different tumor type, namely epithelial, germ cell, and stromal. Epithelial tumors can be benign, borderline or malignant. Malignant epithelial tumors can be further classified as serous, clear cell, mucinous, or endometroid (ACS 2018c). BMI is the main potential confounder in the association between acetaminophen use and ovarian cancer. Hormone therapy is also a risk factor, although its association with acetaminophen exposure is unknown (NCI 2019b).

Cohort studies

Eleven cohort studies evaluated the association between acetaminophen use and ovarian cancer (Barnard et al. 2018; Fairfield et al. 2002; Friis et al. 2002; Lacey et al.

2004; Lipworth et al. 2003; Merritt et al. 2018; Nagle et al. 2015; Pinheiro et al. 2009; Rodriguez et al. 1998; Setiawan et al. 2012; Trabert et al. 2019) (Appendix Table B3).

In the NHS and NHSII studies, Barnard et al. (2018) observed positive but not statistically significant increases in risk of epithelial ovarian cancer with heavy acetaminophen use (≥2500 tablet-days) (HR, 1.41; 95% CI, 0.99–2.00, p for trend = 0.10) and with greater duration of use (≥10 years) (HR, 1.12; 95% CI, 0.87–1.46, p for trend = 0.12) after adjustment for multiple factors. A strength of this study is that it incorporated a latency period of 2-4 years to limit the potential for reverse causation. When the latency period was lengthened to 8-10 years, the associations for acetaminophen were weaker. A limitation was that exposure assessment was conducted through self-administered questionnaires.

A positive association was observed with ovarian cancer mortality in Lipworth et al. (2003) (SMR, 2.3; 95% CI, 1.9–2.8). This association was attenuated when stratified by latency period (SMR, 0.9; 95% CI, 0.3-2.1 for five or more years of latency), suggesting the possibility of protopathic bias since women may take acetaminophen for early symptoms of the disease before knowledge of their cancer status (see Section 3.1 for additional details).

No associations were observed between acetaminophen use and risk of overall ovarian cancer in the Breast Cancer Detection Demonstration Project Follow-up Study (Lacey et al. 2004), NHS (Nurses' Health Study) cohorts (Pinheiro et al. 2009), Multiethnic Cohort (MEC) study (Setiawan et al. 2012), or in a Danish cohort (Friis et al. 2002). No associations were observed between acetaminophen use and risk of invasive ovarian cancer (Fairfield et al. 2002) or ovarian cancer-specific death (Merritt et al. 2018) in the NHS cohorts. No associations were observed between acetaminophen use and risk of ovarian cancer mortality in the Cancer Prevention Study II (CPS-II) (Rodriguez et al. 1998) or overall mortality in ovarian cancer patients in an Australian study (Nagle et al. 2015). Lacey et al. (2004), Friis et al. (2002) and Fairfield et al. (2002) had limited power to detect an association due to small numbers of cases.

Trabert et al. (2019) examined the association of acetaminophen with multiple histologic subtypes of ovarian cancer in the Ovarian Cancer Cohort Consortium, a pooled analysis comprised of up to 13 prospective cohort studies from North America and Europe. Acetaminophen exposure was assessed through self-reported questionnaires in all cohorts, and cancer cases were identified through either cancer registries or medical record review. Daily use of acetaminophen was associated with an increased risk of ovarian cancer overall (HR, 1.28; 95% CI, 1.00–1.65, 71 exposed cases) and the serous subtype (HR, 1.70; 95% CI, 1.14–2.55, 26 exposed cases), and was not statistically significant in the endometrioid subtype (HR, 1.85; 95% CI, 0.75–4.57, 6

exposed cases). Non-significant increased risks were observed with the mucinous and clear cell subtype, but there were very few cases (four or fewer). Dose-response analyses were conducted for several exposure metrics, but p-values for tests for trend were not reported. Several of the studies (Brasky et al. 2014; Gallicchio et al. 2007; Pinheiro et al. 2009) and study populations used in the other cohort studies (e.g., NHS, VITAL) were included in this pooled analysis.

Case-control studies

Acetaminophen use was assessed in 13 case-control studies of ovarian cancer (Ammundsen et al. 2012; Baandrup et al. 2014; Cramer et al. 1998; Hannibal et al. 2008; Hannibal et al. 2018; Lo-Ciganic et al. 2012; Meier et al. 2002; Moysich et al. 2001; Peres et al. 2016; Pinheiro et al. 2010; Rosenberg et al. 2000; Schildkraut et al. 2006; Wu et al. 2009) and one pooled analysis of 12 population-based case-control studies (Trabert et al. 2014) (Appendix Table B4). The pooled analysis (Trabert et al. 2014), which included several previously unpublished results on acetaminophen from case-control studies of ovarian cancer, reported no association between acetaminophen use and ovarian cancer overall (OR = 0.99; 95% CI = 0.88–1.12; I^2: 40.0%[4]) nor by histological subtypes of ovarian cancer, nor by dose, duration, or frequency of acetaminophen use. In general, these studies, including the large pooled analysis, showed no or inverse association between acetaminophen use and risk of ovarian cancer or its histological subtypes. All of these studies adjusted for several covariates, although the covariates included differed by study. Several of the studies also assessed dose-response, and found null or inverse associations that were not statistically significant (p-value for trend >0.05)

Meta-analyses

A now-outdated meta-analysis that included 8 studies (4 cohort studies, 4 case-control studies) published between 1998 and 2004 showed a protective effect of acetaminophen on ovarian cancer risk (Bonovas et al. 2006). The meta RR for regular compared to non-use was 0.70 (95% CI, 0.51–0.95).

Summary of ovarian cancer findings

Eleven cohort studies and 13 case-control studies evaluated the association between acetaminophen use and ovarian cancer. The results from the cohort studies were inconsistent, however a pooled analysis comprised of up to 13 prospective cohort studies from North America and Europe found that daily use of acetaminophen was associated with increased risk of ovarian cancer overall, serous, endometrioid, mucinous and clear cell subtypes. In the pooled analysis of cohort studies, dose-

[4] See footnote 3 for definition of I^2

response analyses were conducted, but the highest exposed categories had the fewest exposed subjects and did not observe the highest risks. Generally, the case-control studies and a large pooled analysis of case-control studies showed no or mostly non-significant inverse associations between acetaminophen use and risk of ovarian cancer or its subtypes.

Cervical cancer

Acetaminophen use was not associated with cervical cancer in a cohort study (Friis et al. 2002) and in a single case-control study of cervical cancer (Friel et al. 2015) (Appendix Table B5). In the case-control study there was no evidence for a dose-response association with the number of tablets used per week, the years of use, or cumulative use (tablets/week and years of use).

Uterine endometrial cancer

Acetaminophen use was not associated with endometrial cancer in three of the four cohort studies (Setiawan et al. 2012; Viswanathan et al. 2008; Walter et al. 2011a), a pooled analysis of the Epidemiology of Endometrial Cancer Consortium (Webb et al. 2018), and a meta-analysis (Ding et al. 2017) (Appendix Table B6). A Danish cohort found a non-significant increase (Friis et al. 2002) (see Section 3.1.1 for study details). The three case-control studies of endometrial cancer (Bodelon et al. 2009; Moysich et al. 2005; Neill et al. 2013) showed some increases in some categories of acetaminophen use, but none of the elevations are statistically significant.

Prostate cancer

Cohort studies

Six independent cohort studies examined the association between acetaminophen use and prostate cancer (Friis et al. 2002; Garcia Rodriguez and Gonzalez-Perez 2004b; Jacobs et al. 2011; Lipworth et al. 2003; Murad et al. 2011; Platz et al. 2005; Veitonmaki et al. 2014; Walter et al. 2011a) (Appendix Table B7).

In a Danish cohort, acetaminophen use was not associated with prostate cancer incidence (SIR, 0.8; 95% CI, 0.5–1.3) (Friis et al. 2002; Jacobs et al. 2011; Platz et al. 2005; Walter et al. 2011a) but was associated with increased prostate cancer mortality (Lipworth et al. 2003) (SMR, 3.7; 95% CI, 3.4–4.1). This association was attenuated when stratified by latency period (SMR, 1.6; 95% CI, 0.9–2.6 for five or more years of latency), suggesting there was likely protopathic bias; however the interpretation of this study is also limited by the use of mortality data (prostate cancer is not always fatal, and therefore there could be an under-ascertainment of cases) (see Section 3.1.1 for study details). Veitonmaki et al. (2014) found increased risks of prostate cancer in current

users of acetaminophen for overall risk, localized cancer, metastatic cancer, and high-grade cancer. Risks were higher for metastatic and high-grade cancer. This study found similar associations with other pain relievers, such as prescription NSAIDs and cox-2 selective inhibitors (coxibs). For these two reasons, the authors hypothesize that the risk elevation was due to protopathic bias due to treatment of symptoms of undiagnosed prostate cancer, particularly pain due to metastases.

Other studies found either no association or a decreased risk of prostate cancer with acetaminophen use (Friis et al. 2002; Jacobs et al. 2011; Platz et al. 2005; Walter et al. 2011a). Walter et al. (2011a) and Jacobs et al. (2011) found decreased risks of aggressive prostate cancer with acetaminophen use. One nested case-control study found a non-significantly increased risk with any acetaminophen use (OR, 1.15; 95%CI, 0.86–1.53; 67 exposed cases) (Murad et al. 2011). Another nested-case control study conducted within the GPRD found an inverse association with increasing years of use (trend-test *p*-value: 0.02) and in the highest category of acetaminophen dose (OR, 0.59; 95% CI, 0.38–0.93; 25 exposed cases for >2000 mg/d among current users for more than a year) (Garcia Rodriguez and Gonzalez-Perez 2004b). Garcia Rodriguez and Gonzalez-Perez (2004b) assessed acetaminophen use through a prescription database, while Murad et al. (2011) assessed acetaminophen use through self-report.

Case-control studies

Two case-control studies of prostate cancer found no association with regular acetaminophen use (Nelson and Harris 2000; Salinas et al. 2010; Wright et al. 2016) (Appendix Table B7).

Summary of prostate cancer findings

The majority of the cohorts (Friis et al. 2002; Jacobs et al. 2011; Platz et al. 2005; Walter et al. 2011a), including a nested case-control study (Garcia Rodriguez and Gonzalez-Perez 2004b), and a single case-control study (Salinas et al. 2010; Wright et al. 2016) found either no association or a decreased risk of prostate cancer with acetaminophen use. Three studies found decreased risks of aggressive prostate cancer with acetaminophen use (Jacobs et al. 2011; Veitonmaki et al. 2014; Walter et al. 2011a).

3.1.2.6 Skin cancer

Skin cancers are the most common type of cancer. Non-melanoma (squamous cell carcinoma [SCC], basal cell carcinoma [BCC]) and melanoma are the main types of skin cancer. Melanoma is much less common than the other types, although it is the cause of most deaths from skin cancer (NCI 2019d). Exposure to ultraviolet radiation

and its proxies (e.g. number of sunburns, fair skin, hair, etc.) is a major risk factor (ACS 2016; Canadian Cancer Society 2019).

Cohort studies

Seven cohort studies evaluated the association between acetaminophen use and skin cancer (Cahoon et al. 2012; Friis et al. 2002; Gamba et al. 2013; Jeter et al. 2012; Pandeya et al. 2019; Walter et al. 2011a; Wysong et al. 2014) and its subtypes (Appendix Table B8).

A Danish cohort found an increased risk of ever prescribed acetaminophen with non-melanoma skin cancer (SIR, 1.3; 95% CI, 1.1–1.6) but no association with malignant melanoma (SIR, 0.6; 95% CI, 0.2–1.3) (Friis et al. 2002). This study calculated SIRs in comparison to the general population and did not control for important covariates, such as sun exposure (see Section 3.1.1 for study details).

Jeter et al. (2012) evaluated risk of skin cancers in 92,125 women in the Nurses' Health Study. After multivariable adjustment, current use was associated with a slightly increased risk of BCC (RR, 1.05; 95% CI, 1.00–1.10), while past use was not (RR, 1.02; 95% CI, 0.96–1.07). Positive dose-response associations with increasing days per week and tablets per week of acetaminophen used were observed in some categories, although trend tests for frequency and duration of use were not statistically significant. This study also found no or inverse association with current and past acetaminophen use and SCC and no association with malignant melanoma. Dose-response analyses also showed no associations or significant trends of increasing risk ($p>0.05$).

Cahoon et al. (2012) evaluated risk of BCC with use of acetaminophen in 58,213 men and women in the United States Radiologic Technologists cohort. After a mean follow-up time of 5.5 years in cases and 8.9 years in non-cases, the study found that any acetaminophen use was significantly positively associated with BCC after adjustment for age, sex, and UV exposure (HR, 1.14; 95% CI, 1.04–1.25). When stratified by average days of use per month, only the lowest category (0-4 days/month) was significantly associated with BCC (HR, 1.18; 95% CI, 1.07–1.3). Higher days of use per month (15-21 and ≥22 days/month) were positively associated with BCC, but were not statistically significant. The *p* for trend was 0.08.

In a cohort from Queensland, Australia, Pandeya et al. (2019) did not find an association with BCC or SCC among infrequent and frequent users of acetaminophen among low risk participants (no history of skin cancer excision and at most five actinic lesions treated).

Wysong et al. (2014) was a prospective cohort study of 54,728 postmenopausal Caucasian women in the Women's Health Initiative Observational Study (WHI OS)

between 1993 and 1998. After adjusting for multiple covariates, there was no association between acetaminophen use and risk of non-melanoma skin cancer.

The details of Walter et al. (2011a) are described in Section 3.1.1. This study found either no or a decreased association of prior use of acetaminophen with malignant melanoma in men and women, but did not adjust for UV exposure. Gamba et al. (2013) evaluated melanoma risk in postmenopausal women in the WHI OS. Regular acetaminophen use was not associated with risk of melanoma. Only one risk estimate was reported for acetaminophen use. The primary focus of the study was aspirin and non-aspirin NSAID use, and no subgroup or sensitivity analyses were conducted with acetaminophen.

Case-control studies

One case-control study of non-melanoma skin cancer found statistically significant reduced risks for BCC and SCC with acetaminophen use, adjusted for age, gender, number of cigarettes smoked per day, skin type, lifelong number of painful sunburns and lifelong cumulative number of hours of sun exposure (Torti et al. 2011). Risk ratios were attenuated towards the null for past acetaminophen use and away from the null for current use. For BCC, a reduced odds ratio was found with acetaminophen use, especially among current users (OR = 0.56; 95% CI 0.33–0.97) and those who reported a longer duration of use (>7 years) (OR = 0.54; 95% CI 0.29–1.03). Similarly, acetaminophen use was associated with reduced risk of SCC among current users (OR = 0.56, 95% CI = 0.33–0.97) and users of >7 years (OR 0.68 95% = CI 0.37–1.23). *P*-values for trend were not presented.

Summary of skin cancer findings

Overall, the associations between acetaminophen use and several types of skin cancer, including malignant melanoma and non-melanoma skin cancer, were either null or inconsistent. For non-melanoma skin cancer, a Danish cohort (Friis et al. 2002) found a significantly increased risk while a prospective cohort from the US (Wysong et al. 2014) found no association with acetaminophen use. Wysong et al. (2014), but not Friis et al. (2002), adjusted for UV exposure. Two other cohorts (Cahoon et al. 2012; Jeter et al. 2012) found increased risk of BCC (the majority of which were non-statistically significant), but dose-response analyses were not consistent. However, another cohort (Pandeya et al. 2019) did not find an association with BCC among infrequent and frequent users of acetaminophen. For SCC, two cohorts found null or inverse associations with acetaminophen use (Jeter et al. 2012; Pandeya et al. 2019). One case-control study of non-melanoma skin cancer found statistically significant reduced risks for BCC and SCC with acetaminophen use, adjusted for age, gender, number of

cigarettes smoked per day, skin type, lifelong number of painful sunburns and lifelong cumulative number of hours of sun exposure (Torti et al. 2011).

Of the four studies that evaluated the relationship between acetaminophen use and malignant melanoma, none found an association (Friis et al. 2002; Gamba et al. 2013; Jeter et al. 2012; Walter et al. 2011a).

The results for skin cancer were inconsistent and there were few studies for each type of cancer.

3.1.2.8 Colorectal cancer

Colorectal cancer is the third most common cancer diagnosed in both men and women in the US, with an estimated 101,420 new cases of colon cancer and 44,180 new cases of rectal cancer to occur in 2019. Potential confounders in the association between acetaminophen use and colorectal cancer are BMI, smoking, and heavy alcohol use (ACS 2018a).

Cohort studies

Thun et al. (1993) found an increased risk of rectum cancer mortality with high frequency of acetaminophen use; however, there were only four exposed cases (RR, 3.08; 95% CI, 1.11–8.54) (Appendix Table B9). Friis et al. (2002) did not find an association of acetaminophen prescription with colon or rectal cancer (see Section 3.1.1 for study details), and Friis et al. (2009) did not find an association with colorectal cancer with any acetaminophen use and with increasing frequency of use. Walter et al. (2011a) did not find an association of prior use of acetaminophen with colorectal cancer (see Section 3.1.1 for details). Garcia Rodriguez and Huerta-Alvarez (2001) found a significant positive association with colorectal cancer for those who used acetaminophen for less than one year, but no association in those who used the drug for greater than one year. This suggests that protopathic bias occurred, since the finding attenuated with a greater period of time between acetaminophen use and diagnosis of cancer. Cea Soriano et al. (2016a) reported three nested case-control study designs from a cohort of a UK primary care database. No associations were observed between acetaminophen use and colorectal cancer in the limited results pertaining to acetaminophen that were reported.

Case-control studies

Six case-control studies examined the association between acetaminophen use and colorectal cancer (Hardell et al. 1996; Harris et al. 2008; Logan et al. 1993; Meier et al. 2002; Muscat et al. 1994; Peleg et al. 1996) (Appendix Table B9). All but two of the

case-control studies of colon/colorectal cancer found no association with acetaminophen use. A Swedish case-control study (Hardell et al. 1996) reported an OR of 2.2 (95% CI, 0.5–9.2) among women who used acetaminophen, after adjustment for age and job-related physical activity. Peleg et al. (1996) reported an OR of 1.28 (95% CI, 0.53–3.09) for adenocarcinoma among men and women who had consumed a cumulative acetaminophen dose of 500 g or more.

Summary of colorectal cancer findings

The association between acetaminophen use and colorectal cancer was assessed in six cohort studies and five case-control studies. The majority of studies reported no association.

3.1.2.9 Other sites

The data from cohort and case-control studies from a number of other cancer sites were too sparse to draw conclusions. They are presented by organ site below.

Brain cancer

One cohort did not find an association between prescriptions for acetaminophen and brain cancer (Friis et al. 2002). Two of the three case-control studies of brain cancer showed some increases with acetaminophen use (Egan et al. 2016; Sivak-Sears et al. 2004; Stålberg et al. 2010). Egan et al. (2016) reported an increase in meningioma (OR, 1.85; 95% CI, 1.29–2.65; 72 exposed cases) and a non-statistically significant increase in risk of glioma (OR, 1.2; 95% CI, 0.93–1.54; 182 exposed cases) associated with acetaminophen use. Stålberg et al. (2010) observed that prenatal acetaminophen exposure was associated with an increased risk of childhood brain cancer that was not statistically significant (OR, 1.7, 95% CI, 0.6–5.4; 8 exposed cases) (Appendix Table B10).

Respiratory tract cancer

Cohort studies

Acetaminophen prescriptions were associated with lung cancer in Friis et al. (2002) (SIR, 1.6; 95% CI, 1.2–2.0) (see Section 3.1.1 for details). Some positive associations were observed in Walter et al. (2011a), but did not reach statistical significance and attenuated from low to high use (see Section 3.1.1 for details). Acetaminophen use was not associated with fatal lung cancer in one study (Bittoni et al. 2017). Lipworth et al. (2003) found an increased risk of respiratory tract cancer mortality in people who were prescribed acetaminophen (SMR, 3.4; 95% CI, 3.1–3.6 for men and women combined). This study did not control for smoking and was limited by the use of death certificates to

ascertain outcome, which may not reflect cancer incidence. One nested case-control study reported an association between acetaminophen use and lung cancer (RR, 1.34; 95% CI, 1.07–1.68), which attenuated after controlling for smoking and occupational training (i.e., unskilled worker; skilled worker; bachelor's degree; master's degree or higher) (RR, 1.11; 95% CI not reported) (Olsen et al. 2008) (Appendix Table B11).

Case-control studies

Four case-control studies of lung cancer and acetaminophen use have been published to date (Erickson et al. 2018; Harris et al. 2007; Lim et al. 2012; Van Dyke et al. 2008); all were adjusted for smoking and age (Appendix Table B11). Two studies reported positive associations with metrics of acetaminophen intake (Erickson et al. 2018; Harris et al. 2007), one of which was statistically significant, whereas the other two studies reported no association between acetaminophen intake and lung cancer (Lim et al. 2012; Van Dyke et al. 2008). The study by Lim et al. (2012) stratified by smoking status and found regular acetaminophen use (at least 2 times a week for at least a month or more) was not associated with lung cancer risk in nonsmokers (OR, 1.02; 95% CI, 0.47–2.21; 10 exposed cases), whereas there was a non-statistically significant elevation in risk only among smokers (OR, 2.58; 95% CI: 0.62–10.78; 10 exposed cases) (Lim et al. 2012).

Gastrointestinal tract cancer

Cohort studies

Friis et al. (2002) found no association of acetaminophen prescription with oral/pharyngeal cancer or stomach/gastric cancer, but found an increased risk of esophageal cancer (SIR, 2.5; 95% CI, 1.2–4.7) (see Section 3.1.1 for study details). In the Multiethnic Cohort study, Epplein et al. (2009) found no association with duration of acetaminophen intake and stomach cancer.

Walter et al. (2011a) did not find an association of acetaminophen use with gastrointestinal cancer overall in men, women, or men and women combined (see Section 3.1.1 for details). Lipworth et al. (2003) found an increased risk of digestive tract cancer mortality in people who were prescribed acetaminophen. However, this study was limited by the use of death certificates to ascertain outcome, which may not reflect cancer incidence, particularly for low fatality disease (Appendix Table B12).

Case-control studies

Neither of the two case-control studies of esophageal cancer (Anderson et al. 2006; Sadeghi et al. 2008) observed significant associations with acetaminophen use (Appendix Table B12). Anderson et al. (2006) observed no significant relationships between acetaminophen use and Barrett's esophagus or esophageal adenocarcinoma

compared with controls: OR (95% CI), 1.09 (0.63-1.91) and 0.82 (0.45-1.52), respectively. Sadeghi et al. (2008) also observed no association between frequent use of acetaminophen and esophageal cancer. There were no case-control studies of stomach cancer available.

Pancreatic cancer

Two cohort (Friis et al. 2002; Walter et al. 2011a) and two case-control studies (Kho et al. 2016; Tan et al. 2011) did not find an association of acetaminophen prescription or use with pancreatic cancer (Appendix Table B13).

All sites

Two cohort studies evaluated the risk of cancer at all sites (Appendix Table B14). Walter et al. (2011a) did not find an association between high use of acetaminophen and total cancer risk in men, women, or overall. Low use of acetaminophen was associated with a non-statistically significant increased risk of total cancer in men (HR, 1.18; 95% CI, 0.99–1.31) (see Section 3.1.1 for details). Friis et al. (2002) observed an increased risk of all cancers combined in men (SIR, 1.21; 95% CI, 1.05–1.38) but not women (SIR, 1.03; 95% CI, 0.92–1.16). A significantly increased risk was observed in individuals prescribed 5-9 acetaminophen prescriptions (SIR, 1.50; 95% CI, 1.20–1.80). Individuals prescribed ten or more prescriptions did not have an increased total cancer risk (SIR, 1.00; 95% CI, 0.80–1.10) (see Section 3.1.1 for details). There were no case-control studies of all cancers combined.

3.2 Carcinogenicity Studies in Animals

A review of the literature on carcinogenicity studies of acetaminophen in experimental animals identified ten studies conducted in mice, and seven studies conducted in rats. Acetaminophen was administered in feed in all studies.

There are also a number of additional studies conducted in mice, rats, and hamsters, utilizing a variety of experimental study designs involving administration of acetaminophen with carcinogens and other modifying factors to investigate acetaminophen's ability to promote or otherwise effect the development of tumors or pre-neoplastic lesions (e.g., liver foci). Additional studies investigating effects on tumor development were limited by short duration, e.g., 25 weeks (one study), 26 weeks (one study), 30 weeks (two studies), 32 weeks (two studies), 36 (one study), 40 weeks (two studies), 44 weeks (one study), 47 weeks (one study), 48 weeks (one study), 52 weeks (three studies), and many were also limited by use of small group sizes. In addition, some studies were non-informative due to observations of 100% tumor incidence in both the carcinogen-alone, and carcinogen plus acetaminophen groups. Additional studies investigating effects on pre-neoplastic lesion development were limited by short duration, e.g., 3.5 weeks (two studies), 8 weeks (three studies), 9 weeks (two studies), 10 weeks (one study), 13 weeks (one study), 18 weeks (one study), and 24 weeks (one study), and many were also limited by use of small group sizes. These additional studies are briefly summarized in Appendix C.

This section is organized as follows:

- An overview of the available carcinogenicity studies in mice and rats is provided in Table 14, which presents key design elements for each study, organized by species, strain, and sex.
- Carcinogenicity studies conducted in mice are summarized in Table 15, which presents information on several aspects of study design, dosing, tumor findings (site, type, and incidence), and other relevant issues. Findings from the mouse studies are then briefly discussed in the text.
- Carcinogenicity studies conducted in rats are summarized in Table 16, which presents information on study design, dosing, tumor findings (site, type, and incidence), and other relevant issues. Findings from the rat studies are then briefly discussed in the text.

Table 14. Overview of acetaminophen animal carcinogenicity studies

Study No.	Species	Strain	Sex (M,F)	Number of animals/group	Route of Administration	Dose (mg/kg-day) [a]	Exposure Duration	Reference
1	Mouse	B6C3F1	M	60 [b]	Feed	0, 90, 450, 1000	103 weeks	NTP (1993)
2			F	60 [b]		0, 110, 600, 1200		
3			M	30-120		0, 600, 1200	70 weeks	Hagiwara and Ward (1986)
4			M	50-55		0, 460, 920 [c]	134 weeks	Amo and Matsuyama (1985)
5			F	50-55		0, 360, 720 [c]		
6		IF	M	52-60		0, 250, 500	18 months	Flaks and Flaks (1983)
7			F	52-60		0, 250, 500		
8		Swiss [d]	M [e]	20		0, 1320 [f]	11 months	Weisburger et al. (1973)
9			F	20		0, 1430 [d]		
10		ABC-A	F	30		0, 130, 615, 1210	Lifetime (mean survival < 40 weeks) [g]	Wright (1967)
11	Rat	F344/N	M	60 [b]	Feed	0, 30, 150, 300	103 weeks	NTP (1993)
12			F	60 [b]		0, 35, 160, 320		

Study No.	Species	Strain	Sex (M,F)	Number of animals/group	Route of Administration	Dose (mg/kg-day) [a]	Exposure Duration	Reference
13		F344 /DuCrj	M	50		0, 195.4, 402.1[h]	104 weeks	Hiraga and Fujii (1985)
14			F	50		0, 335.7, 688 [h]		
15		Leeds	M	40-50		0, 300, 600	18 months	Flaks et al. (1985)
16			F	40-50		0, 300, 600		
17		S-D	M	30		0, 206.6 [i]	117 weeks	Johansson (1981)

M: Male; F: Female; mg/kg-day: milligrams per kilogram per day; F344: Fischer 344; S-D: Sprague-Dawley

[a] Values are as reported by study authors unless specified.

[b] Ten animals per group were sacrificed at 15 months.

[c] Doses were calculated by OEHHA using total acetaminophen intake and bodyweight data provided by Amo and Matsuyama (1985).

[d] Authors identified the mice as "NIH General purpose" mice, which is a strain derived from Swiss mice from the Rockefeller Institute and given to the NIH in 1935 (Rice and O'Brien 1980).

[e] Due to severe mortality due to fighting, the combined tumor data are from three separate experiments.

[f] Doses were calculated by OEHHA using average body weight and food consumption values in Gold et al. (1997).

[g] This study is considered uninformative because of limited study design, conduct, and reporting. In addition, the only tissue examined histopathologically was the mammary gland.

[h] Average daily intake during the 104 weeks of exposure was reported by the authors.

[i] Calculated by OEHHA based on information provided by Johansson (1981).

3.2.1 Carcinogenicity studies conducted in mice

Ten carcinogenicity studies of acetaminophen have been conducted in mice, all with administration in feed (Table 15). Five studies were conducted in B6C3F1 mice (two in females, three in males) (Amo and Matsuyama 1985; Hagiwara and Ward 1986; NTP 1993), two studies in IF mice (one in females, one in males) (Flaks and Flaks 1983), two studies in Swiss mice (one in females, one in males) (Weisburger et al. 1973), and one in female ABC-A mice (Wright 1967). The study by Wright (1967) was determined to be an inadequate test of carcinogenicity due to limited study design, duration, and reporting. The studies of Weisburger et al. (1973) were determined to have limited ability to detect carcinogenic effects, based on small group size and less-than-lifetime duration. In addition, Weisburger et al. (1973) presented combined data from three separate experiments in male mice, due to severe fighting-related mortality in the study.

As shown in Table 15 and discussed below, increases in tumors were observed in treated animals in the Amo and Matsuyama (1985) study in female B6C3F1 mice (hepatocellular adenomas and carcinomas (combined), pituitary adenomas), and in the Flaks and Flaks (1983) studies in male (hepatocellular adenoma, carcinoma, and combined adenoma and carcinoma) and female IF mice (hepatocellular adenoma and combined adenoma and carcinoma); tumors were also observed in treated male Swiss mice (hepatocellular adenoma and carcinoma (combined) and urinary bladder papilloma) in the study by Weisburger et al. (1973).

Table 15. Summary of study design, exposure and tumor incidences in mouse bioassays of acetaminophen

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
National Toxicology Program (1993) **Animal:** Mouse B6C3F1 Male (M) **Age at start of exposure:** 8-9 week (wk) **Study duration:** 103 wk **Control:** Concurrent **N =** 60 per group (10 for 15-month interim sacrifice)	**Agent and purity:** Acetaminophen >99% **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 600, 3,000, or 6,000 ppm for 103 wk (approximately 0, 90, 450, or 1,000 mg/kg-day)				**Survival:** No significant difference between the control and treated groups. **Body weight:** Body weights of treated mice were lower than controls but the differences were not statistically significant. **Other comments:** The incidence of thyroid follicular cell hyperplasia was increased in exposed mice in a dose-dependent manner (0/49, 6/49, 12/50, 15/50). Follicular cell tumors were not increased by treatment. Renal tubule hyperplasia occurred in one low dose and in two high dose males, and renal tubule adenoma occurred in one low dose and one high dose male, with none in controls. NTP concluded there was no evidence of carcinogenic activity in this study.
National Toxicology Program (1993) **Animal:** Mouse B6C3F1 Female (F) **Age at start of exposure:** 8-9 wk **Study duration:** 103 wk **Control:** Concurrent **N =** 60 per group (10 for 15-month interim sacrifice)	**Agent and purity:** Acetaminophen >99% **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 600, 3,000, or 6,000 ppm for 103 wk (approximately 0, 110, 600, or 1,200 mg/kg-day)				**Survival:** No significant difference between the control and treated groups. **Body weight:** Body weights of treated mice were lower than controls but the differences were not statistically significant **Other comments:** The incidence of thyroid follicular cell hyperplasia was increased in exposed mice in a dose-dependent manner (2/48, 8/50, 11/50, 25/50). Follicular cell adenomas were seen in treated, but not control mice (0/48, 1/50, 1/50, 2/50). Laboratory historical control incidence of follicular cell adenoma ranged from 0-10%, with a mean of 1.3%. NTP concluded there was no evidence of carcinogenic activity in this study.

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Hagiwara and Ward (1986) **Animal:** Mouse B6C3F1 M **Age at start of exposure:** 6 wk **Study duration:** 70 wk **Control:** Concurrent **N =** 30 for control; 60 for low dose; 120 for high dose	**Agent and purity:** Acetaminophen purity not stated **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 5,000, 10,000 ppm for 70 wk (0, 600, or 1,200 mg/kg-day)				**Survival:** Almost half of the high dose animals died prior to 24 wk. At 72 weeks, survival was 16% in high dose, 94% in low dose, and 100% in controls. **Body weight:** BW in the treated groups was reduced in a dose-dependent manner compared to controls (by 13.1% and 49.5% in the low- and high-dose groups at 72 wk, respectively). **Other comments:** A significant increase in liver weight/body weight ratio was observed in both treatment groups compared to control. High dose mice exhibited a high incidence of liver hepatocytomegaly, cirrhosis, lipofuscin, deposition, and necrosis. Most low dose mice exhibited mild heptocytomegaly, and a few low dose mice had focal cirrhosis or necrosis. No counts were provided for the nonneoplastic liver lesions.

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)			Comments
Amo and Matsuyama (1985) **Animal:** Mouse B6C3F1 M **Age at start of exposure:** 8-9 wk **Study duration:** 134 wk **Control:** Concurrent **N =** 50 for control and low dose; 55 for high dose	**Agent and purity:** Acetaminophen >98% **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 3,000, or 6,000 ppm for 134 wk (0, 460, or 920 mg/kg-day) [a]						**Survival:** No significant difference between the control and treated groups. **Body weight:** After approximately 40 wk, body weights of treated mice were lower than controls but the differences were not statistically significant. **Other comments:** Pneumonia was observed in a number of mice and was not treatment related. Enteritis, or inflammation of the small intestine, was reported in four low dose and five high dose mice, with none in controls.
Amo and Matsuyama (1985) **Animal:** Mouse B6C3F1 F **Age at start of exposure:** 8-9 wk **Study duration:** 134 wk **Control:** Concurrent **N =** 50 for control and low dose; 55 for high dose	**Agent and purity:** Acetaminophen >98% **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 3,000, or 6,000 ppm for 134 wk (0, 360, or 720 mg/kg-day) [a]	Liver	Hepatocellular adenoma and carcinoma, combined	**Concentration levels (ppm)** 0 2/49 (4.1%) Trend *p*-value < 0.05	3,000 2/46 (4.3%)	6,000 8/50* (16%)	**Survival:** No significant difference between the control and treated groups. **Body weight:** No significant difference in bw between treated and controls. **Other comments:** Pneumonia was observed in a number of mice and was not treatment related. Enteritis, or inflammation of the small intestine, was observed in one control, one low dose, and four high dose animals.
		Pituitary	Adenoma	2/49 (4.1%) Trend *p*-value < 0.05	3/46 (6.5%)	9/50* (18%)	

Footnotes for the studies by Amo and Matsuyama (1985):
The denominator is the number of animals alive at the occurrence of the first tumor at any site, which was 51 weeks.
*p < 0.05, pairwise comparison with control by Fisher's exact test (performed by OEHHA)
Trend *p*-value: exact trend test (performed by OEHHA)
[a] Doses in mg/kg-day were calculated by OEHHA using data from Amo and Matsuyama (1985). The authors reported that there was no remarkable difference in food intake between the treated and control groups, and calculated the total acetaminophen intake to be 863 and 675 g/kg in high-dose male and female mice, respectively.

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)			Comments
Flaks and Flaks (1983) **Animal:** Mouse IF M **Age at start of exposure:** young adult **Study duration:** 18 month (mo) **Control:** Concurrent **N =** 52 controls; 60 per treated group	**Agent and purity:** Acetaminophen ≥98% **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 5,000, 10,000 ppm for 18 mo (approximately 0, 250, 500 mg/kg-day)	Liver	Hepatocellular adenoma [a]	**Concentration levels (ppm)**			**Survival:** 32 males in the high dose group died with severe liver necrosis within the first 48 hours. Thereafter, survival in all groups was good, with no additional treatment-related decreases. **Body weight:** Dose-related weight loss was observed, this was a decisive factor in determining the time of study termination. **Other comments:** Hepatic histologic changes such as centrilobular cytoplasmic vacuolation, decreased glycogen centrilobular intracellular fat accumulation, and altered liver foci were observed in treated animals at both doses, but not in controls. Focal centrilobular necrosis occurred in 0/52, 1/27, and 7/27 control, low- and high-dose animals, respectively. Foci of cellular alteration were observed in 0/52, 5/57, and 21/27 control, low- and high-dose animals, respectively. Biliary hyperplasia was observed in 6/27 high dose animals only. Necrosis of the adrenal medulla occurred in 11/57 of the low dose mice, 12/27 of the high dose, and none in controls. Focal tubular necrosis of the renal cortex was observed in two high dose animals with none in low dose or controls. Focal alveolar epithelialization in the lungs was found in three low dose animals but not in high dose or controls.
				0	5,000	10,000	
				1/50 (2%)	1/54 (1.9%)	15/23*** (65.2%)	
				Trend *p*-value < 0.0001			
			Hepatocellular carcinoma	0/50	0/54	5/23** (21.7%)	
				Trend *p*-value < 0.001			
			Hepatocellular adenoma and carcinoma, combined	1/50 (2%)	1/54 (1.9%)	20/23*** (87.0%)	
				Trend *p*-value < 0.0001			

Footnotes for the studies by Flaks and Flaks (1983):
Incidence reported by the study authors; the denominator is the number of animals alive at 18 months.
[a] Mice with both adenomas and carcinomas were assigned to the carcinoma category by Flaks and Flaks (1983).
**p < 0.01, pairwise comparison with control by Fisher's exact test (performed by OEHHA)
***p < 0.001, pairwise comparison with control by Fisher's exact test (performed by OEHHA)
Trend p-value: exact trend test (performed by OEHHA)

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)			Comments
Flaks and Flaks (1983) **Animal:** Mouse IF F **Age at start of exposure:** young adult **Study duration:** 18 mo **Control:** Concurrent **N =** 52 controls; 60 per treated group	**Agent and purity:** Acetaminophen ⩾98% **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 5,000, 10,000 ppm for 18 mo (approximately 0, 250, 500 mg/kg-day)	Liver		**Concentration levels (ppm)**			**Survival:** Five mice died with severe liver necrosis within the first 48 hours. Thereafter, subsequent survival in all groups was high. **Body weight:** Dose-related weight loss was observed, this was a decisive factor in determining the time of study termination. **Other comments:** Hepatic histologic changes such as centrilobular cytoplasmic vacuolation, decreased glycogen, and centrilobular intracellular fat accumulation were observed in the treated animals at both doses but not in controls. Focal centrilobular necrosis and altered liver foci occurred in 9/50 and 10/50 high dose animals, respectively, with none in the low dose or controls. Non-hepatic pathological changes were not reported.
				0	5,000	10,000	
			Hepatocellular adenoma [a]	0/48	0/57	7/47** (14.9%)	
				Trend *p*-value < 0.001			
			Hepatocellular carcinoma	0/48	0/57	2/47 (4.3%)	
				Test for trend not significant.			
			Hepatocellular adenoma and carcinoma, combined	0/48	0/57	9/47** (19.1%)	
				Trend *p*-value < 0.0001			

Footnotes for the studies by Flaks and Flaks (1983):
Incidence reported by the study authors; the denominator is the number of animals alive at 18 months.
[a] Mice with both adenomas and carcinomas were assigned to the carcinoma category by Flaks and Flaks (1983).
***p* < 0.01, pairwise comparison with control by Fisher's exact test (performed by OEHHA)
Trend *p*-value: exact trend test (performed by OEHHA)

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)		Comments
Weisburger et al. (1973) **Animal:** Mouse Swiss (NIH general purpose) M **Age at start of exposure:** 6 wk **Study duration:** 11 mo **Control:** Concurrent **N =** 30 controls; 20 treated. Because of severe mortality in all groups due to fighting, the experiment was repeated twice, and the combined tumor data from all three experiments for both control and treated groups are reported.	**Agent and purity:** Acetaminophen purity not stated **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0 or 11,000 ppm (0, 1.1% in feed) daily for 11 mo (approximately 0, 1320 mg/kg-day)			Concentration levels (ppm)		**Survival:** Effect of treatment on survival not reported. Severe mortality in all groups due to fighting. **Body weight:** Not reported. **Other comments**: The authors identified lesions as hepatomas, which is an older term for what is now classified as hepatocellular adenomas. Due to severe fighting-related mortality, the combined tumor data from three separate experiments are reported. 11/26 mice treated with acetaminophen exhibited severe liver cellular degeneration and necrosis, with none in controls.
				0	11,000	
		Liver	Hepatocellular adenomas or carcinomas	0/27	3/26 (11.5%)	
		Urinary bladder	Papilloma	0/27	2/20[a] (10%)	
Weisburger et al. (1973) **Animal:** Mouse Swiss (NIH general purpose) F **Age at start of exposure:** 6 wk **Study duration:** 11 mo **Control:** Concurrent **N =** 30 controls; 20 treated	**Agent and purity:** Acetaminophen purity not stated **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0 or 11,000 ppm (0, 1.1% in feed) daily for 11 mo (approximately 0, 1430 mg/kg-day)					**Survival:** Not reported. **Body weight:** Not reported. **Other comments:** No liver or bladder tumors were observed in control or treated groups. 6/18 mice treated with acetaminophen exhibited liver cysts with severe liver cellular degeneration and necrosis, with none in controls.

Footnotes for the studies by Weisburger et al. (1973): Administered doses were calculated by OEHHA using average body weight and food consumption values in Gold et al. (1997). Incidence reported by the study authors; the denominator is the number of animals alive at two months, unless otherwise noted. [a]Incidence reported in text as 2 out of 20.

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Wright (1967) **Animal:** Mouse albino ABC-A F **Age at start of exposure:** "weaning age" **Study duration:** Lifetime (mean survival < 40 wks) **Control:** Concurrent **N =** 131 controls; 30 per treated group	**Agent and purity:** Acetaminophen US Pharmacopeia quality **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 1,000, 5,000, or 10,000 ppm (0, 0.1, 0.5, 1% in feed) daily for lifetime (0, 130, 615, or 1210 mg/kg-day)				**Survival:** Significant treatment-related mortality at all doses. **Body weight:** Not reported. **Other comments:** This study was originally planned as a five-generation study; however, poor survival in the treated F0 males and females and reduced fertility resulted in the discontinuation of acetaminophen studies beyond the F1 generation. Although males were treated, only mammary tumors were accessed, and only in female mice. Due to the survival issues, incidence of female mammary tumors was reported for all three treatment groups combined (8%); control female mammary tumor incidence was 9.2%.

Discussion of carcinogenicity study findings in mice

Tumors were observed in acetaminophen-treated groups in the carcinogenicity studies conducted for 134 weeks in female B6C3F1 mice (Amo and Matsuyama 1985), in the studies conducted for 18 months in male and female IF mice (Flaks and Flaks 1983), and in the studies conducted for 11 months in male Swiss mice (Weisburger et al. 1973). Tumors were not reported in the studies conducted for 103 weeks in male and female B6C3F1 mice (NTP 1993), in the studies conducted for 70 (Hagiwara and Ward 1986) and 134 weeks in male B6C3F1 mice (Amo and Matsuyama 1985), in the study in female Swiss mice with limited ability to detect an effect (Weisburger et al. 1973), or in the study determined to be an inadequate test of carcinogenicity due to limited study design, duration and reporting in female ABC-A mice (Wright 1967).

In the 134 week study in B6C3F1 female mice, a statistically significant increase in hepatocellular adenoma or carcinoma combined was observed in the high-dose group compared to controls (Amo and Matsuyama 1985). In addition, a statistically significant increase in pituitary adenomas was observed in the high-dose group, with a significant dose-related trend ($p = 0.01$).

In the 18-month study of acetaminophen in IF strain male mice (Flaks and Flaks 1983), statistically significant increases in hepatocellular adenoma, carcinoma, and adenoma and carcinoma combined were observed in the high dose group (500 mg/kg-d), with significant positive trends. Despite significant mortality in the high-dose group within the first 48 hours of the study, 87% of the surviving high-dose males developed liver tumors (20/23). In the 18-month study conducted in IF female mice (Flaks and Flaks 1983), statistically significant increases in hepatocellular adenoma, and adenoma and carcinoma combined were observed in the high dose group (500 mg/kg-d), with significant positive trends. Data on the spontaneous liver tumor incidence in IF mice is sparse. In 12-month studies in IF mice from the same laboratory, no liver tumors were observed in male or female controls (Flaks 1968) and in 15-month studies in IF mice from the same university, no carcinomas were observed in male or female controls and only one adenoma was observed in the male controls (Wood 1969).

In an 11-month study of acetaminophen in male Swiss mice, the incidence of hepatocellular adenomas and carcinomas (combined) was elevated, but not significantly different from controls in treated mice (control: 0/27 vs. treated: 3/26), as was the incidence of urinary bladder papillomas (control: 0/27 vs. treated: 2/20) (Weisburger et al. 1973). Interpretation of these findings is complicated by not only the short study duration, but also the fact that these data represent the combined observations from three experiments (rates of survival were low in each experiment, due to high levels of fighting-related mortality).

Non-neoplastic hepatic effects were reported in acetaminophen-treated mice in the studies by Hagiwara and Ward (1986), Flaks and Flaks (1983), Amo and Matsuyama (1985), and Weisburger et al. (1973). The lesions included centrilobular histopathological findings ranging from macroscopic effects such as cysts, necrosis, and cirrhosis, to foci of cellular alteration and hepatocytomegaly. Cellular effects such as cytoplasmic vacuolation and intracellular fat accumulation were also reported in acetaminophen-treated mice. Overall, there seemed to be no correlation between these non-neoplastic lesions with liver tumor incidence. For example, Flaks and Flaks (1983) stated that the individual occurrence of focal centrilobular necrosis observed in the high-dose acetaminophen treated mice "did not appear to parallel the presence of hepatic neoplasms". Hagiwara and Ward (1986) reported a high degree of hepatic histologic changes such as necrosis and hepatocytomegaly in treated male B6C3F1 but no liver tumors. In the studies by Weisburger et al. (1973), liver cysts with severe liver cellular degeneration or necrosis were observed in 11/26 male and 6/18 female Swiss mice but hepatocellular tumors were observed only in the male study, in three treated mice.

3.2.2 Carcinogenicity studies conducted in rats

Seven carcinogenicity studies of acetaminophen have been conducted in rats, all with administration in feed (Table 16). Three sets of studies (each including one study in males and one study in females) were conducted in Fischer 344 (F344)/N rats (NTP 1993), F344/DuCrj rats (Hiraga and Fujii 1985), and Leeds rats (Flaks et al. 1985), and the seventh study was conducted in male Sprague-Dawley (S-D) rats (Johansson 1981).

As shown in Table 16 and discussed below, increases in tumors were observed in treated animals in the National Toxicology Program (NTP) (1993) study in female F344/N rats (mononuclear cell leukemia (MNCL)) and in the Flaks et al. (1985) studies in male (hepatocellular adenoma, and rare urinary bladder papilloma, and combined urinary bladder papilloma and carcinoma) and female Leeds rats (hepatocellular adenoma, and combined urinary bladder papilloma and carcinoma).

Table 16. Summary of study design, exposure and tumor incidences in rat bioassays of acetaminophen

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
NTP 1993 **Animal:** Rat F344/N M **Age at start of exposure:** 7-8 wk **Study duration:** 103 wk **Control:** Concurrent **N** = 60 per group (10 for 15-month interim sacrifice)	**Agent and purity:** Acetaminophen 99.7% **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 600, 3,000, or 6,000 ppm for 103 wk (approximately 0, 30, 150, 300 mg/kg-day)				**Survival:** No significant difference between the control and treated groups. **Body weight:** No significant difference in bw between treated and controls. **Other comments:** Zymbal's gland carcinoma was observed in treated animals but not in controls (0/50, 0/50, 4/49, 2/48). NTP historical laboratory incidence: 0.3%; range 0-2%. Dose dependent significant increase in hyperplasia of the parathyroid gland was observed (0/42, 4/45, 6/46, 8/45).
NTP 1993 **Animal:** Rat F344/N F **Age at start of exposure:** 7-8 wk **Study duration:** 103 wk **Control:** Concurrent **N** = 60 per group (10 for 15-month interim sacrifice)	**Agent and purity:** Acetaminophen 99.7% **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 600, 3,000, or 6,000 ppm for 103 wk (approximately 0, 35, 160, 320 mg/kg-day)	All organs	Mononuclear cell leukemia (MNCL)	**Concentration levels (ppm)** 0 / 600 / 3000 / 6000 9/48 (18.8%) / 17/50 (34%) / 15/48 (31.2%) / 24/46*** (52.2%) Trend *p*-value < 0.01	**Survival:** No significant difference between the control and treated groups. **Body weight:** No significant difference in bw between treated and controls. **Other comments:** The incidence of MNCL in concurrent controls was within the range of historical controls from the study laboratory and from NTP studies conducted in other laboratories.

Footnotes for the studies by NTP (1993):
Tumor incidence is numbers of tumor-bearing animals per numbers of animals alive at time of first occurrence of tumor. *** $p < 0.001$, pairwise comparison with control by Fisher's exact test (performed by OEHHA). Trend *p*-value: exact trend test (performed by OEHHA).

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Hiraga and Fujii (1985) **Animal:** Rat F344/DuCrj M **Animal age at the beginning of exposure:** 5 wk **Study duration:** 130 wk **Control:** Concurrent **N** = 50 per group	**Agent and purity:** Acetaminophen Japanese pharmacopeia grade **Exposure route:** feed **Exposure concentrations, frequency, and duration:** 0, 4,500 or 9,000 ppm for 104 wk. plus 26 wk control diet (0, 195.4, 402.1 mg/kg-day during the 104 wk of exposure)				**Survival:** No significant difference between the control and treated groups. **Body weight:** Significantly lower mean body weights were observed in low-dose males until wk 12, and high-dose males until wk 21 as compared with the controls. **Other comments:** Two oral cavity tumors were observed in high dose males (one squamous cell carcinoma and one undifferentiated carcinoma) and none in controls. Squamous cell carcinoma of the oral cavity is rare. In addition, one rare squamous cell carcinoma of the tongue and one rare transitional cell carcinoma of the urinary bladder were observed in the high dose group and none in controls.
Hiraga and Fujii (1985) **Animal:** Rat F344/DuCrj F **Animal age at the beginning of exposure:** 5 wk **Study duration:** 130 wk **Control:** Concurrent **N** = 50 per group	**Agent and purity:** Acetaminophen Japanese pharmacopeia grade **Exposure route:** feed **Exposure concentrations, frequency, and duration:** 0, 6,500 or 13,000 ppm for 104 wk. plus 26 wk control diet (0, 335.7, 688 mg/kg-day during the 104 wk of exposure)				**Survival:** No significant difference between the control and treated groups. **Body weight:** Significantly lower mean body weights were observed in high-dose females throughout the study as compared with the controls. No significant difference in bw between low-dose females and controls. **Other comments:** One rare squamous cell carcinoma of the oral cavity and one rare transitional cell carcinoma of the renal pelvis were observed in the high dose group and none in controls.

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)			Comments
Flaks et al. (1985) **Animal:** Rat Leeds M **Age at start of exposure:** Young adult **Study duration:** 18 mo **Control:** Concurrent **N** = 40 controls; 50 per treated group	**Agent and purity:** Acetaminophen >98% **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 5,000, or 10,000 ppm for 18 mo (approximately 0, 300, 600 mg/kg-day)	Liver	Hepatocellular adenoma	**Concentration levels (ppm)**			**Survival:** No significant difference between the control and treated groups. **Body weight:** No significant difference in bw between treated and controls. **Other comments:** Hyperplasia of the bladder epithelium was significantly increased in the treated animals (0/40, 12/48, 12/49). Bladder calculi were increased significantly in a dose-dependent manner (1/40, 13/48, and 17/49). The author stated there was no clear correlation between animals with bladder calculi and animals with bladder epithelial hyperplasia.
				0	5000	10000	
				0/40	1/48 (2.1%)	9/45** (20%)	
				Trend *p*-value < 0.001			
		Urinary bladder	Papilloma	0/40	3/48 (6.2%)	5/45* (11.1%)	
				Trend *p*-value < 0.05			
			Carcinoma	0/40	0/48	1/45 (2.2%)	
				Test for trend not significant.			
			Papilloma and carcinoma combined	0/40	3/48 (6.2%)	6/45* (13.3%)	
				Trend *p*-value < 0.05			

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)			Comments
Flaks et al. (1985) **Animal:** Rat Leeds F **Age at start of exposure:** Young adult **Study duration:** 18 mo **Control:** Concurrent **N** = 40 controls; 50 per treated group	**Agent and purity:** Acetaminophen >98% **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0, 5,000, or 10,000 ppm for 18 mo (approximately 0, 300, 600 mg/kg-day)	Liver	Hepatocellular adenoma	**Concentration levels (ppm)**			**Survival:** No significant difference between the control and treated groups. **Body weight:** No significant difference in bw between treated and controls. **Other comments:** Hyperplasia of the bladder epithelium was significantly increased in the treated animals (0/40, 12/49, 10/50). There was no apparent treatment related increase in bladder calculi (2/40, 3/49, 3/50). Two rare fallopian tube sarcomas were observed in the high-dose group and none in controls.
				0	5000	10000	
				0/40	0/49	10/49** (20.4%)	
				Trend *p*-value < 0.0001			
		Urinary bladder	Papilloma	0/40	4/49 (8.2%)	2/49 (4.1%)	
				Test for trend not significant.			
			Carcinoma	0/40	1/49 (2.0%)	1/49 (2.0%)	
				Test for trend not significant.			
			Papilloma and carcinoma combined	0/40	5/49* (10.2%)	3/49 (6.1%)	
				Test for trend not significant.			

Footnotes for the studies by Flaks et al. (1985):
Tumor incidence is number of tumor bearing animals per number of animals examined. *$p < 0.05$, ** $p < 0.01$, pairwise comparison with control by Fisher's exact test (performed by OEHHA). Trend *p*-value: exact trend test (performed by OEHHA).

Reference and study design	Exposure	Tumor site	Tumor type	Tumor incidence (%)	Comments
Johansson (1981) **Animal:** Rat Sprague-Dawley M **Age at start of exposure:** 6 wk **Study duration:** 117 wk **Control:** Concurrent **N** = 30 per group	**Agent and purity:** Acetaminophen 99.5-99.7% **Exposure route:** Feed **Exposure concentrations, frequency, and duration:** 0 or 5,350 for up to 117 wk (0, 206.6 mg/kg-day)				**Survival:** No significant difference between the control and treated groups. **Body weight:** No significant difference in bw between treated and controls. **Other comments:** The authors reported the incidence of urinary bladder tumors (2/30, 4/30) was associated with severe urinary tract infection in both the control and treated groups.

Discussion of carcinogenicity study findings in rats

Tumors were observed in acetaminophen-treated groups in the carcinogenicity studies conducted for 103 weeks in female F344/N rats (NTP 1993) and for 18 months in male and female Leeds rats (Flaks et al. 1985), but not in the studies conducted for 103 weeks in male F344 rats (NTP 1993), for 130 weeks in male and female F344/DuCrj rats (Hiraga and Fujii 1986), or for 117 weeks in male S-D rats (Johansson 1981).

In the NTP two-year studies of acetaminophen (NTP 1993), statistically significant increases in mononuclear cell leukemia (MNCL) were observed in female F344/N rats in the high-dose group compared to controls, with a positive dose-related trend. Among all females with MNCL, the proportion of animals dying before week 100 increased with dose (2/9 or 22% in controls; 4/17 or 24% in low-dose; 7/15 or 47% in mid-dose; 14/24 or 58% in high-dose group). In controls with MNCL, the leukemia was often observed only in the spleen and liver, with infrequent involvement of more than one additional organ, while in treated females with MNCL there was an increase in multiple organ involvement (defined as spleen and liver, plus two or more additional organs) [3/9 (33%) in controls; 16/17 (94%) in the low-dose; 12/15 (80%) in the mid-dose; 21/24 (88%) in the high-dose].

The control incidence of MNCL, 9/48 (18.8%), was similar to the laboratory historical control incidence of 16.5% (66/399; range 6–28%) and the historical control incidence reported for all NTP studies available at that time (20.8%, 425/2043; range 6–40%) (NTP 1993).

NTP (1993) noted the following about the MNCLs:

> "On average, leukemias were detected one month earlier in the high-dose group than in the controls, suggesting a shortening of neoplasm latency. In addition there was an increase in the extent of multiple organ involvement in the organ distribution of mononuclear cell leukemia in groups of exposed female rats compared to controls."

NTP concluded that there was "equivocal evidence" of carcinogenic activity in female rats, "based on increased incidences of MNCL". In reaching this conclusion, NTP noted the "generally high and variable background rate of this neoplasm in Fischer rats, and the lack of concordance of this study result with a lifetime study of acetaminophen in Fischer rats in Japan" (NTP 1993).

In 18-month feed studies of acetaminophen in Leeds rats, statistically significant increases in the incidences of hepatocellular adenomas were observed in both the male rat study, and the female rat study, with positive dose-related trends (Flaks et al. 1985). In males, statistically significant increases in urinary bladder transitional cell papilloma

and transitional cell papilloma and carcinoma combined were also seen in the high-dose group, with positive dose-related trends. In females, a statistically significant increase in urinary bladder transitional cell papilloma and carcinoma combined was seen in the mid-dose group.

Flaks et al. (1985) reported that no spontaneous tumors were observed at any site in the 40 untreated male and 40 untreated female Leeds rats in these 18-month studies. Other publications from the same laboratory corroborate the extremely low spontaneous incidence of liver and bladder neoplasms in Leeds rats, e.g. no liver tumors observed among 40 untreated controls in an 18-month study in male Leeds rats (Flaks and Flaks 1982), no liver tumors reported in untreated controls in a 20-month study in male Leeds rats (Flaks 1978), Flaks et al. (1985) discussed the reported differences in the rates of spontaneous liver neoplasms between rats of the Leeds and F344 strains, and suggested that the differences may be due to metabolic differences.

In order to put the control incidence of hepatocellular adenomas reported for Leeds rats in context with other strains, OEHHA reviewed information available from NTP on the historical control incidence of hepatocellular adenomas observed in NTP studies of F344, Wistar Han, and S-D rats. The review identified studies conducted in males and females of each strain, in which the control incidence of hepatocellular adenoma was 0% at 2 years. Specifically, among 20 sets of feed studies in F344 rats conducted between 1984 and 1994, the overall incidence of hepatocellular adenoma was 2.3% (range 0-10%) in males and 0.4% (range 0-4%) in females (NTP 1999); among seven sets of studies by any route in Wistar Han rats conducted from 2007 to 2009, the overall incidence of hepatocellular adenoma was 1.2% (range 0-6%) in males and 1.7% (range 0-6%) in females (NTP 2016a); and among four sets of studies by any route in S-D rats conducted from 2007 to 2012, the overall incidence of hepatocellular adenoma was 0.4% (range 0-2%) in males and 4.6% (range 0-8%) in females (NTP 2017).

With regard to the control incidence of urinary bladder transitional cell tumors reported for Leeds rats, it is important to note that regardless of strain, the spontaneous occurrence of urinary bladder tumors is rare in rats (Frith et al. 1995). In the Flaks et al. (1985) study, no bladder tumors were observed in controls. In the NTP historical control database of two-year studies, among 20 sets of feed studies in F344 rats conducted between 1984 and 1994, the control incidence of bladder papilloma or carcinoma combined was 0.3% (3/991) in males and 0.2% (2/989) in females; among seven sets of studies by any route in Wistar Han rats conducted from 2007 to 2009, the combined incidence was 0.29% in male (1/349) and 0.29% in females (1/349), and among five studies of male and 13 studies of female S-D rats conducted from 1998 to 2012, the combined incidence was 0% (0/289) in males and 0.14% (1/705) in females.

3.3 Other Relevant Data

3.3.1 Pharmacokinetics and metabolism

Hundreds of studies have investigated the pharmacokinetics and metabolism of acetaminophen in humans and animals, and the subject has been reviewed in depth in multiple publications over the past 40 years (for example, Prescott (1980), Levy (1981), Forrest et al. (1982), Hinson (1983), Jackson et al. (1984), NTP (1993), IARC (1999)). More recent reviews include Bessems and Vermeulen (2001), Hinson et al. (2004), Josephy (2005), Bond (2009), McGill and Jaeschke (2013), Raffa et al. (2014), Mazaleuskaya et al. (2015), Ramachandran and Jaeschke (2018), Ramachandran and Jaeschke (2019), Zhao and Pickering (2011), and several others. OEHHA did not review all available studies on the pharmacokinetics and metabolism of acetaminophen. Using review articles as an initial guide, OEHHA focused on human *in vivo* and *in vitro* studies that included data on absorption, disposition, pharmacokinetics, metabolism, and elimination. OEHHA also reviewed a number of human and animal studies that provide relevant information on (i) enzymes involved in acetaminophen metabolism, (ii) human polymorphisms in some of these key enzymes, and (iii) reactive metabolites of acetaminophen. We excluded clinical studies that exclusively investigated overdoses, pain management, comparison of drug formulations, and drug-drug interactions. Animal studies were limited to those conducted in rodents. Since humans and animals share many of the reported metabolic pathways for and metabolites of acetaminophen, as well as similar toxic effects, data from animal studies are included when human data are unavailable or incomplete. Overall, OEHHA reviewed over 400 studies on acetaminophen pharmacokinetics and metabolism.

3.3.1.1 Absorption

The absorption of acetaminophen is rapid and occurs within 30-60 minutes after oral ingestion. It has a high oral bioavailability (88%), and reaches the peak blood concentrations within 90 minutes after ingestion (Mazaleuskaya et al. 2015). Acetaminophen has been detected in plasma samples from human subjects that received an oral dose of 20 mg/kg as early as 3 minutes after dosing (Clements et al. 1978). Rectal absorption, measured in infants, is slower and reaches maximum concentrations in approximately 100 minutes (Hansen et al. 1999). There was no difference in bioavailability of acetaminophen after oral versus rectal administration (Forrest et al. 1982).

Acetaminophen is absorbed from the small intestine after oral or intraduodenal administration, with the majority of absorption occurring in the proximal portion of the

small intestine (Forrest et al. 1982; Hansen et al. 1999; Holmer Pettersson et al. 2004; Kennedy and van Rij 2006; Raffa et al. 2014). The rate of absorption depends on gastric emptying, specific drug formulations, and intake of food. Gastric emptying is the rate-limiting step, and the intake of food tends to slow the rate of absorption (Clements et al. 1978; Divoll et al. 1982; Heading et al. 1973; Ibanez et al. 2006; McGilveray and Mattok 1972; Raffa et al. 2014). Absorption from the small intestine occurs by passive diffusion (Raffa et al. 2014).

3.3.1.2 Distribution and pharmacokinetics

Following oral absorption, acetaminophen is rapidly and evenly distributed throughout most tissues and fluids, including saliva, breast milk, and cerebrospinal fluids (Berlin et al. 1980; Forrest et al. 1982; Prescott 1980; Retaco et al. 1996). Acetaminophen is detected in the cerebrospinal fluid as early as 20 minutes after a single *i.v*. dose of 2 g propacetamol (a pro drug of acetaminophen), reaching a maximum concentration at 4 hours, with an elimination half-life of 3.2 hours (Bannwarth et al. 1992).

Little binding to plasma proteins and a high volume of distribution (0.9 L/kg) (Prescott 1980) suggest that distribution of acetaminophen is uniform throughout tissues and fluids (Forrest et al. 1982; Graham et al. 2013; Morris and Levy 1984; Prescott 1980). The peak plasma concentration after a therapeutic dose of 1 g is approximately 20 – 30 mg/L after oral or *i.v.* administration (Graham et al. 2013). Plasma concentrations increase with dose and time, and peak plasma concentrations (C_{Max}) can be reached within 25 minutes but can take up to 2 hours; plasma half-life ($T_{1/2}$) ranges from 1.5–3 hours (Buniva et al. 1977; Clements et al. 1978; Divoll et al. 1982; Gregoire et al. 2007; Holmer Pettersson et al. 2004; Kennedy and van Rij 2006; McGill and Jaeschke 2013; McGilveray and Mattok 1972; Prescott 1980; Rawlins et al. 1977).

Repeated *i.v.* dosing with acetaminophen over 24 hours (total dose 5 g) resulted in a plasma C_{max} of 67.9 µg/ml and subsequent doses reached maximal plasma concentrations of 37.8–45 µg/ml (Gregoire et al. 2007). Plasma clearance following *i.v.* administration is biphasic, with a rapid decrease during the first 60–90 minutes, followed by a slower elimination phase over the subsequent 4–5 hours (Prescott 1980; Rawlins et al. 1977). Overall plasma clearance rates ranged from 264–505 milliliter per minute (ml/min) (Rawlins et al. 1977).

Acetaminophen crosses the placenta, and levels measured in fetal cord blood closely mirror maternal levels (Nitsche et al. 2017). After an oral dose of 1 g, the plasma levels reached a C_{Max} of 12.3 µg/ml in maternal and 11.2 µg/ml in fetal blood, with a $T_{1/2}$ in maternal and fetal blood of 84 and 82 minutes respectively, indicating that maternal blood levels of acetaminophen are predictive of, and correlated with, fetal levels.

Maternal and fetal levels were comparable at 30 minutes after dosing, indicating rapid placental transfer.

In lactating women, acetaminophen is distributed into breast milk with a mean C_{max} of 27.8 µmol/L compared to mean plasma levels of 37 µmol/L (Bitzen et al. 1981). Acetaminophen is present in breast milk within 15 minutes after maternal dosing and reaches a maximum concentration within 1–2 hours (Berlin et al. 1980; Bitzen et al. 1981). The mean elimination time from breast milk ranges between 1.35–3.35 hours (Berlin et al. 1980; Bitzen et al. 1981).

3.3.1.3 Metabolism

The metabolism of acetaminophen is largely similar in humans and laboratory animals, with many of the same metabolites detected in both humans and animals. Some acetaminophen metabolites identified in animals have not yet been investigated in humans (Bessems and Vermeulen 2001). In animals, species, strain, and gender differences in metabolic pathways are thought to account for the observed variability in susceptibility to acetaminophen toxicity (Gregus et al. 1988; Hoivik et al. 1995; Hu et al. 1993; IARC 1999a). In rodents, susceptibility to acetaminophen is correlated with quantities of oxidative metabolites produced, with highly susceptible animal species (mice, hamsters) metabolizing 27-42% of acetaminophen via oxidation, and less susceptible species oxidizing smaller amounts, about 5-7% (Gregus et al. 1988; IARC 1999a). In humans, factors that affect susceptibility to acetaminophen toxicity include genetic polymorphisms associated with key enzymes involved in metabolic activation and detoxification, and a variety of conditions that affect the level of reduced glutathione (GSH) in the body, including obesity, liver steatosis, starvation, fasting, and alcohol consumption, all of which lead to depletion of GSH in key tissues and organs (Caparrotta et al. 2018; Court et al. 2001; Court et al. 2017; de Morais et al. 1992; Josephy 2005; Mazaleuskaya et al. 2015; Zhao and Pickering 2011).

Metabolism of acetaminophen occurs primarily in the liver, and to a lesser extent in the kidney and intestine (Bessems and Vermeulen 2001; Prescott 1980). The majority of acetaminophen is metabolized via conjugation, either glucuronidation or sulfation, and is catalyzed by uridine 5'-diphospho-glucuronosyltransferase (UDP) - glucuronosyltransferases and sulfotransferases, respectively (Mazaleuskaya et al. 2015). The conjugated metabolites are rapidly excreted and hence these pathways are considered detoxification pathways. Other metabolic pathways involve oxidation catalyzed by cytochrome P450 (CYP) and other enzymes, or deacetylation. Several genotoxic and electrophilic metabolites are formed during acetaminophen metabolism, including quinones and semi-quinones, free radicals, and *p*-aminophenol (PAP). Several metabolic reactions also lead to the formation of reactive oxygen species

(ROS). The pathways involved in acetaminophen metabolism are discussed in more detail below, and shown in Figure 5.

Conjugation pathways

As discussed in numerous reviews (Bessems and Vermeulen 2001; Bond 2009; Forrest et al. 1982; Hinson et al. 2004; IARC 1999a; Josephy 2005; Mazaleuskaya et al. 2015; McGill and Jaeschke 2013; NTP 1993; Prescott 1980; Raffa et al. 2014; Ramachandran and Jaeschke 2018), acetaminophen is extensively metabolized in the liver, primarily via conjugation of the phenolic group with either glucuronic acid or sulfate, which allows for fast urinary excretion. Conjugation with either glucuronic acid or sulfate accounts for approximately 80-85% of acetaminophen metabolites (Mitchell et al. 1974).

Glucuronidation of acetaminophen is carried out by UDP-glucuronosyltransferases (UGTs), primarily UGT1A1 and 1A6, resulting in acetaminophen-4-glucuronide, which accounts for approximately 50-70% of metabolites (Gelotte et al. 2007; Josephy 2005; McGill and Jaeschke 2013; Mutlib et al. 2006; Zhao and Pickering 2011). Glucuronidation of acetaminophen has been shown to be inducible by repeated dosing with acetaminophen (Gelotte et al. 2007). Inter-individual variability of acetaminophen conjugation by UGTs can be substantial, with a 15-fold difference observed in one study (Court et al. 2001). Reduced glucuronidation may increase the toxicity of acetaminophen, possibly by a shift to oxidative metabolism. Humans with reduced glucuronidation ability (for example, Gilbert's syndrome) have decreased clearance of acetaminophen and can have a nearly 5% increase in oxidative acetaminophen metabolism (de Morais et al. 1992). Homozygous and heterozygous Gunn rats, which are severely and moderately deficient in glucuronyl transferase, show significant liver and kidney toxicity after acetaminophen administration (de Morais and Wells 1989).

Sulfation of acetaminophen is carried out by sulfotransferases (SULT) (SULT1A1, 1A3/4, and 1E1, 2A1) to form acetaminophen-4-sulphate (Gelotte et al. 2007; Josephy 2005; McGill and Jaeschke 2013; Zhao and Pickering 2011). Sulfation accounts for approximately 25-35% of urinary metabolites after a therapeutic dose of acetaminophen. Sulfation of acetaminophen in fetal liver is carried out by SULT1E1 and 1A3/4; postnatally, sulfation of acetaminophen is primarily carried out by SULT1A1 and 2A1 (Mazaleuskaya et al. 2015). Sulfation becomes saturated at high acetaminophen doses due to limited availability of inorganic sulfate and/or reduced sulfotransferase activity, while glucuronidation may not be saturated even after an overdose (Gelotte et al. 2007; Josephy 2005; Mazaleuskaya et al. 2015; Xie et al. 2015).

Oxidation pathways: NAPQI formation and GSH conjugation

About 5-10% of a therapeutic dose of acetaminophen is metabolized via the cytochrome P-450 (CYP) oxidative pathway (Forrest et al. 1982; Gelotte et al. 2007; IARC 1999a) leading to the formation of the reactive metabolite, *N*-acetyl-*p*-benzoquinone imine (NAPQI) (Bessems and Vermeulen 2001; Jollow et al. 1973; Miner and Kissinger 1979; Mitchell et al. 1973; Mitchell et al. 1974; Potter et al. 1973; van de Straat et al. 1988). NAPQI is an electrophilic and reactive metabolite that forms covalent adducts with nucleophiles such as glutathione (GSH) and protein thiols (Albano et al. 1985; Bond 2009; Chen et al. 1999). It is formed via CYP enzymes in a sequential 2-electron oxidation reaction (Hoffmann et al. 1990; Potter and Hinson 1987a, 1987b, 1989; van de Straat et al. 1988) and can be reduced back to acetaminophen in the presence of NADPH *in vitro* (Dahlin et al. 1984). NAPQI forms DNA adducts and causes DNA strand breaks in mammalian and non-mammalian cells (see Section 3.3.3 Genotoxicity) (Dybing et al. 1984; Hasegawa et al. 1988; Klopcic et al. 2015; Rogers et al. 1997).

Acetaminophen can also be oxidized in a one-electron reaction to the semiquinone *N*-acetyl-*p*-benzosemiquinone imine (NAPSQI), which can disproportionate into NAPQI or acetaminophen, or form an acetaminophen dimer (3,3′-biacetaminophen) or larger polymers (Potter and Hinson 1987a, 1987b, 1989). Other one-electron oxidation reactions can result in the formation of free radicals, including the *N*-acetyl-*p*-aminophenoxy radical (from acetaminophen) and the *p*-aminophenoxy radical (from PAP, another acetaminophen metabolite). Both types of radicals have been observed *in vitro* (Fischer et al. 1985; Josephy et al. 1983; Mason and Fischer 1986; Potter and Hinson 1987a, 1987b; West et al. 1984). In addition, ROS formation has been observed during these oxidation reactions, suggestive of redox cycling (Fig. 5) (Foreman and Tarloff 2008; Mirkovic et al. 2011; Rosen et al. 1983).

The formation of the *p*-aminophenoxy radical is thought to occur via deacetylation of either acetaminophen or NAPQI to form PAP, which is then further oxidized to form the radical (Fischer et al. 1985). Further oxidation or disproportionation of the *p*-aminophenoxy radical may yield another reactive metabolite, *p*-benzoquinone imine (Fowler et al. 1991; Josephy et al. 1983).

Metabolism of NAPQI may also yield the reactive and genotoxic compound *p*-benzoquinone (See Section 3.3.3 Genetoxicity) (Dahlin et al. 1984; Eastmond 1993; IARC 2018; Pascoe et al. 1988). *p*-Benzoquinone was detected in incubations of acetaminophen with purified CYPs from phenobarbital treated rats (Dahlin et al. 1984). Indirect evidence of its formation *in vivo* comes from studies in mice that identified two acetaminophen metabolites in urine that are thought to arise from the conjugation of *p*-benzoquinone with GSH, namely (S-(2,5-dihydroxyphenyl)-cysteine (hydroquinone

[HQ]-cysteine) and S-(2,5-dihydroxyphenyl)-N-acetyl-cysteine (HQ-mercapturate) (Pascoe et al. 1988). Redox cycling reactions involving *p*-benzoquinone can also generate ROS (IARC 2018; Song and Buettner 2010).

Several human CYP enzymes can oxidize acetaminophen to NAPQI, including CYP2E1, 1A2, 2A6, and 3A4 (Gelotte et al. 2007; Mazaleuskaya et al. 2015; McGill and Jaeschke 2013; Patten et al. 1993; Raucy et al. 1989). Two P450 isozymes, CYP2E1 and CYP1A2, catalyze the majority of acetaminophen activation in human liver microsomes. Chronic alcohol consumption and other factors may affect the hepatic levels of CYP2E1 (Raucy et al. 1989).

While both CYP2E1 and CYP2A6 can catalyze the oxidation of acetaminophen to NAPQI, they catalyze this reaction at different rates. Based on experiments with purified human CYP enzymes, CYP2E1 is thought to be the main enzyme responsible for catalyzing this reaction. In these experiments, CYP2E1 selectively oxidized acetaminophen to the reactive metabolite, NAPQI, whereas CYP2A6 primarily oxidized acetaminophen to 3-hydroxy-acetaminophen (discussed further, below) (Chen et al. 1998). Support for a prominent role of CYP2E1 also stems from animal studies in which Cyp2e1 knockout (null) mice were less susceptible to acetaminophen-induced liver toxicity than wild-type mice, and CYP2E1 humanized mice were susceptible (Cheung et al. 2005; Lee et al. 1996). The differences in acetaminophen metabolism between wild-type and Cyp2e1 null mice were reduced at higher doses of acetaminophen, suggesting that the relative contribution of CYP2E1 to the formation of NAPQI and its thiol conjugates decreases with increasing doses (Chen et al. 2008). At therapeutic doses, NAPQI is detoxified by covalently binding with GSH in the liver to form 3-(glutathionyl) acetaminophen (acetaminophen-GSH) (Bond 2009; Mazaleuskaya et al. 2015; McGill and Jaeschke 2013; Mitchell et al. 1974; Moldeus 1978). Conjugation of NAPQI with GSH can occur either spontaneously or via glutathione-S-transferases (GST), with GSTP1 considered the most efficient enzyme to catalyze this reaction (Coles et al. 1988; Dragovic et al. 2014; Mazaleuskaya et al. 2015).

Acetaminophen-GSH is subsequently metabolized to 3-(cysteinyl) acetaminophen (acetaminophen-CYS), which can then form 3-thiomethyl acetaminophen-4-sulfate, acetaminophen-3-mercapturate, 3-thiomethylacetaminophen, 3-thiomethyl acetaminophen-4 glucuronide, and methanesulfinylacetaminophen (Andrews et al. 1976; Gelotte et al. 2007; Klutch et al. 1978; Wilson et al. 1982).

Additional metabolites of the oxidative and GSH conjugation pathways that have been identified in rodents *in vivo* include S-(5-acetylamino-2-hydroxyphenyl)mercaptopyruvic acid (SAMP); 3,3'-biacetaminophen (an acetaminophen-dimer); and an unidentified

benzothiazine compound (Chen et al. 2008), and S-(2,5-dihydroxyphenyl)-cysteine and S-(2,5-dihydroxyphenyl)-N-acetyl-cysteine) (Pascoe et al. 1988).

At high enough doses of acetaminophen (e.g., 300 mg/kg bw or greater in some strains/studies of adult mice and rats) the glucuronidation and sulfation pathways can become saturated and glutathione can be depleted, resulting in the covalent binding of NAPQI to cellular proteins, particularly at sulfhydryl groups on cysteine components of proteins (Albano et al. 1985; Bond 2009; IARC 1999a; Ramachandran and Jaeschke 2017).

Recent evidence from *in vitro* experiments indicates that NAPQI can bind to, and inhibit, glutathione synthase, which could lead to an inhibition of GSH synthesis and compromise the detoxification of this reactive metabolite (Walker et al. 2017).

Other metabolic pathways

Catechol pathway. Oxidative metabolism of acetaminophen by CYP enzymes can also lead to formation of the catechols 3-hydroxy-acetaminophen (and the sulfate conjugate 3-hydroxy-acetaminophen-3-sulfate) and 3-methoxy-acetaminophen[5] (and its glucuronide conjugate 3-methoxy-acetaminophen-4-glucuronide) (Chen et al. 2008; Chen et al. 1998; Forte et al. 1984; Gelotte et al. 2007; Harvison et al. 1988; Hinson et al. 1980; Knox and Jurand 1977; Ladds et al. 1987; Wilson et al. 1982). Using purified human CYP enzymes, Chen et al. (1998) demonstrated that CYP2A6 selectively oxidized acetaminophen to 3-hydroxy-acetaminophen. Each of the catechols and their sulfate or glucuronide conjugates have been identified in human urine (Gelotte et al. 2007; Knox and Jurand 1977; Ladds et al. 1987). Formation of these catechols in humans has been shown to increase with increasing doses of acetaminophen (Jetten et al. 2012), and both catechols have been shown to be hepatotoxic in mice and in isolated mouse hepatocytes (Forte et al. 1984; Holme et al. 1991).

Deacetylation pathway. N-deacetylation of acetaminophen results in the formation of PAP, which has been detected in the urine of humans and rodents, as well as in incubations with perfused rat kidneys, and in incubations with microbes isolated from the cecum of rats (Clark et al. 1986; Gemborys and Mudge 1981; Newton et al. 1982b; Smarr et al. 2017; Smith and Griffiths 1974). PAP is nephrotoxic in rodents (Calder et al. 1979; Crowe et al. 1979; Gartland et al. 1989; Gartland et al. 1990; Hoivik et al. 1995; Klos et al. 1992; Möller-Hartmann and Siegers 1991; Newton et al. 1982a; Newton et al. 1983; Newton et al. 1985; Newton et al. 1986) (see Section 3.3.3 Genotoxicity, and section on kidney metabolism below in this section). Sex and species

[5] Hydroxylation/methoxylation of the ring carbon at the 2 position has also been reported (Andrews et al. 1976).

differences exist with regards to PAP's nephrotoxicity (see section on kidney metabolism below) (Calder et al. 1979; Gemborys and Mudge 1981; Hu et al. 1993; Mudge et al. 1978; Mugford CA 1997; Newton et al. 1982a; Newton et al. 1983; Newton et al. 1985). PAP is genotoxic (see section 3.3.3.2 on genotoxicity) and *in vitro* studies have shown that it can be oxidized to the reactive *p*-aminophenoxy free radical by horseradish peroxidase (HRP), prostaglandin H synthase (PGES)[6], or alkaline auto-oxidation (Josephy et al. 1983). Further oxidation or disproportionation of the *p*-aminophenoxy free radical may yield *p*-benzoquinone imine (see section on kidney metabolism below) and may generate ROS by redox cycling and further oxidation reactions (Josephy et al. 1983).

Arachidonic acid conjugation. Once formed, PAP can also be conjugated with arachidonic acid via fatty acid amide hydrolase (FAAH) to form the therapeutically active compound N-arachidonylphenolamine (AM_{404})[7] (Hogestatt et al. 2005). In these studies by Hogestatt et al. (2005), AM_{404} and PAP were detected in the brain and spinal cord of rats after *i.p.* injections of acetaminophen. AM_{404} was not detected in mice lacking FAAH activity. *In vitro*, AM_{404} was detected with rat and mouse liver homogenates but could not be detected in rat liver *in vivo*. The authors speculated that AM_{404} may undergo rapid metabolism in that tissue under *in vivo* conditions.

PGES metabolism. PGES, with arachidonic acid as a co-substrate, can catalyze the metabolism of acetaminophen or PAP to reactive metabolites which bind to protein and GSH (Moldeus et al. 1982; Potter and Hinson 1987a). The enzyme is present in high concentrations in the kidney medulla in rodents (Smith and Wilkin 1977). *In vitro* experiments have shown that PGES can catalyze one- and two-electron oxidations of acetaminophen to form NAPSQI and NAPQI, respectively, as well as one-electron oxidation of acetaminophen to form acetaminophen polymers and, in the presence of GSH, acetaminophen-GSH (Potter and Hinson 1987a, 1989). As mentioned earlier, PGES has also been shown to catalyze the oxidation of PAP to form the *p*-aminophenoxy free radical (Josephy et al. 1983).

Acetaminophen metabolism in the kidney

In humans, long-term exposure to chronic therapeutic or acute high doses of acetaminophen is positively correlated with renal toxicity (Berg et al. 1990; Mazer and

[6] Prostaglandin H synthase (PGES, also known as prostaglandin endoperoxide synthase) has two distinct catalytic activities: a cyclooxygenase activity that forms prostaglandin G2 from arachidonic acid; and a peroxidase activity that reduces prostaglandin G2 to prostaglandin H2 (Marshall and Kulmacz 1988; Bessems and Vermeulen 2001). The pharmacological activity of acetaminophen is based on its inhibition of the cyclooxygenase activity (Bessems and Vermeulen 2001; Mazaleuskaya et al. 2015).

[7] AM_{404} is a potent activator of transient receptor potential vanilloid type 1 ($TRPV_1$), an affinity ligand of the cannabinoid receptor type 1 (CB_1), and an inhibitor of cellular uptake of the endogenous cannabinoid anandamide. $TRPV_1$ and CB_1 receptors are part of the pain and thermoregulatory pathways.

Perrone 2008; Sandler et al. 1989). Renal tubular necrosis related to acetaminophen administration has also been observed in rodents (Emeigh Hart et al. 1991a, b; Emeigh Hart et al. 1996; Newton et al. 1982a; Newton et al. 1983; Newton et al. 1985). The exact mechanisms of kidney toxicity are unclear, but may include the direct oxidation of acetaminophen to a reactive metabolite by CYP enzymes in either kidney or liver, with possible transport of the reactive metabolite to the kidney, and/or alternatively may include the deacetylation of acetaminophen to PAP and its further metabolism with GSH (Calder et al. 1979; Crowe et al. 1979; Fowler et al. 1991; Gartland et al. 1989; Gartland et al. 1990; Klos et al. 1992). CYP-oxidation, deacetylation, and GSH conjugation, as well as species, sex, and strain differences, and their respective roles in kidney toxicity are discussed below.

In CD-1 mice, nephrotoxicity likely involves CYP enzyme activation without prior deacetylation, and may be sex-specific (Emeigh Hart et al. 1991b). Male but not female CD-1 mice exhibit CYP-dependent nephrotoxicity and selective protein covalent binding after acetaminophen exposure. Following pre-treatment with testosterone, female CD-1 mice were also sensitive to acetaminophen-induced nephrotoxicity (Hoivik et al. 1995). Similarly, castrated male CD-1 mice were more resistant to acetaminophen induced kidney toxicity compared to intact male controls, but castration did not alter the level of protein binding or toxicity observed in the liver in these animals (Emeigh Hart et al. 1994). Testosterone treatment resulted in increased activation of renal, but not hepatic, Cyp2e1 enzyme *in vitro* (Hoivik et al. 1995). These studies suggest that Cyp2e1 activity may be induced by testosterone in renal, but not liver tissues, in CD-1 mice. Sex-related differences in kidney toxicity based on Cyp2e1 activity were also observed in another mouse strain, C3H/HeJ (Hu et al. 1993).

In male CD-1 mice, acetaminophen-CYS, a downstream metabolite of NAPQI and acetaminophen-GSH, was associated with kidney (but not liver) toxicity when administered prior to a non-toxic dose of acetaminophen, but not when administered by itself (Stern et al. 2005). Furthermore, administration of a single dose of acetaminophen-CYS resulted in a rapid depletion of renal but not hepatic GSH. Thus, acetaminophen-CYS acts synergistically with acetaminophen and has organ specific toxicity in this mouse model (Stern et al. 2005).

In rats, both deacetylation of acetaminophen, as well as oxidation and conjugation reactions of acetaminophen may be involved in nephrotoxicity (Mugford and Tarloff 1995; Mugford CA 1997; Newton et al. 1985). The association of either type of metabolic activation, i.e. oxidative metabolic activation of acetaminophen versus deacetylation of acetaminophen to PAP, was examined in two strains of rats treated with cycloheximide, a protein synthesis inhibitor. Fischer rats are susceptible to acetaminophen-induced kidney toxicity, whereas SD rats are less susceptible. Using

specific ^{14}C acetyl- and ring-labeled acetaminophen, Fischer rats had four times as much binding of ring-labeled acetaminophen to renal cortical protein than acetyl-labeled acetaminophen, whereas there was no isotope difference in protein binding in SD rats (Newton et al. 1985). No difference in hepatic protein labeling with either acetyl- or ring-labeled acetaminophen was observed in either strain. The authors concluded that deacetylation of acetaminophen to PAP is a requisite step in kidney toxicity in Fischer rats (Newton et al. 1985).

Deacetylation of acetaminophen to PAP occurs to some extent in the liver, with subsequent transport via systemic circulation to the kidney, based on studies in Fischer rats where biliary cannulation partially protected animals from PAP-induced nephrotoxicity (Gartland et al. 1990). Formation of urinary PAP increased with increasing doses of administered acetaminophen (Newton et al. 1982b). PAP may be accumulated in the kidney by organic cation transport systems which are concentrated in the proximal tubules in rats. Cellular absorption of PAP may be required for toxicity, based on a study where co-incubation of PAP with a transport inhibitor (tetraethylammonium bromide) protected rat kidney cortical cells from PAP-induced toxicity (Klos et al. 1992).

The extent of acetaminophen deacetylation to PAP may be reduced when oxidative metabolism of acetaminophen is increased, for example by treatment of animals with CYP inducers. Pretreatment of Fischer rats with CYP inducers (polybrominated biphenyls and β-naphthoflavone) reduced nephrotoxicity, and pretreatment with 3-methylcholanthrene increased hepatic necrosis and covalent binding of metabolites to hepatic but not renal proteins (McMurtry et al. 1978; Newton et al. 1982a). The authors concluded that the reduced nephrotoxicity is possibly a result of enhanced oxidative acetaminophen metabolism in the liver (Newton et al. 1982a). Increased oxidation of acetaminophen would reduce the amount of substrate available for direct deacetylation of acetaminophen to PAP.

The nephrotoxicity of and some of its metabolites has been observed in many rodent studies (Calder et al. 1979; Crowe et al. 1979; Fowler et al. 1991; Fowler et al. 1993, 1994; Gartland et al. 1989; Gartland et al. 1990; Klos et al. 1992; Newton et al. 1982a; Newton et al. 1983; Newton et al. 1985). PAP induced proximal nephropathy with elevated urinary enzymes, blood urea nitrogen, glucose, and urine total protein, indicating functional defects in the proximal tubule of the kidney (Gartland et al. 1989). PAP, but not its *ortho*- or *meta*-analogues, was nephrotoxic, suggesting stereo-specificity for biological activity (Newton et al. 1982a).

The nephrotoxicity of PAP may be due to the formation of reactive metabolite(s) via different metabolic mechanisms (Calder et al. 1979; Fowler et al. 1993, 1994; Klos et al.

1992). PAP depleted GSH levels in the kidney, supporting the formation of a reactive metabolite that readily binds to GSH (Crowe et al. 1979). Animals treated with radiolabeled PAP had extensive protein binding of ring-labeled PAP in the kidney but to a much lesser extent in the liver (Crowe et al. 1979). Protein binding was inhibited with ascorbate, GSH, N_2, and NADPH, suggesting the formation of an oxidative and reactive PAP metabolite (Calder et al. 1979). Increasing doses of PAP lead to an increased incidence of kidney lesions where both the cortical and the juxta-glomerular nephrons are affected (Fowler et al. 1991). Likely reactive metabolites formed from PAP are the *p*-aminophenoxy radical and *p*-benzoquinone imine (Fowler et al. 1993). PAP can readily undergo oxidation by HRP, PGES, or autoxidation *in vitro*, forming a *p*-aminophenoxy free radical (Josephy et al. 1983). Further oxidation or disproportionation of the radical is likely to yield *p*-benzoquinone imine. PAP has been shown to generate ROS in porcine cell cultures and in a cell-free system (Foreman and Tarloff 2008; Mirkovic et al. 2011).

In addition to these reactive oxidative metabolites, several glutathione metabolites of PAP have also been associated with kidney toxicity. While GSH conjugation is generally considered a detoxification pathway with conversion of the glutathione-S-conjugates to corresponding mercapturic acids and subsequent excretion, GSH conjugation and downstream metabolism by γ-glutamyltransferase[8] (GGT) can also mediate toxicity (Anders et al. 1988; Fowler et al. 1993, 1994; Gartland et al. 1990; Klos et al. 1992; Stern et al. 2005; van Bladeren 1988). GGT cleaves PAP-GSH to form cysteinylglycine- and/or cysteinyl-conjugates of PAP (PAP-CYS), which may undergo further metabolism to form the proximate toxic metabolite(s) (Fowler et al. 1994).

Glutathione-dependent bioactivation of PAP has been observed in multiple studies. Thus, GSH depletion with buthionine sulphoximine protected rats from nephrotoxicity caused by PAP, suggesting a reactive PAP-glutathione (PAP-GSH) metabolite may be responsible for nephrotoxicity (Gartland et al. 1990). Dose-dependent renal necrosis resulted after *ip* injections of PAP-GSH (4-amino-3(glutathione-S-yl)phenol) in Fischer rats, whereas the sulfate conjugate (PAP–o-sulfate) caused no histological or functional alteration to the kidney (Fowler et al. 1991). PAP-GSH was also shown to increase

[8] The name γ-glutamyl transferase was preferred by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology for this enzyme, E,C.2.3.2.2. Older names include γ-glutamyl transpeptidase or glutathionase. GGT hydrolyzes the γ-glutamyl bond of extracellular glutathione, initiating cleavage into glutamate, cysteine, and glycine and carries out transpeptidation reactions (Whitfield 2001; Hanigan 2014; Zhang et al. 2005). GGT plays a key role in GSH homeostasis by breaking down extracellular GSH and providing cysteine, the rate-limiting substrate, for intracellular *de novo* synthesis of GSH.

levels of blood urea nitrogen and urinary excretion of glucose, protein and GGT in Fischer rats (Fowler et al. 1994).

PAP-GSH and two corresponding 3,5-disubstituted and 2,3,6-trisubstituted thio-adducts, namely 4-amino-2,5-bis(glutathione-S-yl)phenol, and 4-amino-2,3,5 (or-6)-tris(glutathion-S-yl-)phenol were identified as biliary metabolites after *i.p.* injection of PAP (Klos et al. 1992). All three metabolites caused toxicity to isolated rat renal cortical cells (Fowler et al. 1991; Klos et al. 1992). Prior administration of acivicin, a GGT inhibitor, greatly reduced nephrotoxicity from PAP-GSH in Fischer rats, suggesting that PAP nephrotoxicity may be induced via a GGT-dependent pathway (Fowler et al. 1993, 1994). Overall, these results indicate that glutathione conjugation of an oxidative metabolite of PAP is not a detoxification process.

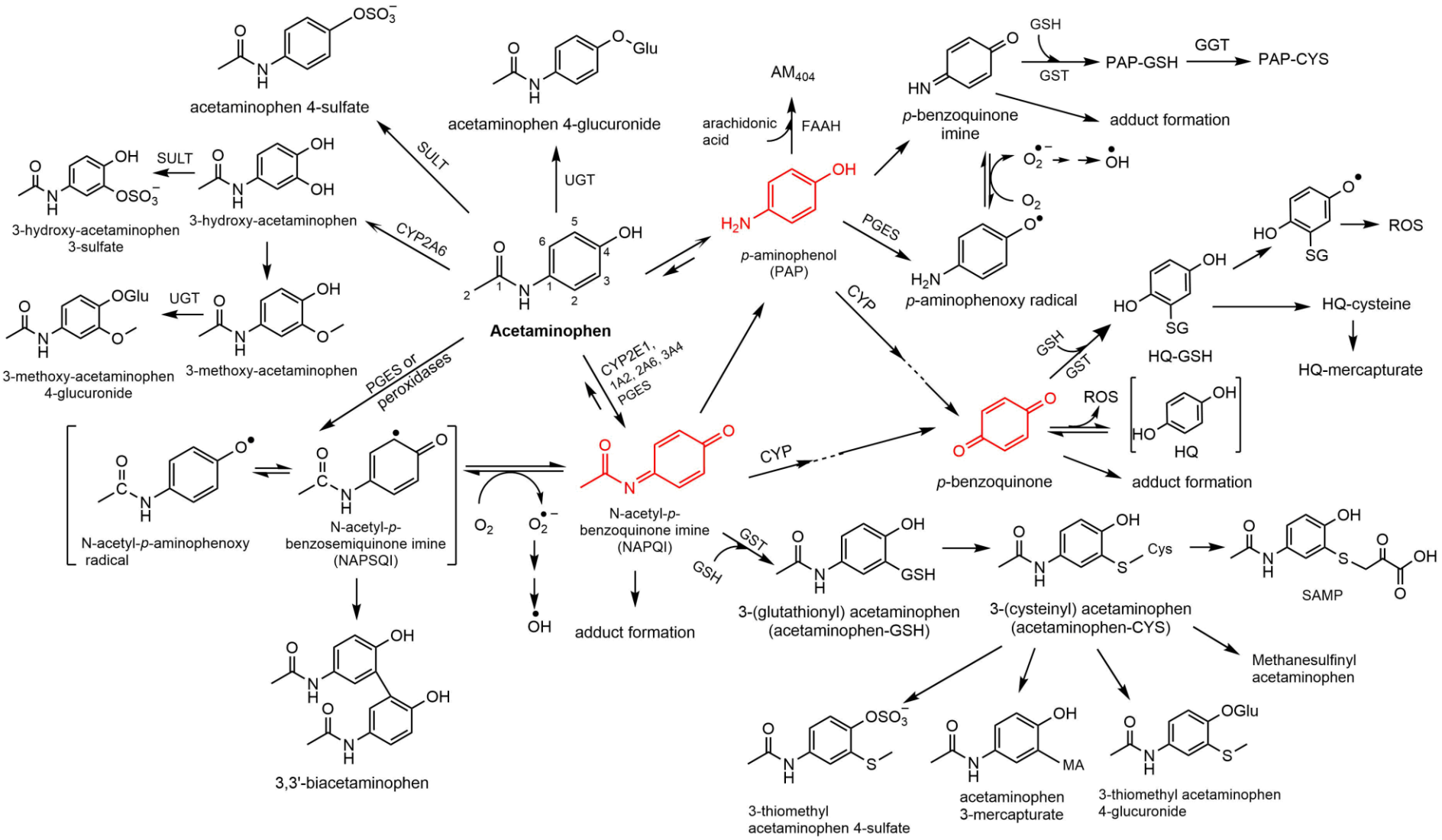


Figure 5. The proposed metabolism of acetaminophen in humans and animals

3.3.1.4 Elimination

Acetaminophen is extensively metabolized, and the primary route of excretion is via urine. Other routes of excretion are feces, bile, breast milk, saliva, and expired air.

Urinary excretion

The majority of acetaminophen is excreted in urine, mainly as products from the glucuronidation, sulfation, and glutathione conjugation pathways. In adults, about 85-90% of the administered dose of acetaminophen is excreted in urine within 24 hours, with the majority excreted in the first 8-12 hours (Buniva et al. 1977; Mitchell et al. 1974). Renal excretion of acetaminophen involves glomerular filtration and passive re-absorption (Morris and Levy 1984). The mean renal clearance rate of acetaminophen in subjects given 20 mg/kg was 13 mL/min (Prescott 1980). These same authors reported higher renal clearance rates (normalized by body surface area) for acetaminophen glucuronide (100 mL/min/1.73 m^2) and acetaminophen sulfate (161 mL/min/1.73 m^2) than for acetaminophen (6.32 mL/min/1.73 m^2) (Morris and Levy 1984).

The major acetaminophen-related substances excreted in the urine are acetaminophen (2.8–4%), acetaminophen glucuronide conjugate (50–66%), and acetaminophen sulfate conjugate (25–35%). Minor metabolites present in the urine include *PAP* (Clark et al. 1986; Gemborys and Mudge 1981; Newton et al. 1982b; Smarr et al. 2017), the catechol pathway metabolites 3-hydroxy-acetaminophen (and its sulfate) and 3-methoxy-acetaminophen (and its glucuronide) (3.8–5.4%), 2-hydroxy-acetaminophen sulfate (not shown in Figure 5), and various thiol metabolites (8.8–10.2%) (e.g. 2.6–3.2% acetaminophen-CYS, and 3–4% acetaminophen-mercapturate, methanesulfinylacetaminophen and methylthio-acetaminophen combined) (Andrews et al. 1976; Forrest et al. 1982; Gelotte et al. 2007; IARC 1999a; Kamali et al. 1987; Ladds et al. 1987; Mrochek et al. 1974; Wilson et al. 1982). Two additional minor metabolites, the cysteine and the N-acetyl-cysteine conjugates of hydroquinone (S-(2,5-dihydroxyphenyl)-cysteine and S-(2,5-dihydroxyphenyl)-N-acetyl-cysteine), have not been assessed in humans, but have been identified in the urine of male BALB/c mice (Pascoe et al. 1988).

Breastfed infants whose mothers took acetaminophen excrete acetaminophen, acetaminophen-4-glucuronide, acetaminophen-4-sulfate, acetaminophen-CYS and mercapturic acid conjugates in urine although only acetaminophen, but not its metabolites, was detected in breast milk, suggesting that infants can metabolize acetaminophen (Berlin et al. 1980; Notarianni et al. 1987). Neonates excrete significantly greater proportions of unchanged acetaminophen and lesser proportions of acetaminophen-4-sulfate compared to older subjects aged 11-80 years (Notarianni et al. 1987). The rate of glucuronidation in neonates is roughly half that of adults (6.6 L/h/70

kg versus 11.8 L/h/70 kg). Metabolites of the oxidative pathway (cysteine and mercapturic acid conjugates) may or may not be present in infant urine (Notarianni et al. 1987; van der Marel et al. 2003).

Fecal excretion

The majority of studies of fecal excretion, which plays a minor role in the elimination of acetaminophen, have been conducted in laboratory animals, with few studies conducted in humans.

In one study, a human subject received 650 mg of ^{13}C and ^{15}N radiolabeled acetaminophen orally; the fecal recovery of ^{13}C and ^{15}N was 0.9% and 1.1%, respectively (Browne et al. 1998). Metabolites observed in feces included the parent compound acetaminophen, acetaminophen-CYS, acetaminophen-4-glucuronide and 2-hydroxyacetaminophen sulfate (Goedert et al. 2014).

Experiments in Wistar rats showed that only very small amounts of acetaminophen (0.2–0.7% of the administered dose) are excreted in feces (Smith and Griffiths 1976). A study with Balb/c mice identified two fecal metabolites from the oxidative pathway, acetaminophen-CYS and the mercapturic acid metabolites (Hoffmann et al. 1990).

Biliary excretion

The majority of studies of biliary excretion have been conducted in laboratory animals, primarily rodents, with few studies conducted in humans.

Bile is a minor excretion pathway for acetaminophen in humans. In one study bile samples were collected overnight (mean: 11.8 hours) from 10 subjects who had received 1 g of acetaminophen (Jayasinghe et al. 1986). Only a small amount of unchanged acetaminophen was recovered in the bile. The metabolites identified in bile were acetaminophen-4-glucuronide and acetaminophen-CYS. The cysteine conjugate recovered in bile represented the largest portion; acetaminophen-mercapturate and acetaminophen-4-sulfate were below the detection level of <1 µg/ml (Jayasinghe et al. 1986).

In another study by Siegers et al. (1984), patients had T-tubes inserted in the common bile duct, after which they ingested 1 g of acetaminophen. In these patients, biliary excretion accounted for 2.6% of the oral dose within 8 hours. Substances identified in bile included the parent molecule acetaminophen (5.7 mg), acetaminophen-4-glucuronide (3.6 mg), acetaminophen-4-sulfate (3.6 mg), and acetaminophen-CYS conjugate (16.3 mg). Acetaminophen-GSH was not detected, and the authors speculated that acetaminophen-GSH may be rapidly metabolized to the cysteine conjugate by GGT, which metabolizes reduced glutathione.

Biliary excretion of acetaminophen and its metabolites has been studied in rats, mice, hamsters, rabbits, and guinea pigs (Ghanem et al. 2005; Gregus et al. 1988; Hinson et al. 1982; Hjelle and Klaassen 1984; Siegers et al. 1983; Watari et al. 1984). In rats, 28.7% of the total dose was excreted into the bile within 8 hours post *i.v.* administration (Siegers et al. 1983). The glucuronide conjugate is the major biliary metabolite in rats (ranging from 10.5 - 21.4%, depending on dose), whereas biliary excretion of acetaminophen or acetaminophen-sulfate remains small (0.58 - 1.89% for acetaminophen and 1.8 - 7% for acetaminophen-sulfate) (Hjelle and Klaassen 1984; Watari et al. 1983).

When comparing rats, hamster, mice, guinea pigs and rabbits, rats had the highest biliary excretion of acetaminophen glucuoride and -sulfate, followed by guinea pig, rabbit, mouse, and hamster, with rat and guinea pig excreting up to 50 fold more acetaminophen glucuronide compared to the other species. Conversely, mice and hamster excreted significantly more acetaminophen-CYS compared to the remaining species (Gregus et al. 1988).

Biliary excretion experiments conducted in rats suggest that reabsorption and enterohepatic circulation occur, and that some metabolites are hydrolyzed by microflora prior to enterohepatic circulation (Grafstrom et al. 1979; Siegers et al. 1983; Watari et al. 1983; Watari et al. 1984). As mentioned earlier, an *in vitro* study demonstrated that acetaminophen was deacetylated to PAP by microflora from rat caecum (Smith and Griffiths 1974).

Excretion into breast milk

Acetaminophen is excreted into breast milk, as shown in nursing mothers who received oral doses of 650 mg (Berlin et al. 1980) and 1 g (Notarianni et al. 1987). Metabolites of acetaminophen have not been detected in breast milk (Notarianni et al. 1987).

Salivary excretion

Salivary excretion of acetaminophen occurs, and is dependent on the plasma concentration, with a correlation coefficient of 0.99 obtained between plasma and saliva levels (Idkaidek and Arafat 2014). Measured C_{max} concentrations of acetaminophen over a 10 hour period were 6.8 µg/mL in saliva and 5.5 µg/mL in plasma (Berlin et al. 1980; Kamali et al. 1987). Metabolites of acetaminophen have not been assessed in saliva.

Expired air

Excretion of acetaminophen into expired air has been measured in Wistar rats after oral administration of acetaminophen, where 5.5-6.5% of [acetyl-^{14}C]-labeled

acetaminophen was de-acetylated by microbial microflora in rats and released as CO_2 in expired air (Smith and Griffiths 1976). No human studies regarding expiration of acetaminophen or its metabolites into air were identified.

3.3.1.5 Summary

In conclusion, acetaminophen is rapidly absorbed and distributed throughout the body and largely excreted via urine, with some biliary and little fecal excretion occurring. Metabolism of acetaminophen occurs mainly in the liver and kidney and occurs via multiple pathways, including glucuronidation, sulfation, oxidation via CYPs and other enzymes, and deacetylation. Multiple metabolites of acetaminophen may participate in redox cycling reactions, resulting in the formation of ROS. Acetaminophen-CYS and PAP have been implicated in acetaminophen's toxicity to the kidney in mice and rats, respectively. Several genotoxic and electrophilic metabolites of acetaminophen are formed, including NAPQI, NAPSQI, *p*-benzoquinone, *p*-benzoquinone imine, PAP, and the *N*-acetyl-*p*-aminophenoxy and *p*-aminophenoxy radicals.

3.3.2 Factors that modulate acetaminophen metabolism

Many of the enzymes that are involved in acetaminophen metabolism are polymorphic in humans. These include Phase I enzymes, such as CYPs, and Phase II enzymes, such as UGTs, SULTs, and GSTs. Besides genetic polymorphisms, non-genetic factors can also modulate enzyme activity thus affecting acetaminophen metabolism. The phenotypic variability of these enzymes has been observed in liver samples from human donors in *in vitro* experiments. For example, the acetaminophen sulfation activity of SULTs ranged from 93.5 to 1720 pmol/mg/min among 20 donors (den Braver-Sewradj et al. 2018).

This section will mainly focus on factors that can modulate the formation or detoxification of NAPQI, as data on other toxic acetaminophen metabolites is sparse.

Genetic polymorphisms of Phase I enzymes

Studies with human enzymes have shown that several CYP enzymes (CYP2E1, CYP1A2, CYP3A4, and CYP2A6) can carry out the oxidation of acetaminophen to NAPQI (Chen et al. 1998; Patten et al. 1993; Thummel et al. 1993).

CYP2E1

As discussed in Section 3.3.1, CYP2E1 is the main enzyme responsible for the formation of the reactive intermediate NAPQI in the liver.

The polymorphisms of *CYP2E1* have been summarized by the Pharmacogene Variation Consortium (https://www.pharmvar.org/gene/CYP2E1). As of 2019, 13 allelic variants have been characterized (Hayashi et al. 1991; Persson et al. 1993; Uematsu et al. 1991). These variants can carry increased, normal, or decreased enzyme activity, and their prevalence in different human populations can vary (Hayashi et al. 1991; Persson et al. 1993; Uematsu et al. 1991). One study found that carriers of certain *CYP2E1* polymorphisms are more susceptible to acetaminophen toxicity when they are also exposed to alcohol (ethanol) or obesity (McCarver et al. 1998). A separate study has shown that CYP2E1-mediated oxidation of acetaminophen is significantly higher in obese individuals with BMI > 40 (van Rongen et al. 2016).

Besides genetic polymorphisms, the expression of CYP2E1 is regulated transcriptionally by hepatocyte nuclear factor 1α (HNF1α) and its cofactors, and post-transcriptionally via protein stabilization (Gonzalez 2007). CYP2E1 activity is also inducible by a variety of substrates (Gonzalez 2007).

One important player in the modulation of CYP2E1 activity and acetaminophen hepatotoxicity is ethanol. Ethanol is known to be an inducer of CYP2E1 (Koop et al. 1982; Lieber and DeCarli 1970), but it can also inhibit this enzyme (Yang et al. 1991). On the other hand, in the presence of high doses of ethanol, CYP2E1 is thought to be involved in the metabolic activation of ethanol (Guengerich and Avadhani 2018). These different actions of ethanol on CYP2E1 activity may underlie the reported differences in acetaminophen toxicity associated with acute versus chronic alcohol consumption (Banda and Quart 1982; Schiodt et al. 2002).

Caparrotta et al. (2018) reviewed case reports and retrospective case series regarding alcohol use and acetaminophen toxicity, and stated the following two scenarios for chronic vs acute alcohol intake:

> ”Alcohol consumption upregulates the expression of CYP2E1, potentially increasing the amount of paracetamol oxidized to NAPQI…When alcohol is acutely presesent in the body it competes for CYP2E1, thereby potentially reducing the amount of paracetamol oxidized to NAPQI”.

In people consuming alcohol chronically, the main effects are upregulation of CYPs (both CYP2E1 and CYP1A4), and possibly depletion of glutathione, although the evidence for the latter has been conflicting (Riordan and Williams 2002). In the acute setting where an individual takes acetaminophen and alcohol simultaneously, there is an apparent decrease in NAPQI formation, either by direct or indirect inhibition of CYP2E1 (Riordan and Williams 2002).

Court et al. (2014) did not find a correlation between two CYP2E1 genetic variants and acetaminophen-induced acute liver failure (ALF). However, the study is limited because

it only examined two of the many variants, and the phenotypes (*i.e.*, acetaminophen oxidation activity) of these two variants have not been adequately characterized. In mice, Cyp2e1, and to a lesser extent Cyp1a2, seem to be the main enzymes responsible for the acetaminophen-induced hepatotoxicity, as shown by studies using Cyp2e1 and Cyp1a2 knockout models (Lee et al. 1996; Zaher et al. 1998). In the kidney, acetaminophen toxicity can be caused by either direct oxidation by Cyp2e1 to form NAPQI, or deacetylation by *N*-deacetylase to form PAP, downstream free radicals and GSH conjugates. In a *Cyp2e1*-null mouse model, 200 and 400 mg/kg acetaminophen induced significantly less nephrotoxicity than in wild-type mice treated with the same doses (Chen et al. 2008).

In summary, both genetic and non-genetic factors (e.g., obesity, alcohol) can influence an individual's CYP2E1 activity, thus altering one's susceptibility to acetaminophen-induced toxicity.

CYP1A2

CYP1A2 is polymorphic in humans (Koonrungsesomboon et al. 2018). Its role in acetaminophen hepatotoxicity may not be important in the presence of CYP2E1. There are data showing Cyp1a2 had an effect when *Cyp2e1* was also knocked out (Zaher et al. 1998). *Cyp1a2* single knockout offered no protection of acetaminophen hepatotoxicity in mice, as measured by urinary metabolites and protein adducts (Tonge et al. 1998).

CYP3A4 and CYP3A5

CYP3A4 activity is highly variable due to genetic polymorphisms and induction by factors such as diet, medication, or exposure to environmental chemicals (Lamba et al. 2002; Thummel and Wilkinson 1998). Laine et al. (2009) reported that at therapeutic concentration of acetaminophen, CYP3A4 had the highest bioactivation capacity among nine CYP enzymes. The inter-individual variability of CYP3A4 was shown in one study where a CYP3A4 inhibitory antibody reduced acetaminophen oxidative metabolism by CYP3A4 by as low as 6% and as high as 76% in human liver microsomes from four donors (Patten et al. 1993). In a study with hepatocytes from 16 human donors, CYP3A4 activities correlated with acetaminophen cytotoxicity, and the hepatocytes with the highest CYP3A4 activities were the most sensitive to acetaminophen cytotoxicity (Utkarsh et al. 2016). Cyp3a11 (the homologous enzyme in mice of human CYP3A4) has been shown to be responsible for the acetaminophen-induced liver injury in C57/BL6 mice (Li et al. 2018).

CYP3A5 shares considerable substrate specificity with CYP3A4, although CYP3A4 is considered to have higher activity (Smith et al. 2018). CYP3A5 is polymorphic in humans (Hustert et al. 2001). Among patients with ALF, one *CYP3A5* allele (fast

metabolizer phenotype) was over-represented in patients who intentionally overdosed on acetaminophen (OR = 2.3, 95% CI 1.1–4.9; p = 0.034) (Court et al. 2014). Although the capacity of CYP3A5 to oxidize acetaminophen has not been reported, the results from Court et al. (2014) suggest that CYP3A5 can also catalyze the formation of NAPQI, leading to acetaminophen-associated ALF.

CYP2A6

CYP2A6 is a highly polymorphic gene (https://www.pharmvar.org/gene/CYP2A6). Enzyme inhibition studies *in vitro* showed that inhibition of CYP2A6 significantly reduced the amount of NAPQI formation (Hazai et al. 2002). However, it seems CYP2A6 favors the 3-hydroxylation pathway over the oxidation with a 3:1 ratio, compared to a 1:6 ratio for CYP2E1 (Chen et al. 1998). Both experimental data and computational prediction for enzyme regioselectivity indicate that acetaminophen 3-hydroxylation is favored over *N*-oxidation by CYP2A6 (Yang et al. 2014). Therefore, the impact of CYP2A6 polymorphisms on acetaminophen toxicity in humans may not be significant.

Genetic polymorphisms of Phase II enzymes

GSTs

GSTs are a superfamily of Phase II enzymes that can catalyze the conjugation of acetaminophen metabolites with GSH. Cytosolic GSTs are expressed in the liver and many other tissues, although more data is needed on the tissue-specific expression for each class of GST (Hayes and Strange 2000). At low doses, NAPQI is conjugated with GSH and subsequently excreted as mercapturic acid or cysteine derivatives. *In vitro* studies reported that human GSTP1 was the most effective GST isoform in catalyzing the conjugation of NAPQI with GSH (Coles et al. 1988). The balance between the rate of NAPQI formation and its inactivation by conjugation (*e.g.*, to GSH) can determine the rate of protein/DNA adduct formation (Park et al. 2011; Rinaldi et al. 2002). Some studies have suggested that the GSH conjugation of another acetaminophen metabolite, PAP, leads to downstream bio-activation and nephrotoxicity (Klos et al. 1992). However, no studies have been conducted to examine what GST isoform(s) play a role in such conjugation.

Human GSTs are known to be genetically polymorphic (Bolt and Thier 2006; Buchard et al. 2012; Hayes and Strange 2000). One study found no significant difference in acetaminophen conjugation activities among several human GSTP1 variants compared to the wild-type enzyme (Dragovic et al. 2014).

One case-control study identified no association between acetaminophen use and bladder cancer, but found a significant interaction of acetaminophen use with the *GSTP1* I105V genotype (Fortuny et al. 2006). Among the individuals with homozygous

decrease-of-function alleles (*GSTP1* Val/Val), use of acetaminophen was non-significantly associated with increased risk of bladder cancer compared to non-users of the same genotype (OR = 1.8, 95% CI 0.9–3.6, p = 0.008 for interaction between acetaminophen use and this genotype). Subjects who were homozygous for this mutant allele and used acetaminophen regularly for more than four years had an increased risk of bladder cancer (OR = 2.5, 95% CI 0.4–15.6). These results suggest that GSTP1 is an important detoxifying enzyme for acetaminophen metabolites that are GSTP1 substrates, and GSTP1 I105V may be an effect modifier for the association between exposure to acetaminophen and bladder cancer.

UGTs

The UGTs are a superfamily of membrane bound Phase II enzymes that are found in the liver, kidney, and intestinal mucosa, and catalyze the conjugation of acetaminophen with uridine diphosphoglucuronic acid (UDPGA) to form acetaminophen-glucuronide (Fisher et al. 2000). In individuals with little or slow glucuronidation activity exposed to acetaminophen, less of the compound will be detoxified via glucuronidation, increasing the likelihood that acetaminophen undergoes bioactiviation to NAPQI, PAP, or other reactive metabolites.

UGTs are polymorphic in humans (Hu et al. 2016). The 3´-untranslated region (3´-UTR) of *UGT1A* is shared by nine different isoforms (Court et al. 2014). One particular single nucleotide polymorphism (SNP) in this 3´-UTR was associated with higher acetaminophen glucuronidation activity and was under-represented in patients with ALF caused by unintentional acetaminophen overdose, compared to patients with ALF induced by other causes (Court et al. 2013). In humans, one variant *UGT2B15*2* was associated with significantly lower levels of glucuronidation clearance and higher levels of acetaminophen-protein adducts compared to wild-type, with the differences stronger in people who were homozygous for the mutant allele than those that were heterozygous (Court et al. 2017).

UGT1A6, and to a lesser extent UGT1A1 and UGT1A9, were found to be the most active isoforms for acetaminophen glucuronidation (Allegaert et al. 2005). Individuals who express low-capacity isoforms of UGTs (such as UGT1A3 or UGT2B7) were less efficient at forming acetaminophen-glucuronide than individuals with the most active isoforms (Court et al. 2001). The activity of UGT1A6, the main isoform for acetaminophen glucuronidation, varied by 7-fold among the liver microsomes from 20 donors (Fisher et al. 2000). The overall UGT activity was found to be higher in livers from male donors than female donors. This was possibly due to the higher UGT1A6 protein content in livers from males.

SULTs

In humans, approximately 30% of administered acetaminophen is excreted in urine as the sulfate conjugate (Bessems and Vermeulen 2001). The sulfation of acetaminophen is carried out by cytosolic SULTs, with SULT1A1, SULT1A3 and SULT1C4 having the strongest sulfating activity towards acetaminophen (Yamamoto et al. 2015). Acetaminophen-sulfation activity has been detected in cytosol preparations of human intestine, liver, lung, and kidney, in decreasing order (Yamamoto et al. 2015).

SULTs are polymorphic in humans, and the frequencies of certain polymorphisms differ by ethnic groups (Nowell and Falany 2006). For example, common SNPs in SULT1A1 occur at different frequencies in Caucasian, African-American, and Chinese groups (Nagar et al. 2006; Ning et al. 2005). Polymorphisms of SULTs can influence acetaminophen metabolism, with certain variants showing significantly lower enzyme activity toward acetaminophen in humans *in vitro* (Bairam et al. 2018).

Genetic polymorphisms of other enzymes

As mentioned in Section 3.3.1, FAAH is responsible for the formation of acetaminophen's therapeutically active metabolite, AM_{404}. FAAH is polymorphic in humans, and one SNP (C385A) that exists at 25% frequency in a Caucasian population correlates with normal catalytic activity but enhanced sensitivity to proteolytic degradation (Sipe et al. 2002) and phenotypic changes in carriers (Conzelmann et al. 2012; Spagnolo et al. 2016). No studies on the effect of FAAH polymorphisms on acetaminophen metabolism were identified.

Genetic polymorphisms of sulfate transporters

As mentioned above, sulfate conjugation is a major pathway for acetaminophen detoxification. This reaction relies not only on the sulfotransferases, but also on a sufficient supply of the universal sulfate donor, 3´-phosphoadenosine 5´-phosphosulfate (PAPS). The formation of PAPS from inorganic sulfate (SO_4^{2-}) is known as sulfate activation (Markovich 2001). Sulfate transporters such as NaS1 (gene name: *SLC13A1*) and Sat1 (gene name: *SLC26A1*) are important for maintaining adequate concentrations of intracellular SO_4^{2-}, since this anion is hydrophilic.

NaS1 is a Na^+-dependent sulfate transporter expressed on the brush border membrane of renal proximal tubular epithelial cells (Markovich et al. 1993). The *SLC13A1* gene is polymorphic in humans (Tise et al. 2016). Lee et al. (2006) identified a loss-of-function SNP (R12X) and a decrease-of-function SNP (N174S) in the human *SLC13A1* gene, indicating certain populations may be more susceptible to acetaminophen hepatotoxicity due to inadequate sulfate transport. In mice with no expression of NaS1,

acetaminophen induced more severe hepatotoxicity (*e.g.*, increased Alanine transaminase or ALT levels, cellular damage, and liver necrosis) than in wild-type mice (Lee et al. 2006). Since NaS1 is not expressed in the liver, the authors considered the hepatotoxicity in the *Slc13a1*-null mice to be due to the exacerbated GSH depletion caused by hyposulfatemia.

Sat1 is a Na^+-independent sulfate anion transporter expressed on the basolateral membrane of renal proximal tubules, distal ileum of the small intestine, cecum, and proximal colon, and the sinusoidal membrane of hepatocytes (Markovich 2012). The *SLC26A1* gene is polymorphic in humans (Dawson and Markovich 2007; Dawson et al. 2013). Similar to NaS1, mice lacking Sat1 were also more sensitive to acetaminophen-induced hepatotoxicity (Dawson et al. 2010).

Non-genetic factors that affect acetaminophen metabolism

Besides genetic variability, other factors can also affect acetaminophen metabolism. These factors may interfere with or alter enzyme activity, deplete GSH, and/or compete for sulfate conjugation or glucuronidation. For example, malnutrition can lead to reduced GSH as well as reduced CYP2E1 activity (Rumack 2002). A number of potential modulating factors have been reviewed by Caparrotta et al. (2018) and Bessems and Vermeulen (2001). For some of the factors, the evidence is clear, albeit complicated. For example, ethanol binds to CYP2E1 and blocks access of acetaminophen to the enzyme's catalytic site, while long-term alcohol intake induces the activity of the enzyme. On the other hand, many factors are involved in multiple pathways of acetaminophen metabolism and they may not be independent of one another (*e.g.*, bodyweight, calorie intake, and nutritional status), making it difficult to predict the effect of a single factor. Caparrotta et al. (2018) also examined the effects of several drugs on acetaminophen metabolism, and found mixed results. For example, one pharmacokinetic study showed that carbamazepine increases NAPQI formation while three studies showed no effects; however, several clinical case reports suggest that carbamazepine potentiates acetaminophen toxicity. Overall, more research is needed to evaluate many of these factors and their effects on acetaminophen metabolism.

Besides the factors mentioned above, it is worth mentioning that dimethyl sulfoxide (DMSO) has been shown to modulate acetaminophen metabolism. DMSO is a solvent frequently used to deliver chemicals in research because of its unique polar aprotic nature. It is also used as a medication and dietary supplement; for example, it is given intravenously as an prescription medication for interstitial cystitis (bladder pain syndrome), available for use topically as an OTC gel/cream, and occasionally used as an oral supplement by consumers (HSDB 2014). DMSO at 2.5% increased the expression of CYP2E1 in cultured human hepatocytes (Nishimura et al. 2003), while at

concentrations as low as 0.1% DMSO has been shown to inhibit CYP2E1 enzyme activity in human hepatocytes and human liver microsomes *in vitro* (Easterbrook et al. 2001; Hickman et al. 1998). DMSO has been used as a vehicle to deliver acetaminophen both *in vivo* and *in vitro*, despite the fact that it has been shown to inhibit acetaminophen-induced hepatotoxicity in animals (El-Hage et al. 1983; Siegers 1978; Yoon et al. 2006). Besides inhibiting hepatotoxicity, DMSO also inhibits acetaminophen-induced GSH depletion and DNA fragmentation in mice (Jaeschke et al. 2006; Yoon et al. 2006). The inhibitory effect of DMSO has led to controversies in interpreting experimental results (Jaeschke et al. 2006). Earlier proposed mechanisms of how DMSO protects against acetaminophen-induced hepatotoxicity include directly scavenging reactive species generated during acetaminophen metabolism, and inhibiting CYP enzymes. However, the protection by DMSO appeared to be tissue-specific (Jeffery and Haschek 1988), which made the non-selective mechanism of radical scavenging less plausible. Yoon et al. (2006) has proposed that DMSO protects against acetaminophen hepatotoxicity by competitively binding to CYP2E1 and inhibiting the activation of acetaminophen to NAPQI.

In summary, metabolism of acetaminophen varies among individuals, as a result of genetic and non-genetic factors. Individuals with altered function of certain enzymes and transporters discussed above, either caused by genetic or non-genetic factors, may generate more electrophilic and genotoxic metabolites of acetaminophen than others.

3.3.3 Genotoxicity

The genotoxicity of acetaminophen has been well studied and reviewed by a number of research groups and institutions, including IARC (1990, 1999), NTP (1993), Bergman et al. (1996), and Rannug et al. (1995). OEHHA reviewed these evaluations of the genotoxicity studies, as well as additional genotoxicity studies published since these reviews.

Acetaminophen has been tested for a variety of genotoxic endpoints in humans *in vivo*, human cells *in vitro,* animals *in vivo*, animal cells *in vitro*, and non-mammalian species (i.e. mussels, *Drosophila*, plants, and yeasts). It has also been tested for reverse mutations in bacteria, and for DNA adduct formation in acellular systems.

Genotoxic effects of acetaminophen have been observed in humans, animals, and a number of experimental systems over a range of doses, including doses that fall within the range of those used therapeutically.

Overall, the findings from these genotoxicity studies of acetaminophen include evidence of (i) induction of chromosomal aberrations (CAs), sister chromatid exchanges (SCEs), and micronuclei formation (MN), and a decrease in unscheduled DNA synthesis (UDS) in humans *in vivo* (Table 17), (ii) induction of DNA strand breaks, MN, CAs, and SCEs, impairment of DNA excision repair, formation of DNA adducts, and a slight increase in UDS in human cells *in vitro* (Table 18), (iii) formation of DNA adducts, DNA strand breaks, impairment of DNA excision repair, oxidative damage to DNA, MN, CAs, SCEs, and aneuploidy in animals *in vivo* (Table 19), (iv) formation of mutations, oxidative damage to DNA, MN, CAs, and SCEs, impairment of DNA repair, and alterations in UDS in animal cells *in vitro* (Table 20), and (v) DNA strand breaks and MN in mussels, CAs in onion roots, and formation of DNA adducts in acellular systems (Table 21).

3.3.3.1 Studies on the genotoxicity of acetaminophen

Humans in vivo

There are six publications reporting on genotoxicity studies of acetaminophen, conducted in different European populations (See Table 17). All the studies measured genotoxicity endpoints in peripheral blood lymphocytes (PBL); in addition, one study assessed effects in buccal mucosa cells. Endpoints measured in these studies included CAs, SCEs, MN, and UDS. In all but one set of studies individuals served as their own controls, with markers of genotoxicity assessed before and after treatment with acetaminophen. The study by Kirkland et al. (1992) used an age- and gender-matched placebo group as the comparator to the acetaminophen-treated group.

Overall evidence of acetaminophen genotoxicity in humans *in vivo* includes some positive clastogenic effects (*i.e.*, CAs, SCE, and micronuclei formation) and one assay showing a decrease in UDS (Table 17).

As shown in Table 17, the ability of acetaminophen to induce CAs was assessed in PBLs of exposed humans in four studies, and the results were positive in two studies (Hongslo et al. 1991; Kocisova et al. 1988) and negative in the other two studies (Hantson et al. 1996; Kirkland et al. 1992). Acetaminophen induced SCEs in PBL in one study (Hongslo et al. 1991) and had no effect in another study (Kirkland et al. 1992). It is possible that Kirkland et al. (1992) had a reduced ability to detect acetaminophen-related effects on PBL CAs and SCEs due to inter-individual variability between the placebo and acetaminophen- treated groups in “baseline” levels of these markers of clastogenicity.

Additionally, acetaminophen was shown to induce MN in human PBLs (Kocisova and Sram 1990) and buccal mucosa cells (Kocisova and Sram 1990; Topinka et al. 1989). Topinka et al. (1989) also reported that acetaminophen decreased UDS in PBLs. These authors noted that acetaminophen has been shown to interfere with nucleotide excision repair in several mammalian cell types (Brunborg et al. 1995; Hongslo et al. 1993), and suggested that the decrease in UDS observed following acetaminophen treatment was the result of reduced DNA excision repair activity.

Table 17. Genotoxicity studies of acetaminophen in humans

Endpoint	Tissue	Cell type (if specified)	Description of exposed and controls, *e.g.*, doses, regimens, number of subjects, and study location	Response (p-value)*	Reference
Chromosome aberrations	Blood	Peripheral lymphocytes	The study included three groups. The first consisted of 5 healthy male volunteers, treated with a single oral dose of 3 g acetaminophen, the second consisted of 5 patients that received *i.v.* infusions of 1 g acetaminophen every 6 h for 7 d, the third consisted of 5 self-poisoned female patients that had ingested variable doses of acetaminophen, ranging from 10 g to an unknown amount that was much greater than 15 g. Blood was drawn from each individual in groups 1 and 2 before the start of acetaminophen treatment, as well as from individuals in group 1 at 24, 72 and 168 h after the single dose of acetaminophen, from individuals in group 2 at 168 h after the first acetaminophen infusion, and from individuals in group 3 after hospital admission. The study was conducted in Louvain, Belgium.	-	Hantson et al. (1996)
			24 non-smoking volunteers aged 29-45 (mainly among the staff of University of York and their relatives) were assigned to either a placebo or and a treated group, with 12 subjects in each group (age- and gender-matched). Individuals in the treated group ingested 3 g of acetaminophen over 8 h (once every 4 h), while individuals in the placebo group ingested placebo pills on the same schedule as the treated group. Blood was drawn from each volunteer after the final dose was administered. The study was conducted in York, Great Britain.	-	Kirkland et al. (1992)
			10 healthy volunteers ingested 3 g of acetaminophen over 8 h (once every 4 h). Blood was taken before (as a control) and 24 h after the first dose. The study was conducted in Oslo, Norway.	↑ ($p < 0.1$)	Hongslo et al. (1991)
			11 healthy volunteers (8 women and 3 men with a mean age of 37.7± 9.7 yrs) ingested 3 g of acetaminophen over 8 h (once every 4 h). Blood was taken before (as a control) and 24, 72 and 168 h after the first dose. The study was conducted in Prague, Czechoslovakia.	[a]↑ ($p < 0.05$)	Kocisova et al. (1988)
Sister chromatid exchanges	Blood	Peripheral lymphocytes	See study description above, for the chromosome aberrations endpoint.	-	Kirkland et al. (1992)
			See study description above, for the chromosome aberrations endpoint.	↑ ($p < 0.05$)	Hongslo et al. (1991)
Micronuclei	Blood	Peripheral lymphocytes	12 healthy volunteers (9 women and 3 men with a mean age of 37.8± 8.7 yrs) ingested 3 g of acetaminophen over 8 h (once every 4 h). Blood was taken before (as a control) and 24, 72, 168 h after the first dose. The study was conducted in Prague, Czechoslovakia.	[a](↑)	Kocisova and Sram (1990)
	Oral cavity	Buccal mucosa cells	11 healthy volunteers (8 women and 3 men with a mean age of 37.7± 6.1 yrs) ingested 3 g of acetaminophen over 8 h (once every 4 h). Buccal mucosa cells were collected before (as a control) and 24, 72, 168 h after the first dose. The study was conducted in Prague, Czechoslovakia.	[b]↑ ($p < 0.01$)	Topinka et al. (1989)
Unscheduled DNA synthesis	Blood	Peripheral lymphocytes	See study description above, for the micronuclei endpoint.	↓ ($p < 0.01$)	

↑, increased response; (↑), weak increased response; –, no effect; ↓, decreased response; * student's paired *t*-test; [a] evident only at 24 h; [b] evident only at 72 h

Human cells in vitro

As summarized in Table 18, acetaminophen at concentrations ranging from 0.05 to 10 mM has been tested with or without metabolic activation for several genotoxic endpoints related to DNA damage and chromosomal effects in multiple human cell types *in vitro*.

Overall, the evidence of acetaminophen genotoxicity in human cells *in vitro* consists of findings of DNA damage, including increases in DNA strand breaks detected by two different methods (comet assay, phosphorylated histone 2AX [γ-H2AX] staining), one assay showing DNA adduct formation, and another assay showing a slight increase in UDS; findings of impairment of DNA repair; and findings of chromosomal damage, including increases in MN, CAs and SCEs.

Acetaminophen induced DNA strand breaks in a human hepatocellular carcinoma cell line, as measured by the comet assay and by γ-H2AX staining (Bandi et al. 2014), and in liver slices, as measured by the comet assay (Jetten et al. 2014). Acetaminophen did not induce DNA single strand breaks in cultured human skin fibroblasts in the presence of sheep seminal vesicle microsomes (Andersson et al. 1982). In studies with human granulocytes stimulated to undergo the "respiratory burst" by treatment with phorbol myristate acetate (PMA) for 30 minutes, ^{14}C-labelled acetaminophen was incorporated into cellular DNA and RNA, indicating the formation of DNA and RNA adducts (Corbett et al. 1989). In one study in human PBLs, incubation with acetaminophen resulted in a slight increase in UDS (Binkova et al. 1990).

Acetaminophen inhibits ribonucleotide reductase activity (Hongslo et al. 1991), and so its ability to impair nucleotide excision repair in human cells was investigated by Hongslo et al. (1993) and Brunborg et al. (1995). In several different human cell types, acetaminophen was found to delay the repair of single strand DNA breaks (SSBs) induced by treatment with either UV light (mononuclear blood cells, T lymphocytes, B lymphocytes, monocytes, HL-60 cells, fibroblasts) or 4-nitroquinoline n-oxide (NQO) (mononuclear blood cells). In these studies, the effect of acetaminophen on the repair of SSBs was abrogated by the addition of deoxyribonucleotides to the cell medium. Hongslo et al. (1993) and Brunborg et al. (1995) concluded that acetaminophen's ability to delay the repair of SSBs in these studies was the result of impaired nucleotide excision repair due to acetaminophen's inhibition of ribonucleotide reductase.

Chromosomal effects of acetaminophen in human cells exposed *in vitro* have been observed at concentrations ranging from 1 to 1.5 mM. These effects include induction of MN in human amniotic fluid cells (Simko et al. 1998), weak induction of MN in human PBLs (Ibrulj et al. 2007), and induction of CAs and SCEs in human PBLs (Hongslo et al. 1991; Ibrulj et al. 2007; Watanabe 1982).

Table 18. Genotoxicity studies of acetaminophen in human cells *in vitro*

Test endpoint	Cell type or tissue	Concentration (LEC or HIC)	Results without S-9	Results with S-9	Reference
DNA strand breaks [Alkaline elution]	Cultured skin fibroblasts	0.05 mM	NT	- (SSV)	Andersson et al. (1982)
DNA strand breaks [Comet assay]	Liver slices	[a]2.31 mM	+	NT	Jetten et al. (2014)
DNA strand breaks [Comet assay and γ-H2AX staining]	Hepatocellular carcinoma cell line (HuH-7 cells)	10 mM	+	NT	Bandi et al. (2014)
DNA adducts	Granulocytes	10 µM (+PMA)[b]	+	NT	Corbett et al. (1989)
Impairment of nucleotide excision repair	UV-pretreated Mononuclear blood cells	0.1 mM	+	NT	Hongslo et al. (1993)
	UV-pretreated T lymphocytes	0.3 mM	+	NT	
	UV-pretreated B lymphocytes	0.3 mM	+	NT	
	UV-pretreated Monocytes	0.3 mM	(+)	NT	
	UV or 3 mM NQO-pretreated mononuclear blood cells	0.3 mM	+	NT	Brunborg et al. (1995)
	UV-pretreated HL-60 cells	0.3 mM	+	NT	
	UV-pretreated fibroblast cells	0.3 mM	(+)	NT	
Unscheduled DNA synthesis	Peripheral blood lymphocytes	0.05 mM	(+)	NT	Binkova et al. (1990)
Micronuclei	Amniotic fluid cells	1.3 mM	+	NT	Simko et al. (1998)
	Peripheral blood lymphocytes	1.3 mM	(+)	NT	Ibrulj et al. (2007)
Chromosomal aberrations	Peripheral blood lymphocytes	1.3 mM	+	NT	Watanabe (1982)
	Peripheral blood lymphocytes	1.5 mM	+	NT	Hongslo et al. (1991)
	Peripheral blood lymphocytes	1.3 mM	+	NT	Ibrulj et al. (2007)
Sister chromatid exchanges	Peripheral blood lymphocytes	1 mM	+	NT	Hongslo et al. (1991)

+, positive; –, negative; (+), weakly positive; HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested; SSV: sheep seminal vesicle microsomes; NQO: 4-Nitroquinoline N-oxide.
[a]The authors did not report the doses used, instead reporting the "average BMD" observed among liver slices from five individuals; BMDs varied by 64-fold between individuals.
[b]With treatment of granulocytes with phorbol myristate acetate (PMA), which stimulates the respiratory burst.

Animals in vivo

The genotoxicity of acetaminophen has been tested in a number of studies in rats and mice of multiple strains through multiple routes (Table 19). Tissues analyzed for genotoxicity endpoints in these studies include liver, kidney, spleen, bone marrow, peripheral blood, testes and embryos.

Overall, acetaminophen has been shown to form DNA adducts, induce DNA strand breaks, impair nucleotide excision repair, and increase multiple types of chromosomal damage, *i.e.* MN, CAs, SCEs, and aneuploidy in animal studies *in vivo*. Acetaminophen was not found to induce mutations in reporter genes in the livers of transgenic rats (Kanki et al. 2005; Matsushita et al. 2013; Suzuki et al. 2016) or to induce Pig-a gene mutations expressed in red blood cells of rats exposed via the oral route (Suzuki et al. 2016).

Acetaminophen was found to form DNA adducts in liver and kidney of mice exposed via *i.p*. injection in two studies (Hongslo et al. 1994; Rogers et al. 1997), and a third *i.p.* study in mice also reported DNA adduct formation in liver (Dybing et al. 1984). No DNA adducts were detected in two studies in rats exposed via the oral route (Dybing et al. 1984; Hasegawa et al. 1988; Hongslo and Holme 1994; Rogers et al. 1997; Williams et al. 2007). An increase in serum levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG, or 8-oxodG), a marker for oxidative DNA damage, was observed in Kunming mice administered acetaminophen by the oral route for 10 weeks (Wang et al. 2015).

DNA strand breaks were detected in the livers of acetaminophen-treated male B6 mice and ICR mice after single *i.p.* injections of 600 mg/kg bw or 300 mg/kg bw acetaminophen, respectively (Hongslo et al. 1994; Oshida et al. 2008). DNA strand breaks were not detected in the kidney or bone marrow in these studies (Hongslo et al. 1994; Oshida et al. 2008). In addition, DNA strand breaks were not detected in the liver or kidney of acetaminophen-treated male Wistar rats after a single i.p. injection of 600 mg/kg bw acetaminophen (Hongslo et al. 1994).

Acetaminophen can cause impairment of nucleotide excision repair in rodents *in vivo*. Hongslo et al. (1994) showed that NQO-induced DNA-repair synthesis was decreased in the liver, spleen, and kidney of male B6 mice and Wistar rats exposed to acetaminophen via *i.p.* injection 5 minutes before treatment with NQO (mice, 50 mg/kg; rats, 20 mg/kg). Similar to what was observed in *in vitro* studies with human cells (Table 18); acetaminophen increased SSBs and delayed the repair of SSBs in livers, spleens and kidneys of NQO-treated mice and rats. The authors concluded that these effects were the result of impaired nucleotide excision repair due to acetaminophen's inhibition of ribonucleotide reductase.

In mouse studies, acetaminophen tested positive in several chromosomal damage assays (*e.g*., MN, CAs and SCEs) in two strains, BALB/c and Swiss, via multiple administration routes. Increases in MN were observed in the peripheral blood cells of BALB/c mice exposed to acetaminophen via *i.p.* injection or *in utero* (Markovic et al. 2013). Increases in MN were also observed in the bone marrow cells of Swiss mice exposed to acetaminophen via *i.p.* injection (Sicardi et al. 1991). In studies of NMRI mice administered acetaminophen via gavage or *i.p.* injection, no increase in MN was observed in the bone marrow (King et al. 1979). Increases in CAs were observed by three different research groups in the bone marrow of Swiss mice treated with acetaminophen either orally or via *i.p.* injection (Giri et al. 1992; Reddy 1984; Severin and Beleuta 1995). The CAs induced by acetaminophen in mouse bone marrow included gaps, chromatid breaks, acentric fragments, and polyploid metaphases. These types of structural CAs were not statistically significantly increased in the testes of Swiss mice exposed to acetaminophen via the oral route, although other chromosomal abnormalities were observed in the testes, such as polyploidy (Reddy and Subramanyam 1985). A dose-dependent increase in SCEs was observed in the bone marrow of Swiss mice treated with acetaminophen via *i.p.* injection (Giri et al. 1992).

Chromosomal damage has also been observed in rats exposed to acetaminophen. In one oral study acetaminophen increased MN formation in the bone marrow of treated rats (Hazleton Microtest 1993, as cited by Muller and Kasper 1995). In another oral study, administration of acetaminophen to female SD rats for two weeks prior to mating and continuing through the first 11 days after mating resulted in an increase in chromosomal aneuploidy in the embryos of exposed rats, compared to controls (Muller and Kasper 1995; Tsuruzaki et al. 1982). Tsuruzaki et al. (1982) reported that the chromosomal karyotypes of the affected embryos were all mosaics, consisting of monosomy/normal or trisomy/normal cells.

Table 19. Genotoxicity studies of acetaminophen in animals *in vivo*

Endpoint	Species, strain, sex	Tissue analyzed	Dose (LED or HID)	Route, duration, dosing regimen	Results	Reference
Mutation frequency [guanine phosphoribosyl-transferase (gpt) reporter gene]	Rat, gpt delta transgenic Sprague-Dawley, Female	Liver	10,000 ppm	Diet; 13 weeks	-	Kanki et al. (2005)
	Rat, gpt delta transgenic F344/NSlc, Male	Liver	6000 ppm	Diet; 14 weeks	-	Matsushita et al. (2013)
Mutation frequency [Pig-a gene][a]	Rat, Sprague-Dawley, Male	Red blood cells	2000 mg/kg	Gavage; one single dose	-	Suzuki et al. (2016)
DNA adducts	Mouse, B6, Male	Liver	500 mg/kg	*i.p.*; one single injection	(+)	Dybing et al. (1984)
	Rat, F344, Male	Liver	1.5% (diet) or 1 g/kg bw (gavage)	Diet for 1 week or a single gavage dose	-	Hasegawa et al. (1988)
	Mouse, ICR, Male	Liver	300 mg/kg	*i.p.*; one single injection	+	Hongslo et al. (1994)
		Kidney			+	
	Mouse, ICR, Male	Liver	10 mg/kg	*i.p.*; one single injection	+	Rogers et al. (1997)
		Kidney			+	
	Rat, F344, Male	Liver	288 mg/kg	Diet; 9 weeks	-	Williams et al. (2007)
Oxidation of DNA (8-OHdG)[b]	Mouse, Kunming, Male	Serum	400 mg/kg	Gavage; two doses daily for 10 wks	+	Wang et al. (2015)
DNA strand breaks (single strand breaks) [Alkaline elution]	Mouse, B6, Male	Liver	600 mg/kg	*i.p.*; one single injection	+	Hongslo et al. (1994)
		Kidney			-	
	Rat, Wistar, Male	Liver	600 mg/kg	*i.p.*; one single injection	-	Hongslo et al. (1994)
		Kidney			-	
DNA strand break [Comet assay]	Mouse, Crj:CD1(ICR), Male	Bone marrow	300 mg/kg	*i.p.*; one single injection.	-	Oshida et al. (2008)
		Liver			+	
		Kidney			-	
Impairment of nucleotide excision repair	NQO-treated Mouse, B6, Male	Liver	300 mg/kg	*i.p.*; one single injection	+	Hongslo et al. (1994)
		Kidney			+	
		Spleen			+	
	NQO-treated Rat, Wistar, Male	Liver	300 mg/kg	*i.p.*; one single injection	+	
		Kidney			+	
		Spleen			+	
Micronuclei	Mouse, NMRI, Male and female	Bone marrow	3 mmoles/kg or 453 mg/kg	*i.p.* or gavage; 2 doses 24 h apart	-	King et al. (1979)
	Mouse, Swiss, Male and female	Bone marrow	100 mg/kg	*i.p.;* one single injection	+	Sicardi et al. (1991)

Endpoint	Species, strain, sex	Tissue analyzed	Dose (LED or HID)	Route, duration, dosing regimen	Results	Reference
	Rat, no strain specified, NR	Bone marrow	900 mg/kg	Oral; 3 doses within 8 h	(+)	Hazleton Microtest (1993), as cited by Muller and Kasper (1995)
	Mouse, BALB/c, Female	Peripheral blood	60 mg/kg per day	*i.p.*; one per day; 3 consecutive days between days 12 and 14 of pregnancy	(+)	Markovic et al. (2013)
	Mouse, BALB/c, offspring gender is not reported	Peripheral blood	60 mg/kg per day	Transplacental; *i.p.* one per day; 3 consecutive days between days 12 and 14 of pregnancy to dams	+	Markovic et al. (2013)
Aneuploidy	Rat, Sprague-Dawley, Female	Embryos	500 mg/kg per day	Gavage to dams for 2 wks before mating and continued for 11 days after	+	Tsuruzaki et al. (1982)
Chromosomal aberrations	Mouse, Swiss, Male	Bone marrow	2.5 mg (single dose series) or 0.625 mg/day (cumulative dose series)	Oral; single dose or 3 doses 24 h apart (cumulative)	+	Reddy (1984)
	Mouse, Swiss albino, Male	Testes	2.5 mg	Oral; single dose or 3 doses 24 h apart (cumulative)	-	Reddy and Subramanyam (1985)[c]
	Mouse, Swiss albino, Male and female	Bone marrow	200 mg/kg	*i.p.*; one single injection	+	Giri et al. (1992)
	Mouse, Swiss, Male	Bone marrow	800 mg/kg (oral) or 100 mg/kg (*i.p.*)	Oral or *i.p.*; oral 3 times during 8 h or one single *i.p.* injection	+	Severin and Beleuta (1995)
Sister Chromatid Exchange	Mouse, Swiss albino, Male and female	Bone marrow	50 mg/kg	*i.p.*; one single injection	+	Giri et al. (1992)

+, positive; –, negative; (+), weakly positive; HID, highest ineffective dose; LED, lowest effective dose (units as reported); NT, not tested; NR, not reported; *i.p.*, intraperitoneal injection; NQO: 4-nitroquinoline N-oxide

[a] Pig-a gene encodes a catalytic subunit of the N-acetylglucosamine transferase that is involved in the synthesis of glycosylphosphatidylinositol (GPI), which serves as an anchor of specific protein markers on the surface of red blood cells. The *Pig-a* assay detects loss of GPI-anchored protein markers (CD59 in this study) as a measure of mutation frequencies in the *Pig-a* gene.

[b] 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a biomarker of oxidative DNA damage, and can be further converted to 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-oxodG) (Lin et al. 2018). Both 8-OHdG and 8-oxodG are used in scientific studies to represent the same type of DNA damage.

[c] This study also reported induction of polyploidy with both single and cumulative doses.

Animals in vitro

As shown in Table 20, acetaminophen at concentrations ranging from 0.03 to 33.1 mM has been tested with or without metabolic activation for several genotoxic endpoints related to gene mutation, DNA damage and chromosomal effects in multiple rodent cell types *in vitro*.

Overall, acetaminophen has been shown using *in vitro* systems to induce gene mutations in hamster and mouse cells; DNA damage (*e.g*., oxidation of DNA, DNA strand breaks, UDS) in mouse, rat, and hamster cells; impairment of DNA repair in rat and hamster cells; and chromosomal damage (*e.g*., MN, CAs, SCEs) in mouse, rat, and hamster cells.

Acetaminophen increased gene mutations in mouse lymphoma cells (Muller and Kasper 1995; Sasaki 1986; Shimane 1985), and induced small, dose-dependent increases in mutations associated with ouabain and 6-thioguanine (6TG) resistance in Chinese hamster lung V79 cells (Shimane 1985). Acetaminophen did not induce mutations in Chinese hamster ovary K1 (CHO-K1) cells (Sasaki 1986) or C3H/10$T_{1/2}$ Clone 8 mouse embryo cells (Patierno et al. 1989).

Acetaminophen produced oxidative damage in DNA, measured as 8-oxodG, in rat C6 glioma cells (Wan et al. 2004). DNA single strand breaks were slightly increased by acetaminophen in hamster lung V79 cells (Hongslo et al. 1988) and in CHO-K1 cells (Sasaki 1986), but not in a study conducted in rat hepatoma cells (Dybing et al. 1984).

Acetaminophen has been shown in several studies to alter UDS in rodent cells *in vitro*. Acetaminophen was found to increase UDS in six assays tested in mouse or rat hepatocytes (Dybing et al. 1984; Holme and Soderlund 1986), to decrease UDS in rat, hamster, and guinea pig hepatocytes and in hamster lung cells, and to have no effect in one study of rat primary hepatocytes. UDS assays measure DNA repair synthesis, and as discussed by Madle et al. (1994), the results of UDS assays can be impacted by several factors, including detection methods (autoradiography vs. liquid scintillation), specificity of the blockade of replicative DNA synthesis, metabolic capacity of the test system (determined by genetic and environmental factors), and the presence of solvents (DMSO has been shown to affect Cyp2e1 activity).

Acetaminophen can cause impairment of nucleotide excision repair in rodent cells *in vitro*. Hongslo et al. (1988) showed that UV-induced DNA-repair synthesis was decreased in hamster lung cells exposed to 0.1 mM acetaminophen and completed blocked at concentrations greater than 1 mM, as a result of the inhibition of nucleotide excision repair. Similar to what was observed in *in vitro* studies with human cells (Table 18) and *in vivo* studies in mice and rats (Table 19), acetaminophen increased SSBs after UV pre-treatment in rat hepatocytes and in NQO-treated rat testicular cells

(Brunborg et al. 1995). Brunborg et al. (1995) concluded that acetaminophen's ability to delay the repair of SSBs was the result of impaired nucleotide excision repair due to acetaminophen's inhibition of ribonucleotide reductase.

In addition, Wan et al. (2004) reported that acetaminophen significantly impaired the DNA incision activity of 8-oxoguanine DNA glycosylase/AP lyase (Ogg1), a DNA repair enzyme specific for 8-oxodG, in the nuclei of rat glioma cells.

Chromosomal effects of acetaminophen in mouse, rat, and hamster cells exposed *in vitro* have been observed at concentrations ranging from 0.1 mM to >1 mM, with numerous positive findings observed between 0.03 – 0.5 mM. Among twenty-four chromosomal damage assays, acetaminophen increased either MN, CAs or SCEs in twenty-three. The one study that did not observe an effect was an assay for MN in rat primary hepatocytes (Muller-Tegethoff et al. 1995).

Table 20. Genotoxicity studies of acetaminophen in animals *in vitro*

Test endpoint	Species/ cell line	Concentration (LEC or HIC)	Results without S-9	Results with S-9	Reference
Mutation [Ouabain resistance]	Chinese hamster/ lung V79 cells	2.6 mM	(+)	NT	Shimane (1985)
Mutation [Ouabain resistance]	Chinese hamster/ ovary cells (CHO-K1)	0.66 mM	-	NT	Sasaki (1986)
Mutation [Ouabain resistance]	Mouse/ embryo cells, C3H/10T$_{1/2}$ Clone 8	13.2 mM (2 mg/ml)	-	NT	Patierno et al. (1989)
Mutation [6TG resistance]	Chinese hamster/ lung V79 cells	2.6 mM	(+)	NT	Shimane (1985)
Mutation [Tk locus gene]	Mouse/ lymphoma cells	3.3 mM	+	NT	Hazleton Microtest (1992), cited Muller and Kasper (1995)
Oxidation of DNA (8-oxodG)[a]	Rat/ C6 glioma cells	2.5 mM	+	NT	Wan et al. (2004)
DNA strand breaks [Alkaline elution]	Rat/ Reuber hepatoma cells	10 mM	-	NT	Dybing et al. (1984)
	Hamster/ ovary cells (CHO-K1)	33 mM	(+)	NT	Sasaki (1986)
	Hamster/ lung V79 cells	1 mM	(+)	NT	Hongslo et al. (1988)
Unscheduled DNA synthesis	Mouse/ monolayers of primary hepatocytes	5 mM	↑	NT	Dybing et al. (1984)
		2.5 mM (PB-pretreated)[b]	↑	NT	
	Mouse/ monolayers of primary hepatocytes	5 mM	↑	NT	Holme and Soderlund (1986)
		2.5 mM (MC-pretreated)[c]	↑	NT	
		0.5 mM (PCB-pretreated)[c]	↑	NT	
	Rat/ monolayer of primary hepatocytes	7.5 mM	(↑)	NT	Holme and Soderlund (1986)
	Rat/ primary hepatocyte cultures	1.66 mM	↓	NT	Sasaki (1986)
	Rat/ primary hepatocyte cultures	7 mM	No effect	NT	Milam and Byard (1985)
	Hamster / monolayer of primary hepatocytes	5 mM	↓	NT	Holme and Soderlund (1986)
	Hamster/ lung V79 cells	3 mM	↓	NT	Hongslo et al. (1988)
	Guinea pig/ monolayer of primary hepatocytes	7.5 mM	↓	NT	Holme and Soderlund (1986)

Test endpoint	Species/ cell line	Concentration (LEC or HIC)	Results without S-9	Results with S-9	Reference
Impairment of nucleotide excision repair	UV-pretreated Hamster/ lung V79 cells	0.1 mM	+	NT	Hongslo et al. (1988)
	UV-pretreated rat/ hepatocytes	0.3 mM	+	NT	Brunborg et al. (1995)
	NQO-treated rat/ testicular cells	1 mM	+	NT	
Impairment of DNA repair [measured as 8-oxodG incision activity]	Rat/ C6 glioma cells	2.5 mM	+	NT	Wan et al. (2004)[d]
Micronuclei	Rat/ kidney fibroblast cell line, NRK-49F	10 mM	+	NT	Dunn et al. (1987)
	Rat/ primary hepatocytes	1 mM	-	NT	Muller-Tegethoff et al. (1995)
	Chinese hamster/ lung CHL/IU cells	0.1 mM	+	NT	Matsushima et al. (1999)
Chromosomal aberrations	Chinese hamster/ lung fibroblast cells	0.4 mM	+	NT	Ishidate et al. (1978)
	Chinese hamster/ Don-6 (lung) cells	0.5 mM	+	NT	Sasaki et al. (1980)
	Chinese hamster/ ovary cells (CHO-K1)	0.46 mM	+	NT	Sasaki et al. (1983)
	Chinese hamster/ lung V79 cells	0.66 mM	+	+[e]	Shimane (1985)
	Chinese hamster/ ovary cells (CHO-K1)	0.47 mM	+	NT	Sasaki (1986)
	Chinese hamster/ lung cells	0.2 mM	+	NT	Ishidate et al. (1988)
	Mouse/ mammary tumor TA_3H cells	1 mM	(+)	NT	Hongslo et al. (1990)
	Hamster/ lung V79 cells	0.32 mM (-S9, 6 hr); 3.2 mM (+S9, 2 hr)	+	+	Muller et al. (1991)
	Chinese hamster/ ovary cells (CHO)	8.3 mM (-S9, 8-10 hr), 33.1 mM (+S9, 2 hr)	+	(+)	NTP (1993)
Sister Chromatid Exchange	Chinese hamster/ lung V79 cells	0.66 mM	+	+[e]	Shimane (1985)
	Chinese hamster/ ovary cells (CHO-K1)	0.33 mM	+	NT	Sasaki (1986)
	Chinese hamster/ lung V79 cells	1 mM	+	NT	Holme et al. (1988)
	Chinese hamster/ lung V79 cells	3 mM, 10 mM (+hepatocytes)	+	+[e]	Hongslo et al. (1988)

Test endpoint	Species/ cell line	Concentration (LEC or HIC)	Results without S-9	Results with S-9	Reference
	Mouse/ mammary tumor TA_3H cells	1 mM	+	NT	Hongslo et al. (1990)
	Chinese hamster/ ovary cells (CHO)	0.03 mM (-S9, 26 hr), 33.1 mM (+S9, 2 hr)	+	(+)	NTP (1993)

HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested

+, positive; –, negative; (+), weakly positive; ↑, significantly increased; (↑), slightly increased; ↓, significantly reduced

6TG: 6-Thioguanine; 8-oxodG: 8-oxo-Deoxyguanosine; NQO: 4-nitroquinoline N-oxide

[a] 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a biomarker of oxidative DNA damage, and can be further converted to 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-oxodG) (Lin et al. 2018). Both 8-OHdG and 8-oxodG are used in scientific studies to represent the same type of DNA damage.

[b] For induction purposes, phenobarbital (PB) 75 mg/kg in saline was given *i.p.* to mice 72, 48 and 24 h before isolation of liver cells.

[c] Mice were pretreated in the following way: single *i.p.* injection of 80 mg/kg 3-methylcholanthrene (MC) in corn oil 40 h before isolation of liver cells or single *i.p.* injection of 500 mg/kg polychlorinated biphenyls (Aroclor 1254; PCB) in corn oil 5 days before isolation of liver cells.

[d] 8-Oxoguanine DNA glycosylase (Ogg1) exhibits DNA glycosylase/AP lyase activity, which removes 8-oxodG from damaged DNA by both base excision repair and nucleotide incision repair. Nuclear extracts were prepared from C6 glioma cells exposed to DMSO or different concentrations of acetaminophen for 24 h or 48 h. The Ogg1 incision activity assays were conducted with DMSO or acetaminophen-pretreated nuclear extracts and radiolabbelled-oligonucleotides. In addition, Wan et al. (2004) found the Ogg1 protein content was decreased by acetaminophen in rat glioma cells. The authors mentioned that C6 glioma cells contain catalytically active CYP enzymes such as Cyp2e1 and Cyp1a2.

[e] Co-cultured with rat liver cells.

Non-mammalian species and acellular systems

Acetaminophen has been tested in multiple non-mammalian species, including zebra mussels, insects, plants, yeasts, and bacteria, and DNA adduct formation has been investigated in acellular systems.

Studies conducted in mussels, insects, plants, yeasts, and acellular systems are summarized in Table 21.

DNA strand breaks were found in *Dreissena polymorpha*, a freshwater zebra mussel, treated with acetaminophen at concentrations as low as 5 nM for 24- 96 hours. At 96 hours acetaminophen also induced MN formation in this model (Parolini et al. 2010).

Acetaminophen did not induce sex-linked recessive lethal (SLRL) mutations in a study in *Drosophila* (King et al. 1979), or intra-chromosomal recombination in *Saccharomyces cerevisiae* (Brennan and Schiestl (1997).

Reddy and Subramanyam (1981) reported that acetaminophen induced CAs in onion roots treated at room temperature for 2, 6, 12, 18, 24, 48 , or 72 hours.

Using cell-free systems, Rogers et al. (1997) reported the binding of [^{3}H]-acetaminophen to calf thymus DNA, either in the presence of horseradish peroxide

(HRP) and hydrogen peroxide (H_2O_2), or in the presence of rat liver microsomes. The level of DNA binding observed with the HRP-H_2O_2 system was 200-fold greater than that observed with rat liver microsomes. These results are consistent with the hypothesis that peroxidase-mediated metabolism of acetaminophen can produce DNA-reactive radical intermediates. Additionally, acetaminophen formed adducts with purified deoxyribonucleic acid (type I) in the presence, but not the absence of mouse liver microsomes (Dybing et al. 1984).

Plattner et al. (2012) reported the non-enzymatic formation of covalent adducts of acetaminophen to guanosine, as detected by electrochemistry/liquid chromatography /mass spectrometry. These investigators observed that the first step of adduct formation involved the conversion of both guanosine and acetaminophen into radical forms via one-electron-one-proton reactions, and showed that these radicals reacted with each other to form four different guanosine-acetaminophen-2H isomers.

Acetaminophen has been tested in the presence and absence of metabolic activation (*e.g.*, S9) in *Salmonella typhimurium* reverse mutation assays in multiple strains (TA 97, TA 98, TA 100, TA 102, TA 1535, TA 1537 and TA 1538) and in *Escherichia coli* reverse mutation assays in three different strains.

As shown in Table 22, acetaminophen tested negative in each of the reverse mutation assays conducted in *S. typhimurium* and *E. coli*.

Table 21. Genotoxicity studies of acetaminophen in mussels, insects, plants, yeasts and acellular systems

Test endpoint	Test system	Concentration (LEC or HIC)	Results	Reference
		Mussels		
DNA strand breaks [Comet assay]	*Dreissena polymorpha*, zebra mussel	5 nM	+	Parolini et al. (2010)
Micronuclei	*Dreissena polymorpha*, zebra mussel	5 nM	+	Parolini et al. (2010)
		Insects		
Mutations [Sex-linked recessive lethal]	*Drosophila melanogaster*, Berlin K (wild-type) males and Basc females	40 mM (6040 µg/ml)	-	King et al. (1979)
		Plants		
Chromosomal aberrations	*Allium cepa* roots	0.5 % (5 mg/ml or 33 mM)	+	Reddy and Subramanyam (1981)
		Yeast		
Chromosomal damage [DEL recombination assays]	*Saccharomyces cerevisiae* RS112	19.6 mg/ml (130 mM)	-	(Brennan and Schiestl (1997)
		Acellular systems		
DNA adducts	Deoxyribonucleic acid, mouse liver microsomes	0.5 mM (1.7 x 10^4 cpm/nmole) [^{3}H]acetaminophen	(+)	Dybing et al. (1984)
	Calf thymus DNA, HRP, H_2O_2	100 µM (2000 dpm/pmol) [^{3}H]acetaminophen	+	Rogers et al. (1997)
	Calf thymus DNA, rat liver microsomes		+	
	Guanosine	25 µM	+	Plattner et al. (2012)

+, positive; –, negative; (+), weakly positive; HIC, highest ineffective concentration; LEC, lowest effective concentration

HRP, horseradish peroxide

Table 22. Genotoxicity studies of acetaminophen in bacteria

Endpoint	Test system (species, strain)	Assay/Test	Results without S-9	Results with S-9	Concentration (LEC or HIC)	Reference
DNA Mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Reverse mutation	-	-	3,624 µg/plate	King et al. (1979)
	Salmonella typhimurium TA98, TA100		-	-	1,000 µg/plate	Wirth et al. (1980)
	Salmonella typhimurium TA98, TA100		-	-	755 µg/plate	Camus et al. (1982)
	Salmonella typhimurium TA98, TA100, TA1535, TA1537		-	-	10,000 µg/plate	Haworth et al. (1983)
	Salmonella typhimurium TA100		NT	-	5,000 µg/plate	Imamura et al. (1983)
	Salmonella typhimurium TA98, TA100, TA102		NT	-	3,020 µg/plate	Dybing et al. (1984)
	Salmonella typhimurium TA98, TA100, TA 1535, TA1537, TA1538		-	-	5,000 µg/plate	Oldham et al. (1986)
	Salmonella typhimurium TA97, TA98, TA100, TA 1535, TA1537, TA1538		-	-	10,000 µg/plate	Jasiewicz and Richardson (1987)
	Salmonella typhimurium TA98, TA100, TA1535, TA1357		-	-	10,000 µg/plate	NTP (1993)
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538		NT	-	2,500 µg/plate	Burke et al. (1994)
	Escherichia coli K12/343/113	5-MT resistance, reverse mutation assay	-	-	4,530 µg/ml	King et al. (1979)
	Escherichia coli IC188; oxyR⁺ [parent WP2] uvrA/pKM101	Mutotoxitest, reverse mutation assay	-	NT	1500 µg/plate	Martinez et al. (2000)
	Escherichia coli IC203 deficient in oxyR [parent WP2]	Mutotoxitest, reverse mutation assay	-	NT	1500 µg/plate	Martinez et al. (2000)

–, negative; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; 5-MT, 5-methyltryptophan

IC203: a new *Escherichia coli* WP2 tester strain that is highly sensitive to reversion by oxidative mutagens.

3.3.3.2 Studies on the genotoxicity of acetaminophen metabolites

N-Acetyl-p-benzoquinone imine (NAPQI)

N-Acetyl-*p*-benzoquinone imine (NAPQI) is an electrophilic metabolite of acetaminophen (See Section 3.3.1 Pharmacokinetics and metabolism). Nucleic acids, peptides, and proteins are the major macromolecular targets of NAPQI (Bond 2009; Mazaleuskaya et al. 2015; Ramachandran and Jaeschke 2018).

NAPQI has been tested for genotoxicity in a limited number of assays, and the majority of genotoxicity endpoints evaluated in these studies are markers of DNA damage. Specifically, NAPQI has been tested in one *in vitro* assay for DNA strand breaks in human cells, in two *in vitro* assays in animal cells (one for DNA strand breaks and one for UDS), in one set of reverse mutation assays in bacteria, in multiple assays measuring DNA adducts in acellular systems, and in one acellular assay for DNA strand breaks (Table 23).

The findings from these studies include evidence of DNA strand breaks in human leukemia cells *in vitro* (Bender et al. 2004), rat hepatoma cells *in vitro* (Dybing et al. 1984), and purified bacteria plasmid DNA (Bender et al. 2004), and evidence of DNA adduct formation in multiple acellular systems (Dybing et al. 1984; Hasegawa et al. 1988; Klopcic et al. 2015; Rogers et al. 1997). NAPQI did not induce reverse mutations in *Salmonella* or UDS in mouse primary hepatocytes (Dybing et al. 1984).

Bender et al. (2004) reported that 50 µM and 100 µM NAPQI enhanced human topoisomerase IIα-mediated DNA cleavage, a measure of DNA double strand breaks, in human CEM cells exposed *in vitro*. Using a cell-free system, these authors demonstrated that NAPQI is a potent human topoisomerase IIα poison, and found that NAPQI increased the level of topoisomerase IIα-mediated DNA double strand breaks in purified bacteria plasmid DNA in a concentration-dependent manner at concentrations ranging from 10 - 100 µM. NAPQI also induced DNA strand breaks in rat hepatoma cells exposed *in vitro* at concentrations ranging from 0.05 to 0.25 mM (Dybing et al. 1984).

NAPQI did not increase UDS in cultured mouse primary hepatocytes exposed *in vitro*, and it did not induce mutations in the *Salmonella* reverse mutation assay in the two strains tested, TA 98 and TA 102, in the presence or absence of S-9 (Dybing et al. 1984).

In an acellular system, Hasegawa et al. (1988) detected by ^{32}P-postlabeling the formation of at least six different DNA adducts in isolated calf thymus DNA, following incubation with NAPQI. Using [*ring*-^{14}C]-labeled NAPQI, Rogers et al. (1997) also

demonstrated covalent binding of NAPQI to calf thymus DNA. Using [^{3}H] labeled NAPQI, these authors demonstrated the covalent binding of NAPQI to salmon testes DNA, mouse liver nuclei preparations, and mouse liver chromatin preparations, and observed a greater degree of adduct formation occurred in the mouse liver nuclei and chromatin preparations than in purified salmon testes DNA (Rogers et al. 1997). In a quantum chemical study of the reactivity of NAPQI with deoxyguanosine (dG), the formation of a C-N bond between the C3 atom of the quinone imine moiety and the N7 atom of dG with 26.7 kcal/mol estimated binding free energy was reported (Klopcic et al. 2015). The authors suggested that the C3-N7 DNA adduct could be formed under certain circumstances after acetaminophen treatment, such as conditions of long-term GSH depletion and low levels of other thiol proteins.

Table 23. Genotoxicity studies of NAPQI

Test	Tissue, cell line	Concentration (LEC or HIC)	Results without S-9	Results with S-9	Reference
Human cells *in vitro*					
DNA strand breaks [Topoisomerase IIα-DNA complex]	CEM acute lymphoblastic leukemia cells	50 µM	+	NT	Bender et al. (2004)
Animal cells *in vitro*					
DNA strand breaks [alkaline elution]	Rat Reuber H4-II-E hepatoma cells	0.05 mM	+	NT	Dybing et al. (1984)
Unscheduled DNA synthesis	Mouse primary hepatocytes	0.25 mM	-	NT	
Bacteria					
Reverse mutation	Salmonella typhimurium TA98	0.05 mM (+S9), 0.03 mM (-S9)	-	-	Dybing et al. (1984)
	Salmonella typhimurium TA102	0.1 mM	-	NT	Dybing et al. (1984)
Acellular systems					
DNA adducts	Calf thymus DNA	0.115 mg/0.5 ml	+	NT	Hasegawa et al. (1988)
	Calf thymus DNA with or without cysteine	[*ring*- ^{14}C] NAPQI 18 dpm/pmol	+	NT	Rogers et al. (1997)
	Salmon testes DNA	[^{3}H]NAPQI 500 dpm/pmol	+		
	Nuclei preparations from livers of male ICR mice		+		
	Chromatin preparations from livers of male ICR mice		+		
	Deoxyguanosine	Not Reported	+	NT	Klopcic et al. (2015)
DNA strand breaks [Topoisomerase IIα-DNA complex]	Purified topoisomerase IIα and negatively supercoiled pBR322 DNA	10 µM	+	NT	Bender et al. (2004)

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; +, positive; –, negative

PAP

PAP is a metabolite of acetaminophen and has been identified in rodents administered acetaminophen *in vivo* (Gemborys and Mudge 1981; Newton et al. 1982b; Newton et al. 1985) and rodent cells or organs incubated with acetaminophen *in vitro* (Miyakawa et al. 2015; Newton et al. 1982b) (See metabolism Section 3.1.1).

The genotoxicity of PAP has been reviewed by Bomhard and Herbold (2005), US EPA (2005), and EU-SCCS (2011). OEHHA reviewed these reports, as well as additional genotoxicity studies published since the US EPA and EU reviews.

PAP has been tested for a variety of genotoxic endpoints in human cells *in vitro,* animals *in vivo*, and animal cells *in vitro*, for reverse mutations in bacteria, for somatic and sex-linked mutations in *Drosophila*, and for chromosomal damage in plants and yeast.

Overall, the findings from these studies include evidence of DNA strand breaks, DNA adducts, CAs, and SCEs in human cells *in vitro* (Table 24), germ-cell mutations, MN, and CAs in animals *in vivo* (Table 25), forward mutations, DNA strand breaks, CAs, and SCEs in animal cells *in vitro* (Table 26), somatic mutations in *Drosophila,* chromosomal damage in plants and yeast, and reverse mutations in *E. coli* (Table 27).

Human cells *in vitro* (Table 24)

As shown in Table 24, PAP increased DNA strand breaks in a dose-dependent manner up to 50 µM in cultured human skin fibroblasts incubated with sheep seminal vesicle microsomes (Andersson et al. 1982). In human lymphoblastic cells, PAP increased DNA single strand breaks at concentrations ranging from 0.05 mM to 5.0 mM in the absence of S-9 (Hayward et al. 1982). In studies with human granulocytes, PAP formed adducts with DNA and RNA, as indicated by incorporation of ^{14}C-labelled PAP into cellular DNA and RNA (Corbett et al. 1989). Stimulation of the cells to undergo the "respiratory burst" by treatment with phorbol myristate acetate (PMA) for 30 minutes significantly enhanced DNA binding by PAP.

In a study using lymphocytes drawn from one male volunteer, PAP induced CAs in both the presence and absence of Aroclor 1254-induced rat liver S-9 fraction from rats (EU-SCCS 2011). The effect of PAP on SCEs was tested in human peripheral lymphocytes in two studies, with positive findings observed in one (Takehisa and Kanaya 1982) and equivocal findings in the other (Kirchner and Bayer 1982), and in human fibroblasts in one study, with negative findings (Wilmer and Natarajan 1981).

Animals *in vivo* (Table 25)

In treated mice, PAP induced sperm head abnormalities (Topham 1980), and was negative in a dominant lethal test in rats (Burnett et al. 1989).

In studies investigating the ability of PAP to induce MN in rats and mice *in vivo*, increases in MN were observed in mice, in bone marrow cells in three studies (EU-SCCS 2011; Sicardi et al. 1991; Wild et al. 1980), in hepatocytes in one study (Cliet et al. 1989), and in mouse splenocytes in one study (Benning et al. 1994). In the single study conducted in the rat no increases in MN were observed in bone marrow cells (Hossack and Richardson 1977).

PAP was also shown to induce CAs in the bone marrow cells of mice (Mitra and Manna 1971). PAP did not induce SCEs in bone marrow cells of Chinese hamsters following a single *i.p.* injection (Kirchner and Bayer 1982).

Animal cells *in vitro* (Table 26)

In mammalian cells *in vitro*, PAP tested positive in assays for forward mutations in mouse lymphoma L5178Y TK+/- (TK locus) cells in three studies, at concentrations ranging from 1 to 12 µg/ml (Amacher and Turner 1982; Majeska and Holden 1995; Oberly et al. 1984). Tests for mutagenicity at the HPRT locus were negative in mouse lymphoma L5178Y TK+/- cells in one study (Majeska and Holden 1995) and in CHO K1-BH4 cells in two studies (Majeska and Holden 1995; Oberly et al. 1993) at concentrations ranging from 2.5 to 30 µg/ml.

PAP induced DNA strand breaks in both CHO and mouse lymphoma L5178Y cells in studies using the Comet assay, with a dose-dependent trend evident at concentrations ranging from 4 to 30 µg/ml (Majeska and Holden 1995). Another study using the alkaline and hydroxyapatite elution method also reported that PAP induced DNA strand breaks at a concentration of 15 mM in mouse lymphoma L5178Y TK +/- cells in the presence, but not the absence of S-9 (Garberg et al. 1988).

PAP did not induce UDS in cultured rat primary hepatocytes in two studies, when tested at concentrations ranging from 0.5 to 1000 nM (Probst et al. 1981; Thompson et al. 1983).

In one set of studies, PAP was found to induce CAs in both CHO and mouse lymphoma cells in a dose-dependent trend at concentrations ranging from 4-30 µg/ml (Majeska and Holden 1995). In another set of studies, Oberly et al. (1993) reported that PAP induced CAs in mouse lymphoma cells, but not in hamster CHO strain A52/XPRT cells. Assays for sister chromatid exchange (SCE) were positive in Chinese hamster (V79) cells without S-9 at concentrations from 0.01 to 0.1 mM (Holme et al. 1988).

Insects, plants, yeasts, and bacteria (Table 27)

In a wing spot test in *Drosophila melanogaster* (Somatic mutation and recombination test, SMART), PAP increased the number of mutant spots in two different types of wing spots. The increase was more than tenfold compared with controls (Eiche et al. 1990). Eiche et al. (1990) also performed two SLRL assays in *Drosophila melanogaster*, one with PAP application via feeding and one via PAP injection. PAP did not induce SLRL mutations in either assay.

In a study of *Vicia faba* root cells, PAP increased CAs at concentrations ranging from 1 to 4 mM (Kanaya 1990), and in a study with *Saccharomyces cerevisiae,* PAP significantly increased intrachromosomal recombinations at concentrations ranging from 3 to 6 mg/ml (Brennan and Schiestl 1997).

PAP has been tested in several *Salmonella typhimurium* reverse mutation assays, in several strains, and reported to be negative in all but one test, which was conducted in strain TA1535 in the absence of metabolic activation (Wild et al. 1980). PAP has been tested in several *E. coli* reverse mutation assays, in several strains, and found to be positive in three, all in the absence of metabolic activation: WP2 Mutoxitest IC203, WP2 Mutoxitest IC188, and WP2*uvr*A/pKM101 (EU-SCCS 2011; Martinez et al. 2000).

Table 24. Genotoxicity studies of PAP in human cells *in vitro*

Test endpoint	Cell type	Concentration	Results without S-9	Results with S-9	Reference
DNA strand breaks	Cultured skin fibroblasts	25, 50 µM	NT	+[a]	Andersson et al. (1982)
	Lymphoblastic cells	0.05-5 mM	+	NT	Hayward et al. (1982)
DNA adducts	Granulocytes	10 µM (±PMA)[b]	+	NT	Corbett et al. (1989)
Chromosomal aberrations	Lymphocytes	Without S-9: 13, 19 and 25 µg/ml; with S-9: 960.4, 1372 and 1960 µg/ml	+	+	Microtest (1990), as cited by EU-SCCS (2011)
Sister chromatid exchanges	Lymphocytes	0.1-100 µM	+	NT	Takehisa and Kanaya (1982)
		15-60 µM	±	NT	Kirchner and Bayer (1982)
	Fibroblasts	0.005-0.2 mM	-	NT	Wilmer and Natarajan (1981)

NT, not tested; +, positive; –, negative; ±, equivocal

[a]Incubated with microsomes from sheep seminal vesicles (1 mg of protein per ml).

[b]With or without treatment of granulocytes with phorbol myristate acetate (PMA), which stimulates the respiratory burst. Binding was detected in the absence of PMA, and was significantly enhanced by PMA treatment.

Table 25. Genotoxicity studies of PAP in animals *in vivo*

Endpoint	Species, strain, sex	Tissue Analyzed	Dose	Route, duration, dosing regimen	Results	Reference
Mutation, [sperm head abnormality]	Mouse, (CBA x BALB/c) F1; Male	Sperm	25-400 mg/kg bw	*i.p.*; five injections	+	Topham (1980)
Mutation, [dominant lethal test]	Rat, Sprague-Dawley; Male and female	Fetus	50, 150, 520 mg/kg bw	Diet; 20 weeks	-	Burnett et al. (1989)
Micronuclei	Rat, CFY (S-D descendants); Male and female	Bone marrow	1600 mg/kg bw	Gavage; Two doses	-	Hossack and Richardson (1977)
	Mouse, NMRI; Male and female		109, 218, 327, 436 mg/kg bw	*i.p.*; two injections	+	Wild et al. (1980)
	Mouse, Swiss; Male and female		5,50,100, 200 mg/kg bw	*i.p.*; single injection	+	Sicardi et al. (1991)
	Mouse, Swiss OF1; Male and female		170, 250, 500 mg/kg bw	Oral; single dose	+	Centre International de Toxicologie (1992), as cited by EU-SCCS (2011)
	Mouse, CD-1/CR; Male	Hepatocytes	53, 107, 214 mg/kg bw	*i.p.*; two injections	+	Cliet et al. (1989)
	Mouse, CD-1; Male	Splenocytes	53, 107, 214 mg/kg bw	Oral; single dose	+	Benning et al. (1994)
Chromosomal aberrations	Mouse; Unspecified; NR	Bone marrow	0.27, 0.54, 1.09 mg	*i.p.*; two injections	+	Mitra and Manna (1971)
Sister chromatid exchanges	Chinese Hamster; Male	Bone marrow	5 mg/kg bw	*i.p.*; single injection	-	Kirchner and Bayer (1982)

+, positive; –, negative; *i.p.*: Intraperitoneal injection

Table 26. Genotoxicity studies of PAP in animals *in vitro*

Test endpoint	Species/ cell line	Concentration	Results without S-9	Results with S-9	Reference
tk Mutation	Mouse lymphoma L5178Y TK+/− (TK locus) cells	4.6 µg/mL	NT	+	Amacher and Turner (1982)
		1-7 µg/mL	+	NT	Oberly et al. (1984)
		4-12 µg/mL	+	NT	Majeska and Holden (1995)
hprt Mutation	Hamster CHO HPRT strain K1-BH4 cells	2.5-30 µg/mL	-	-	Oberly et al. (1993)
		10-30 µg/mL	-	NT	Majeska and Holden (1995)
	Mouse lymphoma L5178Y TK+/− (HPRT locus) cells	4-12 µg/mL	-	NT	Majeska and Holden (1995)
DNA strand breaks	Mouse lymphoma L5178Y cells	4-12 µg/mL	+	NT	Majeska and Holden (1995)
	Hamster CHO Cells	5-30 µg/mL	+	NT	
	Mouse lymphoma L5178Y TK+/− cells	0.5-15 mM	-	+	Garberg et al. (1988)
Unscheduled DNA synthesis	Rat primary monolayer culture of hepatocytes	0.5-1000 nM	-	NT	Probst et al. (1981)
		0.5-1000 nM	-	NT	Thompson et al. (1983)
Chromosomal aberrations	Hamster CHO strain A52/XPRT cells	2-20 µg/mL	-	-	Oberly et al. (1993)
	Mouse lymphoma L5178Y/TK +/− cells	2-7 µg/mL	+	NT	
	Mouse lymphoma L5178Y cells	4-14 µg/mL	+	NT	Majeska and Holden (1995)
	Hamster CHO Cells	5-30 µg/mL	+	NT	
Sister chromatid exchanges	Hamster lung V79 cells	0.01-0.1 mM	+	NT	Holme et al. (1988)

+, positive; –, negative; NT, not tested; CHO: Chinese Hamster Ovary; TK: Thymidine kinase; HPRT: Hypoxanthine phosphoribosyl transferase

Table 27. Genotoxicity studies of PAP in insects, plants, yeast, and bacteria

Endpoint	Test	Test system	Concentration	Results without S-9	Results with S-9	Reference
			Insects			
Mutation	Somatic mutation and recombination test (SMART)	*Drosophila melanogaster* null	Feeding: 20 mM	+	NT	Eiche et al. (1990)
	Sex-linked recessive lethal (SLRL) test	*Drosophila melanogaster*; female M5 and male KO	Feeding: 130 mM; Injection: 15 mM or 30 mM	-	NT	
			Plant systems			
Chromosomal damage	Chromosomal aberrations	*Vicia faba* null	1-4 mM	+	NT	Kanaya (1990)
			Yeast			
Chromosomal damage	Intra-chromosomal recombination	*Saccharomyces cerevisiae* null, RS112	2-6 mg/ml	+	NT	Brennan and Schiestl (1997)
			Bacteria			
Mutation (*S. typhimurium*)	Ames test/ Reverse mutation	TA1538	10 mM	-	-	Garner and Nutman (1977)
		TA98, 100	10 mM	-	-	Degawa et al. (1979)
			10 mM	-	-	LaVoie et al. (1979)
		TA1535	0.4 µM/plate	(+)	-	Wild et al. (1980)
		TA98, 100, 1537, 1358	0.4 µM/plate	-	-	
		TA98, 100, 1535, 1537, 1538	10 mM	-	-	Thompson et al. (1983)
		G46, C3076, D3052	10 mM	-	-	
		TA97, 98, 100, 102, 1535, 1537, 1538	15-160 µg/plate	-	-	De Flora et al. (1984)
		TA97, 98, 100, 1535, 1537	3-3333 µg/plate	-	-	Zeiger et al. (1988)
		TA1538	Rat urine[a]	-	NT	Burnett et al. (1989)
Mutation (*E. coli*)	Reverse mutation	WP2, WP2 uvrA−	10 mM	-	-	Thompson et al. (1983)
		WP2, WP100, W3110/P3478 Pol, WP67 Pol	up to 2000 µg/mL	NT	-	Mamber et al. (1983)
		WP2, WP67, CM871	15-160 µg/plate	-	-	De Flora et al. (1984)
		K-12 uvrB/recA	up to 14.3 mM	-	-	Hellmer and Bolcsfoldi (1992)
		WP2 Mutoxitest IC203	1,000 µg/plate	+	-	Martinez et al. (2000)
		WP2 Mutoxitest IC188	1,000 µg/plate	(+)	NT	Martinez et al. (2000)
		WP2 Mutoxitest IC204, 206, 208	1,000 µg/plate	-	NT	
		WP2*uvr*A/pKM101	700, 900, 1100, 1300 and 1500 µg/plate	+	NT	Yoshida et al. (1998), as cited by EU-SCCS (2011)

NT, not tested; +, positive; –, negative; (+): weakly positive.
Acetaminophen was present in rat urine. Urine was sampled from male and female Sprague-Dawley rats treated with PAP in the diet at concentrations of 700, 2,000, 7,000 ppm (50, 150, 520 mg/kg) for 12 weeks.

p-Benzoquinone

p-Benzoquinone is a metabolite of acetaminophen that has been identified in the urine of mice administered acetaminophen *in vivo* (Pascoe et al. 1988) and has been detected in mouse liver microsomes incubated with acetaminophen *in vitro* (Dahlin et al. 1984) (See metabolism section 3.1.1).

The genotoxicity of *p*-benzoquinone has been reviewed and summarized by IARC (IARC 1999b, 2018). OEHHA reviewed IARC's evaluation of the genotoxicity studies, as well as additional genotoxicity studies published since the IARC review. *p*-Benzoquinone is an electrophile that readily reacts with peptides, proteins, and DNA (Gaskell et al. 2005; Levay et al. 1991; McDonald et al. 1993), and it has been tested for a variety of genotoxic endpoints in human cells *in vitro,* animals *in vivo*, and animal cells *in vitro*, for reverse mutations in bacteria, for forward and reverse mutations in yeast, and for inhibition of Topoisomerase IIα in an acellular system.

Overall, the findings from these studies include evidence of DNA adducts, DNA strand breaks, clastogenic effects, and genomic instability in human cells *in vitro* (Table 28), weak induction of MN in animals *in vivo* (Table 29), DNA damage, clastogenic effects, mutations, genomic instability, and decreased expression of a critical DNA repair gene in animal cells *in vitro* (Table 30), DNA adducts, inhibition of Topoisomerase IIα in an acellular system, and little evidence of genotoxicity in bacteria or yeast (Table 31).

Human cells *in vitro*

As shown in Table 28, DNA adducts were detected by ^{32}P-postlabelling in human bone marrow cells, myeloid leukemia cells (HL-60) and fibroblast cells treated with *p*-benzoquinone (Bodell et al. 1993; Gaskell et al. 2005; Levay et al. 1991). In human peripheral blood lymphocytes treated *in vitro*, *p*-benzoquinone significantly increased DNA single-strand breaks in the presence of S-9 (Anderson et al. 1995), and in the absence of S-9 (Andreoli et al. 1997) in the comet assay. *p*-Benzoquinone has consistently tested positive for induction of MN in studies in lymphocytes, embryonal liver (HuFoe-15) cells and peripheral blood mononuclear cells (Glatt et al. 1990; Westphal et al. 2009; Yager et al. 1990), and it tested positive for induction of SCEs in T-lymphocytes (Erexson et al. 1985).

p-Benzoquinone also induces effects associated with genomic instability, including DNA cleavage following Topoisomerase IIα inhibition in human CEM and acute lymphoblastic leukemia cells (Lindsey et al. 2005; Lindsey et al. 2004), and histone γ-H2AX, which is an indicator of DNA double strand breaks (DSBs), in HL-60 cells (Ishihama et al. 2008) .

Animals *in vivo*

As shown in Table 29, a single intraperitoneal (*i.p.*) injection of a low dose (6.25 mg/kg bw) of *p*-benzoquinone did not induce dominant lethal mutations in male C3H mice or in

male (C3H × 101) F1 mice (Rohrborn and Vogel 1967). *p*-Benzoquinone induced MN in the bone marrow cells of male CD-1 mice (Ciranni et al. 1988b) and pregnant CD-1 mice (Ciranni et al. 1988a) treated with a single oral dose of 20 mg/kg bw *in vivo*. Additionally, *p*-benzoquinone induced micronuclei in the liver cells of fetuses exposed transplacentally in the same study (Ciranni et al. 1988a). In general, *i.p.* injection causes greater toxicity than the oral route, thus lower doses of *p*-benzoquinone were administered in the *i.p.* injection studies, which reported negative findings for dominant lethal mutations and mouse bone marrow MN induction (Ciranni et al. 1988b; Rohrborn and Vogel 1967).

Animal cells *in vitro*

In mammalian cells exposed *in vitro* (Table 30), *p*-benzoquinone induced mutations at the *hprt* locus in Chinese hamster V79 cells, and DNA strand breaks in mouse bone marrow cells, mouse lymphoma L5178YS cells, and V79 cells (Abdul Hamid et al. 2019; Glatt et al. 1990; Ludewig et al. 1989; Pellack-Walker and Blumer 1986; Yang and Zhou 2010). *p*-Benzoquinone induced MN in V79 and rat intestinal cells, and CA in mouse bone marrow cells, but not SCEs in V79 cells (Abdul Hamid et al. 2019; Glatt et al. 1990; Ludewig et al. 1989; Pellack-Walker and Blumer 1986; Yang and Zhou 2010).

Similar to what was observed in *in vitro* studies with human cells (Table 28), *p*-benzoquinone induced effects associated with genomic instability in mouse cells *in vitro*. Specifically, *p*-BQ statistically significantly increased histone H2AX phosphorylation at serine 139, followed by DNA recombination, in fetal hematopoietic cells (Tung et al. 2012) and in fetal liver cells (Philbrook and Winn 2016). Additionally, *p*-benzoquinone exposure significantly decreased the transcript levels of the critical DNA repair gene, 8-oxo-guanine glycosylase, in mouse fetal liver cells *in vitro* (Philbrook and Winn 2016).

Bacteria, fungi, and acellular systems

As shown in Table 31, *p*-benzoquinone tested positive in one reverse mutation assay in *Salmonella typhimurium* in the absence of S-9 in strain TA100 (Nazar et al. 1981) and negative in a number of other assays of strains TA98, TA100, TA1535 and TA1537 in either the presence or absence of metabolic activation (S-9) (Mortelmans et al. 1986). *p*-Benzoquinone did not cause forward or reverse mutations in *Neurospora crassa* (Reissig 1963).

In a cell free system, the reaction of *p*-benzoquinone with calf thymus DNA was shown to produce three major DNA adducts as detected by ^{32}P-postlabeling procedure (Bodell et al. 1993).

p-Benzoquinone was shown to covalently bind to and directly inhibit the activity of isolated human topoisomerase IIα at concentrations of 10 μM and greater (Baker et al. 2001).

Table 28. Genotoxicity studies of *p*-benzoquinone in human cells *in vitro*[a]

Test endpoint	Cell type	Concentration (LEC or HIC)	Results without S-9	Results with S-9	Reference
DNA adducts	Myeloid leukemia cells (HL-60)	25 µM per 2 h	+	NT	Levay et al. (1991)
	Bone marrow cells	100 µM	+	NT	Bodell et al. (1993)
	Nucleotide excision repair (NER)-proficient and NER-deficient fibroblast cells	5 mM	+	NT	Gaskell et al. (2005)
DNA strand breaks	Peripheral blood lymphocytes	0.5 mM per 4 h	NT	+	Anderson et al. (1995)
		0.2 mM per h	-	-	
		0.3 µg/mL	+	NT	Andreoli et al. (1997)
Micronuclei	Lymphocytes	0.275 µg/mL	+	NT	Yager et al. (1990)
	Embryonal human liver cells (HuFoe-15)	0.01 µg/mL	+	NT	Glatt et al. (1990)
	Peripheral blood mononuclear cells	0.04 µg/mL	+[b]	NT	Westphal et al. (2009)
Sister Chromatid Exchange	T-lymphocytes	0.5 µg/mL	+	NT	Erexson et al. (1985)
Genomic instability/ DNA cleavage [Topoisomerase IIα inhibition]	CEM leukemia cells	10 µM	+	NT	Lindsey et al. (2005)
		25 µM	+	NT	Lindsey et al. (2004)
Genomic instability/ DNA DSBs [Histone γ–H2AX phosphorylation]	Myeloid leukemia cells (HL-60)	3 µM	+	NT	Ishihama et al. (2008)

+, positive; –, negative; NT, not tested

HIC, highest ineffective concentration; LEC, lowest effective concentration (units as reported)

DSBs, DNA double strand breaks; Histone γ-H2AX phosphorylation is an index of DNA DSBs

CEM, a human T lymphoblastic leukemia cell line

[a] Much of the information presented in this table was obtained from IARC (1999b; 2018)

[b] Positive results shown by adding phenylmercapturic acid, which induces the activity of myeloperoxidase.

Table 29. Genotoxicity studies of *p*-benzoquinone in animals *in vivo*[a]

Endpoint	Species, strain, sex	Tissue analyzed	Dose (LED or HID)	Route, duration, dosing regimen	Results	Reference
Mutation, [dominant lethal test]	C3H mice, male	Embryonic or fetal death	HID: 6.25 mg/kg bw	Intraperitoneal (*i.p.*) injection; one single dose	-	Rohrborn and Vogel (1967)
	(C3H × 101) F1 mice, male				-	
Micronuclei	Pregnant female CD-1 mice	Bone marrow	LED: 20 mg/kg bw	Oral; one single dose	(+)	Ciranni et al. (1988a)
	Fetal CD-1 mice	Liver cells	LED: 20 mg/kg bw	Oral (to dam); one single dose *in utero*	(+)	
	CD-1 mice, male	Bone marrow	LED: 20 mg/kg bw	Oral; one single dose	(+)	Ciranni et al. (1988b)
		Bone marrow	HID: 5 mg/kg bw	*i.p.,* injection; one single dose	-[b]	

–, negative; (+), weak positive

HID, highest ineffective dose; LED, lowest effective dose (units as reported)

[a] Much of the information presented in this table was obtained from IARC (1999b; 2018)

[b] i.p. injection caused greater toxicity than oral route.

Table 30. Genotoxicity studies of *p*-benzoquinone in animal cells *in vitro*[a]

Test endpoint	Species/ cell line	Concentration (LEC or HIC)	Results without S-9	Results with S-9	Reference
Mutation [*hprt* locus]	Chinese hamster/ lung V79 cells	0.54 µg/mL	+	NT	Ludewig et al. (1989)
DNA strand breaks	Mouse/ lymphoma L5178YS cells	0.11 µg/mL	+	NT	Pellack-Walker and Blumer (1986)
	Chinese hamster/ lung V79 cells	6.25 µM	+	NT	Yang and Zhou (2010)
	Mouse/ bone marrow cells	1.25 µM	+	NT	Abdul Hamid et al. (2019)
Micronuclei	Chinese hamster/ lung V79 cells	5.4 µg/mL	+	NT	Ludewig et al. (1989)
	Chinese hamster/ lung V79 cells	0.01 µg/mL	+	NT	Glatt et al. (1990)
	Rat/ intestinal IEC-17 and IEC-18 cells		+	NT	
Chromosomal aberrations	Mouse/ bone marrow cells	1.25 µM	+	NT	Abdul Hamid et al. (2019)
Sister Chromatid Exchange	Chinese hamster/ lung V79 cells	11 µg/mL	-	NT	Ludewig et al. (1989)
Genomic instability/ DNA DSBs [Histone γ-H2AX phosphorylation]	CD-1 mouse/ fetal liver cells	25 µM	+	NT	Philbrook and Winn (2016)
	pZK1 transgenic mice/ fetal hematopoietic cells	25 µM	+	NT	Tung et al. (2012)
DNA repair [qRT-PCR reactions for *Ogg1*]	CD-1 mouse/ fetal liver cells	25 µM	+	NT	Philbrook and Winn (2016)

+, positive; –, negative

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested

qRT-PCR, Quantitative Reverse Transcription Polymerase Chain Reaction

DSBs, DNA double strand breaks; Histone γ-H2AX phosphorylation is an index of DNA DSBs

[a] Much of the information presented in this table was obtained from IARC (1999b; 2018)

Table 31. Genotoxicity studies of *p*-benzoquinone in bacteria, fungi and acellular systems[a]

Endpoint	Test	Test system	Concentration (LEC or HIC)	Results without S-9	Results with S-9	Reference
Bacteria						
Mutation	Ames test/ reverse mutation	*Salmonella typhimurium* TA100	LEC: 5 µg/mL	+	NT	Nazar et al. (1981)
	Ames test/ reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1357	HIC: 16.5 µg/mL	-	-	Mortelmans et al. (1986)
Yeast						
Mutation	Forward mutation	*Neurospora crassa* forward mutation to pyrimidine dependence	NR	-	NT	Reissig (1963)
	Reverse mutation	*Neurospora crassa* reverse mutation to *arg*+	NR	-	NT	
Acellular systems						
DNA adducts		Calf thymus DNA	1 mg	+	NT	Bodell et al. (1993)
Genomic instability/ DNA cleavage	Topoisomerase IIα inhibition assay	Purified human enzyme	10 µM	+	NT	Baker et al. (2001)

+, positive; –, negative

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; NR, not reported

[a] Much of the information presented in this table was obtained from IARC (1999b; 2018)

3.3.4 In vitro cell transformation

Cell transformation assays are designed to detect a change in the growth pattern of cells that is indicative of loss of contact inhibition, a phenotype that is characteristic of cancer cells.

Patierno et al. (1989) studied *in vitro* cell transformation of C3H/10$T_{1/2}$ clone 8 mouse embryo fibroblast (10$T_{1/2}$) cells exposed to acetaminophen. These cells are considered to be similar to BALB/3T3 and Swiss/3T3 cells, as they are stable in culture and highly sensitive to post-confluence inhibition of cell division (Reznikoff et al. 1973). C3H/10$T_{1/2}$ cells, together with other immortalized aneuploid mouse cells, represent one of the two major types of systems used for *in vitro* cell transformation assays, the other type being primary diploid cells, such as Syrian Hamster Embryo cells (Creton et al. 2012).

In this study, Patierno et al. (1989) treated 10$T_{1/2}$ cells with acetaminophen at concentrations ranging from 0.5 – 2.0 mg/mL (3.3 to 13 mM) for either 24 hours without S-9 or 3 hours with Arochlor 1254-induced hamster liver S-9. In the absence of S-9 acetaminophen induced a small, but dose-dependent increase in the number of type II morphologically transformed foci. A greater number of type II transformed foci were induced by acetaminophen in the presence of S-9. Similar cell transformation results were observed with the carcinogen phenacetin (of which acetaminophen is a major metabolite). Several metabolites of acetaminophen (and phenacetin) were also tested in C3H/10$T_{1/2}$ cells (NAPQI, PAP, *p*-benzoquinone), and each were found to be inactive in the cell transformation assay. Patierno et al. (1989) characterized the type II foci induced by acetaminophen and phenacetin as atypical (weak) non-neoplastic morphologically transformed cells that “did not exhibit any other classical parameters of neoplastic transformation, such as increased saturation density or anchorage independence.”

3.3.5 Animal tumor pathology

This section describes the relevant pathology details for the tumor types observed in the animal cancer bioassays of acetaminophen.

3.3.5.1 Mice

Hepatocellular tumors

Hepatocellular adenomas and carcinomas were observed in female B6C3F1 mice (Amo and Matsuyama 1985), male and female IF mice (Flaks and Flaks 1983), and in three male Swiss mice (Weisburger et al. 1973) treated with acetaminophen.

In the 134-week study of acetaminophen in female B6C3F1 mice, 2/49 (4%) untreated controls had hepatocellular adenoma or carcinoma (Amo and Matsuyama 1985). In an earlier study from the same laboratory, a similar spontaneous hepatocellular tumor incidence (2/60, 3%) was observed in untreated female mice (Matsuyama and Amo 1983).

Most of the hepatocellular adenomas and carcinomas observed in treated IF mice in the studies of Flaks and Flaks were reported to display a trabecular growth pattern. In these 18-month studies in IF mice, one adenoma and no carcinomas were observed in the male controls and no hepatocellular tumors were observed in the female controls. In earlier studies of 12-months duration conducted in the same laboratory in male and female IF mice, no liver tumors were observed in untreated controls of either sex (Flaks 1968), and in 15-month studies in male and female IF mice conducted at the same university, only one liver adenoma was observed among 42 untreated male controls, and no liver tumors were observed in 31 untreated female controls (Wood 1969). Taken together, these data suggest that hepatocellular adenomas are uncommon and hepatocellular carcinomas are rare in male and female IF mice.

In the 11-month study of acetaminophen in male Swiss mice by Weisburger et al. (1973), the liver tumors observed in the treated males were identified as liver hepatomas and carcinomas. Hepatoma is an older term for lesions that are now classified as hepatocellular adenomas (Frith et al. 1994). Also in 1973, Weisburger and colleagues reported on the spontaneous tumor incidence of hepatocellular adenoma (1/101, 1%) and hepatocellular carcinoma (0/101, 0%) observed in a series of 18-month carcinogenesis studies conducted in male Swiss mice (Prejean et al. 1973).

Hepatocellular adenomas and carcinomas arise from the same cell type, and adenomas can progress to carcinomas (Harada et al. 1999). For this reason, these two tumor phenotypes are combined when evaluating study results (McConnell et al. 1986).

Pituitary tumors

Pituitary adenomas were increased in female B6C3F1 mice treated with acetaminophen (Amo and Matsuyama 1985). Pituitary adenomas are considered to have the potential to progress to carcinomas (Mahler and Elwell 1999). In an earlier study conducted in female B6C3F1 mice in the same laboratory, no pituitary tumors were observed in 60 untreated females (Matsuyama and Amo 1983).

Bladder tumors

Urinary bladder papillomas (cell type not specified) were observed in two male Swiss mice treated with acetaminophen (Weisburger et al. 1973). Urinary bladder papillomas may be transitional cell or squamous cell in origin; both are considered to the potential

to progress to carcinomas (Gaillard 1999). Also in 1973, Weisburger and colleagues reported on the spontaneous tumor incidence of urinary bladder papillomas (1/101, 1%) and carcinomas (2/101, 2%) observed in a series of 18-month carcinogenesis studies conducted in male Swiss mice (Prejean et al. 1973).

3.3.5.2 Rats

Mononuclear Cell Leukemia (MNCL)

MNCL, more recently referred to as large granular lymphocyte leukemia (LGLL), is a common neoplasm in aging F344 rats, and is not usually seen in untreated F344 rats younger than 20 months of age (Rebelatto 2018). MNCL was increased in female F344/N rats treated with acetaminophen in the NTP study (NTP 1993), and was generally more advanced, involving two or more organs in addition to the liver and spleen (e.g., lung, lymph node), while leukemia in control females was mainly confined to the liver and spleen. NTP (1993) noted that "On average, leukemias were detected one month earlier in the high-dose group than in the controls, suggesting a shortening of neoplasm latency".

MNCL is morphologically characterized by cells that resemble large granular lymphocytes, and can spread to multiple organs, including liver, lungs, and spleen, with splenic infiltration being the most consistent hallmark (Stromberg and Vogtsberger 1983). The historical spontaneous incidence of MNCL in 2-year studies in female F344 rats for the study laboratory was 66/399 (16.5%; range: 6-28%) (NTP 1993). The incidence of MNCL in the control female rats in the acetaminophen study was 0% at 15 months and 19% (9/48) at 24 months (NTP 1993). US EPA (2012) noted that several authors have concluded that rat MNCL is similar to human natural killer (NK) cell large granular lymphocytic leukemia (Ishmael and Dugard 2006; Stromberg 1985; Thomas et al. 2007).

Liver tumors

Hepatocellular adenomas, also referred to by the authors as neoplastic nodules, were increased in male and female Leeds rats treated with acetaminophen (Flaks et al. 1985). The tumors had either a trabecular or solid growth pattern, compressed surrounding parenchyma, and were sometimes encapsulated. No liver tumors were observed in the controls in either of these 18-month studies of acetaminophen in male and female Leeds rats (Flaks et al. 1985). Two earlier studies conducted in male Leeds rats in the same laboratory, one of 18-month duration (Flaks and Flaks 1982) and the other of 20-months duration (Flaks 1978), also reported that no liver tumors were observed in untreated controls.

Hepatocellular adenomas are benign hepatocellular neoplasms. Hepatocellular adenomas and carcinomas arise from the same cell type, and adenomas can progress to carcinomas (McConnell et al. 1986). No hepatocellular carcinomas were observed in treated male or female Leeds rats in these 18-month studies by Flaks et al. (1985). A possible reason is the less-than-lifetime study duration, which may not be sufficiently long to observe such progression.

Urinary bladder tumors

Transitional cell papillomas and carcinomas of the urinary bladder epithelium were observed in male and female Leeds rats treated with acetaminophen (Flaks et al. 1985). The papillomas of the transitional epithelium were either localized or appeared as diffuse papillomatosis. The carcinomas showed regional dysplasia and invasion of the underlying muscle, but none had metastasized (Flaks et al. 1985).

Spontaneous occurrence of urinary bladder tumors in rats is rare (<1%) (Frith et al. 1995) and is predominantly associated with advancing age in various strains of rats, including S-D rats, Osborne-Mendel and Oregon rats, Wistar rats and inbred Fischer 344 rats (Kunze and Chowaniec 1990). In the Flaks et al. (1985) study and in another 18-month study of male Leeds rats (Flaks and Flaks 1982), no bladder tumors were reported in controls.

Urinary bladder transitional cell papillomas and carcinomas arise from the same cell type, and papillomas can progress to carcinomas (Kunze and Chowaniec 1990). For this reason, these two tumor phenotypes are combined when evaluating study results (McConnell et al. 1986).

3.3.6 Structure activity considerations

Acetaminophen consists of a benzene ring with one hydroxyl group and an acetamide group at the para position. OEHHA used the US Environmental Protection Agency's (EPA) web-based CompTox Chemicals Dashboard (https://comptox.epa.gov) (Williams et al. 2017) and relevant literature to identify chemicals that share structural similarities with acetaminophen. Structurally similar chemicals were initially identified as those with a similarity score > 0.8. Those identified chemicals were further screened based on the following characteristics: 1) presence of one aromatic ring; 2) one or more amino groups; and 3) testing for genotoxicity or animal carcinogenicity. Additionally, acetaminophen metabolites and data-rich chemicals with lower similarity scores (0.6-0.8) were also considered. The five compounds identified for structure-activity comparison are: phenacetin, aniline, PAP, 2,4-diaminophenol dihydrochloride, and 3-amino-4-ethoxyacetanilide.

Data on the genotoxicity and carcinogenicity of the selected comparison chemicals are briefly discussed below and summarized in Table 32. The sources of this information include the TOXNET databases (https://toxnet.nlm.nih.gov), such as Chemical Carcinogenesis Research Information System (CCRIS), Carcinogenic Potency Database (CPDB), Genetic Toxicology Data Bank (GENE-TOX), and Hazardous Substances Data Bank (HSDB), as well as NTP and IARC documents and Sections 3.2 Carcinogenicity studies in animals and 3.3.3 Genotoxicity of this document.

Phenacetin

Phenacetin is listed as a carcinogen under Proposition 65. It is an IARC Group 1 carcinogen (carcinogenic to humans) (IARC 2012), and is listed as "reasonably anticipated to be a human carcinogen" in the NTP Report on Carcinogens (NTP 2016b). In humans, phenacetin causes cancer of the renal pelvis and of the ureter (IARC 2012). In rodents, phenacetin induced benign and malignant urinary tract tumors in mice and rats of both sexes and nasal cavity tumors in rats of both sexes (IARC 2012). The genotoxicity evidence of phenacetin was summarized by IARC (2012). It was not mutagenic in mouse embryo cells and did not induce sex-linked recessive lethal mutations in *Drosophila*. *In vitro,* phenacetin was mutagenic to *Salmonella* strain TA100 in the presence of hamster liver metabolic activation (IARC 1980), but did not induce mutations in strains TA98, TA1535, and TA1537 with and/or without metabolic activation. Phenacetin induced chromosomal and DNA damage in various experimental systems. Phenacetin induced MN in bone marrow cells in mice and rats, and in peripheral blood cells in rats; CAs in CHO cells, and DNA damage in the kidney in mice and the urinary bladder in rats *in vivo* and in human and rat bladder cells *in vitro*.

Phenacetin did not induce DNA strand breaks in rat hepatocytes after exposure *in vivo* (IARC 2012).

Aniline

Acetaminophen is a major metabolite of aniline (Modick et al. 2014). Aniline is listed as a carcinogen under Proposition 65. It is classified as a B2 carcinogen (probable human carcinogen) by US EPA (US EPA 1988), and in Group 3 (not classifiable as to its carcinogenicity to humans) by IARC (IARC 1987). Aniline induced rare hemagiosarcoma of the spleen, rare fibrosarcoma and sarcoma of the spleen and other organs, and pheochromocytoma in male and female rats (CIIT 1982; NCI 1978b; US EPA 1988). Genotoxicity findings for aniline have been reported in TOXNET databases (2019) such as CCRIS, GENE-TOX, and HSDB and by Galloway et al. (1987). Aniline was mutagenic in Chinese hamster V79 and CHL/IU cells, mouse lymphoma L5178Y cells, and *S. typhimurium* strain TA98 (with and without metabolic activation), but not in *S. typhimurium* strains TA97, TA100, TA102, TA1535, TA1537, or TA1538 with and/or without metabolic activation, or in *E. coli, B. subtilis,* or *S. cerevisiae*. Aniline induced MN and SCEs in bone marrow cells in mice *in vivo*, MN in Chinese hamster CHL/IU cells *in vitro*, CAs in CHO cells, SCEs in human fibroblasts and CHO cells, and DNA damage *in vivo* in rat liver and kidney, but did not induce UDS in rodent hepatocytes *in vitro*.

PAP

PAP was tested in 101-week carcinogenicity studies in male and female S-D rats via gavage (Centre International de Toxicologie, as cited by EU-SCCS 2011). No tumor findings were reported for female rats, and a marginal increase of heterogeneous malignant lymphoma was seen in treated male rats (one in control, one in low-dose, and three in the highest dose). PAP induced sperm mutations in mice *in vivo* in one study, gene mutations in several studies in rodent cells *in vitro,* somatic mutations in *Drosophila*, and tested positive in several *E. coli* reverse mutation assays and weakly positive in one reverse mutation assay in *S. typhimurium* TA1535 (positive in one study and negative in three others), and negative in additional *S. typhimurium* strains tested. PAP produced equivocal results in a dominant lethal test in the rat and was negative in an SLRL mutation assay in *Drosophila*. PAP caused chromosomal damage in human cells *in vitro* (CAs, SCEs), in mice *in vivo* (CAs, MN), in rodent cells *in vitro* (CAs, SCEs), in plants (CAs), and in yeast (intrachromosomal recombination). It did not induce SCEs in hamsters *in vivo*. PAP also caused DNA strand breaks in multiple types of human and rodent cells *in vitro*, and formed DNA adducts in human cells *in vitro*. It did not induce UDS in rat hepatocytes *in vitro*.

2,4-Diaminophenol dihydrochloride

2,4-Diaminophenol dihydrochloride was tested in two-year carcinogenicity studies in male and female F344/N rats and $B6C3F_1$ mice via gavage (NTP 1992). In male mice, NTP concluded there was "some evidence of carcinogenic activity" based on increased incidence of renal tubule adenomas (single and step-sections: 0/50 in control, 6/50 in high-dose). NTP (1992) concluded that there was "no evidence of carcinogenic activity" in the studies in rats or female mice. The genotoxicity findings for 2,4-diaminophenol dihydrochloride were reported by NTP (1992) and Watanabe et al. (1989) as follows: The chemical induced mutations (*tk* -/-) in mouse lymphoma L5178Y cells without S9. In SLRL assays in *Drosophila*, results were equivocal via feed and negative via injection. It was mutagenic in *Salmonella* strain TA98 with S9 metabolic activation, and negative in TA100, TA1535, and TA1537 with or without S9. It did not induce CAs or SCEs in CHO cells in the presence or absence of S9.

3-Amino-4-ethoxyacetanilide

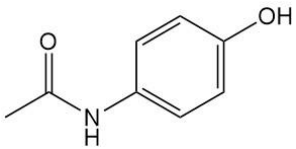
3-Amino-4-ethoxyacetanilide was tested in long-term carcinogenicity studies in male and female F344 rats and $B6C3F_1$ mice (NCI 1978a). In male mice, NCI concluded that 3-amino-4-ethoxyacetanilide was carcinogenic, causing follicular cell carcinomas of the thyroid gland (2/44, 1/49, 0/45, 7/45, in low-dose control, low-dose, high-dose control, and high-dose groups, respectively). In female mice the incidence of pituitary gland tumors (adenoma not otherwise specified [NOS], chromophobe adenoma, and carcinoma NOS, combined) was significantly increased ($p < 0.05$) in the low-dose group compared to the low-dose control group (4/37, 14/43, for low-dose control and low-dose groups, respectively), but not in the high-dose group (3/42, 3/39, for high-dose control and high-dose groups, respectively). NCI concluded these studies were "insufficient to establish the carcinogenicity of 3-amino-4-ethoxyacetanilide" in female mice or in Fischer 344 rats of either sex" (NCI 1978a).

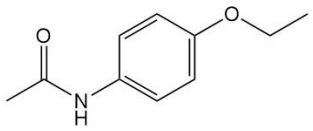
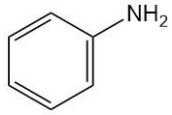
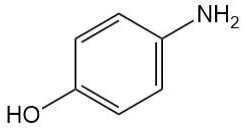
3-Amino-4-ethoxyacetanilide did not induce SLRL mutations in *Drosophila* (Valencia et al. 1985). It induced mutations in *S. typhimurium* strains TA98, TA100, TA1537, and TA1538, but not TA1535, with and without metabolic activation (Dunkel et al. 1985; Haworth et al. 1983). It induced mutations in one assay in *E. coli* strain WP2 *uvrA* with metabolic activation, and was negative in three assays (Dunkel et al. 1985).

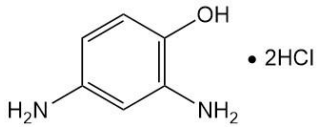
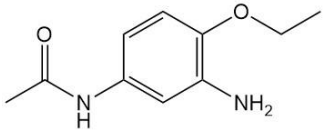
As summarized in Table 32, five chemicals that are structurally related to acetaminophen were considered for comparison. All five comparison chemicals have been tested for carcinogenicity in animals, and two induced tumors at the same sites and in the same species as acetaminophen. Specifically, urinary bladder tumors were observed in male and female mice in studies with phenacetin and with acetaminophen, and pituitary tumors were observed in female mice in studies with 3-amino-4-

ethoxyacetanilide and with acetaminophen. All five comparison chemicals were tested for mutagenicity (in bacteria, *Drosophila*, and/or in mammalian cells) and were positive in at least some of the assays tested. Three of the comparison chemicals, phenacetin, aniline, and PAP were positive in assays detecting chromosomal effects and in assays detecting DNA damage.

Table 32. Structure activity comparison between acetaminophen and five structurally related chemicals

Chemical	Structure	Genotoxicity			Tumors in animal cancer bioassays
		Mutagenicity	Chromosomal effects	DNA damage/ DNA binding	
Acetaminophen (IARC Group 3)		– gene mutation assays in rodents *in vivo*; + in TK+/− rodent cells *in vitro*; – SLRL mutation assay in *Drosophila*; – *Salmonella*; – *E. coli*	+ aneuploidy in rat embryos *in vivo*; + MN in human lymphocytes, rat, mouse bone marrow, mouse peripheral blood and mussels *in vivo*, human and rodent cells *in vitro*; + CAs human lymphocytes, mouse bone marrow *in vivo*, human and rodent cells *in vitro*, and plants; + SCEs in human lymphocytes and mouse bone marrow *in vivo*; human and rodent cells *in vitro*; – DEL recombination assays in yeast	+ DNA strand breaks in mouse liver, + in mussels *in vivo*, + in human and cells *in vitro*; ↓ DNA repair in mice and rats *in vivo*, in human and rodent cells *in vitro*; ↓ UDS in human lymphocytes *in vivo,* rat, hamster, and guinea pig liver cells, and hamster lung cells *in vitro*; ↑ UDS in human lymphocytes, rat and mouse liver cells *in vitro*; + DNA adducts in mouse liver and kidney *in vivo,* human cells *in vitro*, acellular systems (calf thymus DNA, dN-mix and guanosine); + DNA oxidation (8-oxodG) in mouse serum *in vivo*, and in rat C6 glioma cells *in vitro*	Liver: male and female rats and mice Urinary bladder: male and female[a] rats MNCL: female rats Pituitary gland: female mice

Chemical	Structure	Genotoxicity			Tumors in animal cancer bioassays
		Mutagenicity	Chromosomal effects	DNA damage/ DNA binding	
Phenacetin (metabolized to acetaminophen) (P65, IARC Group 1, NTP)		– mutagenicity in mouse embryo cells; – SLRL mutations in *Drosophila*; + *Salmonella* TA100; – *Salmonella* TA1535, TA1537, TA98	+ MN in mouse and rat cells *in vivo*; + CAs in CHO	– DNA strand breaks in rat hepatocytes *in vivo*; + DNA damage in mouse kidney and rat urinary bladder *in vivo* and in human and rat bladder cells *in vitro*	Urinary tract[b]: male and female rats and mice Nasal cavity: male and female rats
Aniline (metabolized to acetaminophen) (P65, IARC Group 3, US EPA B2)		+ L5178+/- mouse lymphoma cells; +Chinese hamster cells; + *Salmonella* TA98; – *Salmonella* TA97, TA100, TA102, TA1535, TA1537, and TA1538; – *E. coli*, *B. subtilis, S. cerevisiae*	+ MN in mouse *in vivo* and Chinese hamster CHL/IU cells *in vitro*; + CAs in CHO cells; + SCEs in mouse *in vivo*, human fibroblasts and CHO cells *in vitro*	+ DNA damage in rat liver and kidney *in vivo*; – UDS in rat hepatocytes *in vitro*	Hemangiosarcoma (r) in spleen: male rats Fibrosarcoma and sarcoma (r) in spleen and multiple organs: male and female rats Pheochromocytoma: male and female rats
***p*-Aminophenol** (a metabolite of acetaminophen)		+ sperm mutation in mice; – dominant lethal test in rat; + gene mutation in rodent cells *in vitro*; + somatic mutation and recombination test (SMART) in *Drosophila*; – SLRL mutation assay in *Drosophila*; – *Salmonella*; + *E. coli*	+ MN in mouse *in vivo;* + CAs in human and rodent cells *in vitro,* mouse and plants *in vivo*; – SCEs in hamster *in vivo*; + SCEs in human and rodent cells *in vitro*; + Intrachromosomal recombination in yeast	+ DNA strand breaks in human and rodent cells *in vitro*; – UDS in rat hepatocytes *in vitro* + DNA adducts in human granulocytes *in vitro*	Equivocal findings of malignant lymphoma in male rats

Chemical	Structure	Genotoxicity			Tumors in animal cancer bioassays
		Mutagenicity	Chromosomal effects	DNA damage/ DNA binding	
2,4-Diaminophenol dihydrochloride		+ *tk* locus in mouse lymphoma cells; ± SLRL mutation assay in *Drosophila*; + *Salmonella* TA98; – TA100, TA1535, TA1537	– CAs and SCEs in CHO cells	NT	Kidney[c]: male mice
3-Amino-4-ethoxyacetanilide		– SLRL mutation assay in *Drosophila*; + *Salmonella* TA98, TA100, TA1537, TA1538; – TA1535; + *E. coli* in one study, – other studies	NT	NT	Pituitary gland[d]: female mice Thyroid gland: male mice

P65: Proposition 65 carcinogen; NTP: NTP Report on Carcinogens "reasonably anticipated to be a human carcinogen"; US EPA B2: Probable human carcinogen; IARC Group 1: Carcinogenic to humans; IARC Group 3: Not classifiable as to its carcinogenicity to humans

+, positive; (+), weakly positive; ±, equivocal; –, negative; ↓, decrease; ↑, increase; NT, not tested; (r), rare.

CAs, chromosomal aberrations; CHO, Chinese hamster ovary; SCE, sister chromatid exchange; SHE, Syrian hamster embryo; UDS, unscheduled DNA synthesis; MN; micronuclei; DEL, deletion; SLRL, sex-linked recessive lethal; $6TG^r$, 6-thioquanine resistant; OUA^r, ouabain resistant; dN-mix, deoxyribonucleic acid mixture.

[a] In the female rat study, urinary bladder papilloma and carcinoma combined was statistically significant only in the mid-dose group.

[b] Urinary tract tumors observed for phenacetin include transitional cell carcinoma of the urinary bladder in male and female rats, urinary bladder carcinomas in female mice, renal cell carcinoma in male rats, and kidney adenomas and carcinomas in male mice.

[c] 2,4-Diaminophenol dihydrochloride increased the incidence of renal tubule adenomas in male mice.

[d] 3-Amino-4-ethoxyacetanilide increased the incidence of pituitary gland tumors (adenoma not otherwise specified [NOS], chromophobe adenoma, and carcinoma NOS, combined) in female mice in the low-, but not the high-dose group.

3.3.7 ToxCast high-throughput screening assays

ToxCast™ is a chemical prioritization research program developed by the US EPA (Dix et al. 2007; Judson et al. 2010; Kavlock et al. 2012). It is a multi-year project that launched in 2007. ToxCast utilizes various *in vitro* and zebrafish systems to identify chemical activity in a battery of high-throughput screening (HTS) assays. As of 2019, more than 9,000 chemicals have been tested, and there are more than 1,000 high-throughput assays in the ToxCast database.

This section highlights the HTS assays in which acetaminophen and two metabolites, *p*-benzoquinone and PAP, were active. OEHHA searched the US EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard, accessed on 5/13/2019), and obtained chemical activity data for the three chemicals.

Data on acetaminophen

Quality Control (QC) information shows that the purity of acetaminophen used in the Tox21 assays (a subset of the ToxCast assays) was over 90%. No QC information is available from other ToxCast assay platforms. The CompTox Chemicals Dashboard reported that acetaminophen was active in five of the 636 ToxCast assays in which it was tested. The inactivity of acetaminophen in the ToxCast assays may be due to the lack of metabolic activation in the testing systems.

For all five active assays, there was a flag for potential overfitting/borderline active issues. Therefore, these results will not be discussed further here.

Data on p-benzoquinone

Information on *p*-benzoquinone chemical purity is available for the Tox21 assays, which tested two different samples of *p*-benzoquinone. QC values are provided for T_0, the beginning of the testing, and T_4, the end of the 4-month testing period. The QC grade for one sample (Tox21_202020) used in the Tox21 assays was “B: MW [molecular weight] confirmed with 75-90% purity” at T_0 and “Ac: caution low concentration” (5-30% of expected value) at T_4. The QC grade for the other sample (Tox21_302970) used in the Tox21 assays was “F: incorrect molecular weight” at T_0 and “Ac: caution low concentration” (5-30% of expected value) at T_4.

Overall, the CompTox Chemicals Dashboard reported 105 active assays out of 556 tested assays. Among these actives, 74 assays are labeled with flags. The flags include: Borderline active, Hit-call potentially confounded by overfitting, less than 50% efficacy, only highest concentration above baseline, noisy data, or a combination of the

above. OEHHA excluded the 74 flagged assays, as well as three additional active assays with inadequate chemical QC (*i.e.*, those performed with Tox21_302970), resulting in a total of 28 active assays for further consideration. These 28 active assays are summarized in Table 33.

Table 33. Active ToxCast assays for *p*-benzoquinone

Biological process/ Intended target family	Assay endpoint component name	Target gene name	AC_{50}
Cell adhesion molecules	BSK_3C_Eselectin_down	selectin E	7.07
Cell adhesion molecules	BSK_3C_HLADR_down	major histocompatibility complex, class II, DR alpha	10.8
Cell adhesion molecules	BSK_3C_VCAM1_down	vascular cell adhesion molecule 1	15
Cell adhesion molecules	BSK_4H_VCAM1_down	vascular cell adhesion molecule 1	19.9
Cell adhesion molecules	BSK_hDFCGF_CollagenIII_down	collagen, type III, alpha 1	13.3
Cell adhesion molecules	BSK_hDFCGF_VCAM1_down	vascular cell adhesion molecule 1	9.84
Cell adhesion molecules	BSK_KF3CT_ICAM1_down	intercellular adhesion molecule 1	17.2
Cell adhesion molecules	BSK_LPS_VCAM1_down	vascular cell adhesion molecule 1	24.6
Cell cycle	BSK_3C_Proliferation_down	null	5.51
Cell cycle	BSK_CASM3C_Proliferation_down	null	15.2
Cell cycle	BSK_hDFCGF_Proliferation_down	null	2.93
Cell cycle	BSK_SAg_PBMCCytotoxicity_down	null	8.18
Cell cycle	BSK_SAg_Proliferation_down	null	3.23
Cytokine	BSK_3C_MCP1_down	chemokine (C-C motif) ligand 2	10.6
Cytokine	BSK_3C_uPAR_down	plasminogen activator, urokinase receptor	9.77
Cytokine	BSK_BE3C_IP10_down	chemokine (C-X-C motif) ligand 10	23.3
Cytokine	BSK_hDFCGF_IP10_down	chemokine (C-X-C motif) ligand 10	12.4
Cytokine	BSK_hDFCGF_MCSF_down	colony stimulating factor 1 (macrophage)	11.7

Biological process/ Intended target family	Assay endpoint component name	Target gene name	AC_{50}
Cytokine	BSK_hDFCGF_PAI1_down	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	14.6
Cytokine	BSK_KF3CT_IL1a_down	interleukin 1, alpha	12.3
Cytokine	BSK_KF3CT_IP10_down	chemokine (C-X-C motif) ligand 10	14.8
Cytokine	BSK_LPS_TNFa_down	tumor necrosis factor	15
DNA binding	ATG_NRF2_ARE_CIS_up	nuclear factor, erythroid 2-like 2	51.9
GPCR	NVS_GPCR_hOpiate_mu	opioid receptor, mu 1	9.55
Growth factor	BSK_KF3CT_TGFb1_down	transforming growth factor, beta 1	9.62
Nuclear receptor	ATG_ERa_TRANS_up	estrogen receptor 1	30
Protease inhibitor	BSK_hDFCGF_TIMP1_down	TIMP metallopeptidase inhibitor 1	10.1
Protease inhibitor	BSK_KF3CT_TIMP2_down	TIMP metallopeptidase inhibitor 2	9.3

AC_{50}: Active Concentration$_{50}$, the micromolar (μM) concentration that induces a half-maximal assay response.
GPCR: G-protein-coupled receptors.

In 2014, the IARC working group for Monograph Volume 112 mapped the ToxCast assay end-points available at that time to the key characteristics of carcinogens (IARC 2017; Smith et al. 2016). IARC has since updated its mapping of the ToxCast/Tox21 assays and OEHHA has obtained IARC's latest table (Houck, K., email communication, May 24, 2018), which maps a total of 299 assay end-points to seven of the ten key characteristics of carcinogens.

To facilitate understanding of cancer pathway-related activities of *p*-benzoquinone in the ToxCast/Tox21 assays, OEHHA utilized IARC's mapping table to map the 28 active ToxCast assays for *p*-benzoquinone to the key characteristics of carcinogens. Six of

the 28 active assays were mapped to three of the key characteristics of carcinogens, as follows:

- *Induces oxidative stress* – *p*-benzoquinone was active in one assay measuring oxidative stress via the activation of nuclear factor erythroid 2-like 2 (NFE2L2 or Nrf2), an oxidative stress-related transcription factor (ATG_NRF2_ARE_CIS_up).
- *Modulates receptor-mediated effects* – *p*-benzoquinone was active in one assay targeting Estrogen receptor alpha (ERα) (ATG_ERa_Trans_up).
- *Alters cell proliferation, cell death or nutrient supply* – *p*-benzoquinone was active in four assays measuring inhibition of cell proliferation (BSK_3C_Proliferation_down, BSK_CASM3C_Proliferation_down, BSK_hDFCGF_Proliferation_down, and BSK_SAg_Proliferation_down).

Data on PAP

A search for PAP in the CompTox Chemicals Dashboard identified 122 active assays out of 211 tested assays. All of these were Tox21 assays. Among the 122 active assays, 62 had no flags, and were thus considered true "actives". Of the 62 true actives, 15 assays were performed with PAP samples associated with serious QC issues and were thus not considered further[9]. Of the resulting 47 active assays, 34 were excluded because they either measured cytotoxicity as the assay endpoint (n=31), or the Active Concentration$_{50}$ (AC_{50}) of the endpoint measured in the assay was higher than the AC_{50} for cytotoxicity associated with the test substance in that particular assay system. The resulting 13 active assays for PAP are summarized in Table 34 below.

[9] The QC grades for the four PAP samples used in these assays are as follows: Tox21_113242, incorrect molecular weight (MW) at T_0 and T_4; Tox21_113477, incorrect MW at T_0; Tox21_113477_1, MW confirmed with no purity information at T_0; Tox21_201030, MW confirmed with 50-75% purity at T_0. OEHHA considered the QC issues to be serious for the first three samples listed here, and did not include assays associated with their use in this table.

Table 34. Active Tox21 assays for PAP

Biological process/ Intended target family	Assay endpoint component name	Target gene name	AC_{50}
DNA binding	TOX21_AhR_LUC_Agonist	Aryl hydrocarbon receptor (AhR)	17.4
DNA binding	TOX21_AP1_BLA_Agonist_ratio	AP-1	16.6
DNA binding	TOX21_SSH_3T3_GLI3_Antagonist	GLI family zinc finger 3	10
Histones	TOX21_H2AX_HTRF_CHO_Agonist_ratio	H2A histone family, member X	189
Nuclear receptor	TOX21_AR_BLA_Antagonist_ratio	Androgen receptor (AR)	12.9
Nuclear receptor	TOX21_AR_LUC_MDAKB2_Antagonist	AR	29.5
Nuclear receptor	TOX21_ERa_LUC_BG1_Antagonist	Estrogen receptor (ER) alpha	74.3
Nuclear receptor	TOX21_ERb_BLA_Antagonist_ratio	ER beta	26.6
Nuclear receptor	TOX21_ERR_Antagonist	Estrogen-related receptor alpha	27.8
Nuclear receptor	TOX21_PGC_ERR_Antagonist	Estrogen-related receptor alpha	21.2
Nuclear receptor	TOX21_PPARd_BLA_antagonist_ratio	Peroxisome proliferator-activated receptor delta	54.1
Nuclear receptor	TOX21_RORg_LUC_CHO_Antagonist	RAR-related orphan receptor C	34.1
Nuclear receptor	TOX21_VDR_BLA_antagonist_ratio	Cytochrome P450, family 24, subfamily A, polypeptide 1	55.4

AC_{50}: Active Concentration$_{50}$, the µM concentration that induces a half-maximal assay response.

Similar to the data analysis approach for *p*-benzoquinone, OEHHA used IARC's mapping table to map the 13 active Tox21 assays for PAP to the key characteristics of carcinogens. Twelve of the 13 active assays were mapped to three of the key characteristics of carcinogens, as follows[10]:

- *Is genotoxic* – PAP was active in one assay measuring the histone variant H2AX when phosphorylated on serine 139 (TOX21_H2AX_HTRF_CHO_Agonist_ratio). According to the Tox21 Data Browser, H2AX phospho-Ser139 serves as a

[10] OEHHA consulted the Tox21 Data Browser website (https://tripod.nih.gov/tox21/assays/) for description of these Tox21 assays.

sensitive biomarker for the detection of DNA double-strand breaks and localizing the site of DNA repair.

- *Modulates receptor-mediated effects* – PAP was active in 10 assays targeting various receptors. Of these 10 assays, only one assay was in the agonist mode and showed that PAP was an Aryl Hydrocarbon Receptor (AhR) agonist. The other nine assays were in the antagonist mode and showed the chemical to be an antagonist for Androgen Receptor (AR), ERα, Estrogen Receptor beta (ERβ), Estrogen Related Receptor alpha (ERRα), Peroxisome Proliferator-Activated Receptor delta (PPARδ), RAR-related Orphan Receptor C (RORC), and Vitamin D Receptor (VDR).
- *Alters cell proliferation, cell death or nutrient supply* – PAP was active in one assay measuring activation of the transcription factor Activator Protein-1 (AP-1). AP-1 is an important regulator of cell proliferation, differentiation, apoptosis, and angiogenesis.

In conclusion, acetaminophen was not considered to be active in any of the ToxCast/Tox21 HTS assays in which it was adequately tested. Two acetaminophen metabolites, *p*-benzoquinone and PAP, were considered to be active in 28 and 13 ToxCast/Tox21 HTS assays, respectively. The active assays for *p*-benzoquinone were linked to the following three key characteristics of carcinogens: inducing oxidative stress, modulating receptor-mediated effects, and altering cell proliferation, cell death, or nutrient supply. The active assays for PAP were linked to: genotoxicity, modulating receptor-mediated effects, and altering cell proliferation, cell death, or nutrient supply.

3.3.8 ROS production and oxidative stress

Oxidative stress refers to a condition of an imbalance between the production and elimination of reactive oxygen and nitrogen species (ROS, RNS). Oxidative stress may contribute to carcinogenic processes by causing DNA mutations, chromosomal damage, genomic instability, and altered cell cycle regulation (Reuter et al. 2010).

Oxidative stress induced by acetaminophen may cause lipid peroxidation, mitochondrial damage, and damage to proteins and DNA (Jaeschke et al. 2012). Many *in vivo* and *in vitro* studies conducted in humans and animals have reported evidence of acetaminophen-induced oxidative stress. For example, a review by Wang et al. (2017) identified over 80 papers on this topic. Acetaminophen-induced oxidative stress markers include 8-oxodeoxyguanosine (8-oxodG, a marker of oxidative DNA damage that is linked to mutagenesis and carcinogenesis), production of ROS and RNS, malondialdehyde (MDA, a marker of lipid peroxidation), decreased antioxidant enzyme activities such as superoxide dismutase (SOD) or catalase (CAT), decreased GSH, increased glutathione disulfide (GSSG), and decreased GSH/GSSG ratio (Wang et al. 2017). What follows is not meant to be a comprehensive review of all published

studies, but rather a summary of examples of study findings illustrative of acetaminophen-induced oxidative stress.

Two human studies were identified that investigated the effects of acetaminophen on markers related to ROS production and oxidative stress. In a study of young healthy adult volunteers (9 men, 6 women; average age: 21 years old) administered therapeutic doses of acetaminophen (1 g four times per day; i.e., 4 g/day) for 14 days, a statistically significant decrease in total antioxidant capacity in serum was observed at day 14, compared to day 0 (Nuttall et al. 2003). In a study in children 10 years of age and under, Kozer et al. (2003) reported statistically significant differences in median levels of erythrocyte glutathione peroxidase and glutathione reductase activities in febrile children taking therapeutic doses of acetaminophen for up to 10 days (dose range: 30-75 mg/kg/day), as compared to levels in untreated heathy children.

In vitro studies using human cell lines have also investigated the effects of acetaminophen on markers related to ROS production and oxidative stress. Acetaminophen was shown to increase levels of ROS in U937 (histiocytic lymphoma) cells (Jamil et al. 1999), SK-MEL-28 (melanoma) cells (Vad et al. 2009) and mesenchymal stem cells (Yiang et al. 2015), and to increase levels of MDA in HepG2 (hepatocellular carcinoma) cells (Jannuzzi et al. 2018). Acetaminophen was also shown to reduce antioxidant enzyme activities (i.e., CAT, glutathione peroxidase, and glutathione reductase) in HepG2 cells (Jannuzzi et al. 2018) and to decrease levels of GSH in SK-MEL-28 (Vad et al. 2009) and HepG2 cells (Jannuzzi et al. 2018).

In vivo evidence from animal studies includes the observation of statistically significantly increased levels of 8-OHdG (or 8-oxodG) in the serum of acetaminophen treated mice (Wang et al. 2015), as well as increased levels of ROS in the renal tissue of acetaminophen treated rats (Ghosh et al. 2010) and increased hepatic H_2O_2 in acetaminophen treated mice (Pu et al. 2016). Increased levels of MDA were reported in the liver (Lahouel et al. 2004)and renal tissue of exposed rats (Ghosh et al. 2010), and in the plasma and liver of treated mice ((Mladenovic et al. 2009; Pu et al. 2016) [measurements in liver only]). Decreased antioxidant enzyme activities (e.g., SOD, CAT, glutathione peroxidase) or decreased levels of GSH were reported in the liver homogenates of treated rats (Lahouel et al. 2004; Marotta et al. 2009) and mice (Pu et al. 2016; Wang et al. 2015), and in the renal tissue of treated rats (Ghosh et al. 2010; Kandemir et al. 2017). Most of these animal studies were conducted with relatively high doses of acetaminophen, but markers of oxidative stress were also seen in studies employing lower doses. For example, administration of a single acetaminophen dose of 150 mg/kg to rats resulted in increased levels of MDA, decreased activities of SOD, CAT, and glutathione peroxidase, and decreased levels of GSH (Marotta et al. 2009) and administration of a single acetaminophen dose of 200 mg/kg to mice resulted in

increased levels of ROS and MDA, and decreased GSH and GSH/GSSG (Pu et al. 2016).

Similar findings were reported in studies using animal cells *in vitro*. For example, statistically significant time- and dose-dependent increases in 8-oxodG were reported in the nuclear DNA of rat C6 glioma cells treated with acetaminophen at concentrations ranging from 2.5 - 5 mM (Wan et al. 2004). In addition, increased levels of ROS and RNS were reported in rat C6 glioma cells (Wan et al. 2004) and in mouse hepatocytes exposed to acetaminophen (Banerjee et al. 2017). Acetaminophen was found to reduce levels of GSH and increase levels of GSSG in mouse hepatocytes (Banerjee et al. 2017).

3.3.9 Transcriptomic, proteomic, and metabolomic (Omics) studies

"Omics" or "cross-omics" analysis (i.e., transcriptomics, proteomics, and metabolomics) provides an approach to understanding the potential mechanisms of acetaminophen toxicity at the molecular level without the occurrence of liver toxicity by evaluating changes to the transcriptome (i.e., expression level of mRNA or miRNA in a given cell or tissue), the proteome (analysis of the entire protein complement of a cell line, tissue, or organism), or the metabolome (i.e., measurement of the metabolite pool within a cell) at therapeutic doses. "Omics" studies with acetaminophen have been conducted in rodents and humans, along with several reviews of the topic (for example, (Beger et al. 2015; Coen 2015; O'Connell and Watkins 2010)). Here we highlight findings relevant to carcinogenicity from omics studies conducted in humans and animals. Studies of high-dose treatments and those showing overt signs of acute toxicity were excluded, *e.g.* >4 g in a 24-hour period in humans, ≥300 mg/kg bw administered as a bolus dose in rodents.

Transcriptomic studies

Human

Fannin et al. (2010) used microarrays to study the effects of a single 4 g dose of acetaminophen on the peripheral blood transcriptome in six healthy adults. Blood samples were taken before dosing and 6, 12, 18, 24, 48, 72, and 96 hours after dosing. The study found many genes differentially expressed with acetaminophen at multiple time points, but the effects among subjects were most consistent at 48 hours. They used Ingenuity Pathways Analysis to analyze the differentially regulated genes and the more stringent Gene Set Analysis to confirmation the results, and identified several enriched pathways, including downregulation of oxidative phosphorylation, mitochondrial function, and ubiquinone biosynthesis.

Jetten et al. (2012) assessed the response to therapeutic doses of 0.5, 2, or 4 g acetaminophen at 1, 7, or 25 hours (4 g dose group analyzed at 25 hours only) in healthy human volunteers using blood transcriptomics (mRNA and miRNA). No effects of acetaminophen toxicity could be detected with clinical biomarkers. Transcriptomic analysis of whole blood mRNA expression found no significantly regulated biological pathways at the 0.5 g dose at any time point. The authors grouped the significantly affected pathways observed at the 2 and 4 g doses into six different categories of cell processes: immune response, lipid metabolism, cholesterol transport, apoptosis and survival/oxidative stress/DNA damage, drug metabolism/pain, and other processes. Transcriptomic analysis of whole blood mRNA expression analysis demonstrated differential effects at 2 g versus 4 g of acetaminophen. At the 2 g dose level (25 hour), immune response pathways (including "macrophage inhibitory factor mediated glucocorticoid regulation", "signaling of several interleukins, CD40", and "T-helper17 cell related processes") were the main altered pathways found, suggesting that a low-dose acetaminophen exposure may trigger an immune response. This is in contrast to the findings at the 4 g dose level (25 hour), which involved changes in "apoptosis and survival/oxidative stress/DNA damage" and "cholesterol transport", with the down-regulation of oxidative phosphorylation being the most significant finding at this dose. This response is thought to be due to oxidative stress and mitochondria dysfunction induced by reactive metabolites. Transcriptomics analysis of miRNA at the 4 g dose level (25 hour), with confirmation by RT-PCR, showed three differentially expressed miRNAs, whose 89 target genes were grouped by cell processes. "Immune response" and "apoptosis and survival/oxidative stress/DNA damage" were the most dominant cell processes affected by these 89 target genes.

Fannin et al. (2016) examined the effect of 1 g acetaminophen every 6 hours for 7 days on the blood transcriptome in 42 volunteers. After five days, 30% of adults (termed "responders") had increased ALT levels. The authors stated that they found unique peripheral blood transcriptome signatures (PBTS) that can distinguish between the responders, non-responders, and individuals given a placebo. Functional analysis of responders revealed an increased expression of genes involved in T type 2 helper cells (T_h2 cell)-mediated and innate immune responses which was absent in non-responders. Using functional analysis of differentially expressed probes the authors concluded that "non-responders" had an abundance of genes that have been associated with hepatic immune tolerance (tolerogenic/anti-inflammatory responses), whereas the genes for responders were largely associated with pro-inflammatory responses.

Animal

Ruepp et al. (2002) treated male CD-1 mice with 150 mg/kg acetaminophen via *i.p.* injection, and collected liver samples 15 min or 2 hours after treatment. Microarrays

and quantitative RT-PCR were used for transcriptomic analysis. Several cancer-related genes were found to be upregulated by acetaminophen at 2 hours, including c-fos, EGR-1, c-myc, TNF-α, and GM-CSF, with the induction of GM-CSF also seen at 15 min. The authors suggested that the increased expression of these genes was mediated by ROS and cellular pro-apoptotic signals.

Williams et al. (2004) also did a transcriptomic analysis for effects of acetaminophen on gene expression in mice. They treated male CD-1 mice with 1 mmol/kg (151 mg/kg) acetaminophen via *i.p.* injection, and collected liver mRNA 1, 4, and 24 hours after treatment. Genes upregulated by acetaminophen at 1 hour include antioxidant-related genes (HSP 70; HSP 105; HSP 40; HO-1; Rho GTPase activator), a GSH-related gene (lactoyl glutathione lyase), metabolism-related genes (CDC-like kinase; Cyp2a4; pyruvate dehydrogenase β; *S*-adenosylmethionine decarboxylase-1), transcription-related genes (ATF4, a Jun proto oncogene; PCNA), immune-related genes (cathepsin E; INFγ-induced GTPase) and an apoptosis-related gene (growth arrest specific 5). The authors suggested the metabolism-related genes may be biomarkers of oxidative stress, and the immune-related gene cathepsin E may be an early marker of cellular dysfunction/oxidative stress. Compared to 1 hour, there were fewer genes upregulated and more genes downregulated at 4 and 24 hours, but the cause for this shift remains to be investigated.

Human in vitro studies

The Human Carcinogenome Project (https://carcinogenome.org) reports transcriptomic data in HepG2 cells treated with low-dose acetaminophen (1.25 – 20 µM). This project uses hallmark gene sets for pathway enrichment analysis. Hallmark gene sets are defined by the Molecular Signatures Database (MSigDB, http://software.broadinstitute.org/gsea/msigdb) as “coherently expressed signatures derived by aggregating many MSigDB gene sets to represent well-defined biological states or processes”. Several of the most enriched hallmark gene sets affected by treatment with acetaminophen include c-myc targets, the oxidative phosphorylation pathway, the p53 pathway, angiogenesis, G2M cell cycle checkpoint, and E2F (a family of transcription factors) targets.

Proteomic studies

Human in vitro studies

Bruderer et al. (2015) treated 3D human liver microtissues consisting of primary human hepatocytes and primary human non-parenchymal cells with acetaminophen (ranging from 4.6 µM to 3.3 mM). Their proteomics analysis showed dose-dependent increases in numbers of up-regulated and down-regulated proteins compared to controls. They

identified a list of enriched pathways such as bile acid and glutathione biosynthesis, as well as induction of enzymes in phase I, II, and III metabolism. Specifically, acetaminophen induced GSTs, eight cytochromes such as CYP1A2, 2E1, and 3A4, glucuronidation enzymes such as UGT1A1, 1A6, and 1A7, and metabolite transporters such as the multidrug resistance-associated protein 2 (MRP2) for biliary secretion. The transporter for basolateral excretion (MRP3) was significantly down regulated. Furthermore, the authors identified protein adducts of NAPQI on four mitochondria-related proteins that were detected at physiologically relevant, non-toxic concentrations (13.7 µM acetaminophen).

Animal

Ruepp et al. (2002) (see description above) analyzed liver samples from male CD-1 mice treated with 150 mg/kg acetaminophen, performing 2 dimensional (2D)-differential gel electrophoresis of mitochondrial protein for proteomic analysis. These investigators found 20 differentially regulated mitochondrial proteins, which showed a pattern of decreased cellular energy production. For example, levels of aldehyde dehydrogenase, thiolase, and subunits of ATP synthase were decreased. Decreases in aldehyde dehydrogenase and thiolase may lead to impaired fatty acid β-oxidation.

Metabolomic studies

Human

Jetten et al. (2012) (see description above) conducted metabolomics analyses in healthy human volunteers using blood/urine samples. No effects of acetaminophen toxicity could be detected with clinical biomarkers, but metabolomic analyses of urine and serum samples indicated that increasing doses of acetaminophen resulted in increased oxidative metabolism (i.e., cysteine-, hydroxyl-, and methoxy- metabolites); additionally, five novel metabolites (glucuronides, sulfates, and a tryptophan) were identified. These results mirror those seen after higher acetaminophen intake and indicate that “omics” techniques are able to identify responses at low acetaminophen doses.

Animal

Chen et al. (2009) performed serum metabolomics analysis in acetaminophen treated wild-type and *Cyp2e1*-null mice. Acetaminophen was given at 200 and 400 mg/kg by *i.p.* injection to wild-type and *Cyp2e1*-null mice. Here we report the findings from the 200 mg/kg dose groups. In both wild-type and *Cyp2e1*-null mice, 200 mg/kg acetaminophen significantly reduced the serum levels of triglycerides, compared to untreated controls.

Summary

Overall, these omic studies in humans suggest that therapeutic doses of acetaminophen can cause gene-expression changes in oxidative stress and immune-modulated processes. Findings from omics studies conducted in animals suggest that acute responses to acetaminophen include changes related to oxidative stress, mitochondrial damage, impaired cellular respiration and energy production, and immune-modulated processes.

4. KEY CHARACTERISTICS

The key characteristics of carcinogens (IARC 2019a; Smith et al. 2016) were used to organize the data relevant to carcinogenicity from mechanistic studies of acetaminophen. These studies provide evidence on four of the 10 key characteristics of carcinogens, namely, being electrophilic or forming electrophilic metabolites, being genotoxic, altering DNA repair or causing genomic instability, and inducing oxidative stress (Table 35).

Table 35. Ten key characteristics of carcinogens

Characteristic	Example of relevant evidence
1. Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (*e.g.,* epoxide, quinone), formation of DNA and protein adducts
2. Is genotoxic	DNA damage (DNA strand breaks, DNA–protein cross-links, UDS), intercalation, gene mutations, cytogenetic changes (*e.g.,* CAs, MN)
3. Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (*e.g.,* topoisomerase II, base-excision or double-strand break repair)
4. Induces epigenetic alterations	DNA methylation, histone modification, microRNA expression
5. Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (*e.g.,* DNA, lipids)
6. Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7. Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8. Modulates receptor-mediated effects	Receptor inactivation/activation (*e.g.,* ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9. Causes immortalization	Inhibition of senescence, cell transformation
10. Alters cell proliferation, cell death, or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell cycle control, angiogenesis

Source: Smith et al. (2016); IARC (2019a)
AhR, aryl hydrocarbon receptor; ER, estrogen receptor; PPAR, peroxisome proliferator–activated receptor. Any of the 10 characteristics in this table could interact with any other (e.g., oxidative stress, DNA damage, and chronic inflammation), which when combined provides stronger evidence for a cancer mechanism than would oxidative stress alone.

The evidence for each of the four key characteristics of carcinogens is enumerated below.

Is electrophilic or can be metabolically activated

Acetaminophen can be metabolized by CYP enzymes, PGES, and other peroxidases to form electrophilic compounds. Electrophiles are electron-seeking molecules that

commonly form addition products (i.e., adducts) with cellular macromolecules, including DNA, RNA, lipids, and proteins (Smith et al. 2016). Electrophilic metabolites of acetaminophen include NAPQI, NAPSQI, *p*-benzoquinone imine, *p*-benzoquinone and the *N*-acetyl-*p*-aminophenoxy and *p*-aminophenoxy free radicals. NAPQI-DNA adducts have been detected in several acellular systems (Hasegawa et al. 1988; Klopcic et al. 2015; Rogers et al. 1997). *p*-Benzoquinone, which readily reacts with peptides, proteins, and DNA (Gaskell et al. 2005; Levay et al. 1991; McDonald et al. 1993), has been shown to form DNA adducts *in vitro* in human HL-60 cells (Levay et al. 1991), human fibroblast cells (Gaskell et al. 2005) and human bone marrow cells, and in an acellular system (Bodell et al. 1993).

Is genotoxic

Genotoxic effects of acetaminophen have been observed in humans, animals, and a number of experimental systems over a range of doses, including doses that fall within the range of those used therapeutically (see Section 3.3.3.1). In exposed humans, acetaminophen induced CAs, MN, and SCEs, and decreased UDS, and in human cells exposed *in vitro,* acetaminophen induced CAs, MN, SCEs, and DNA strand breaks, formed DNA adducts and slightly increased UDS. Acetaminophen has been shown to increase MN, CAs, SCEs, and aneuploidy and to form DNA adducts, oxidative DNA damage and DNA strand breaks in animals exposed *in vivo*, and in animal cells exposed *in vitro,* acetaminophen induced gene mutations, MN, CAs, and SCEs, increased oxidation of DNA, and altered UDS. In addition, acetaminophen is metabolized to the genotoxic metabolites NAPQI, *p*-benzoquinone, and PAP (see Section 3.3.3.2).

Alters DNA repair or causes genomic instability

Carcinogens may alter the processes that control repair of DNA damage or DNA replication to cause DNA damage indirectly (Smith et al. 2016). As discussed in Section 3.3.3, acetaminophen inhibits ribonucleotide reductase, which results in impairment of nucleotide excision repair, as demonstrated in animals *in vivo* and in several *in vitro* studies in human and animal cells. In addition, acetaminophen decreased protein expression and activity of the DNA repair enzyme Ogg1 in animal cells *in vitro*. The acetaminophen metabolite *p*-benzoquinone also decreased gene expression of *Ogg1* in animal cells *in vitro*. Acetaminophen and *p*-benzoquinone have been shown to increase γ-H2AX, a phosphorylated form of H2AX, which is a marker of DNA double-strand breaks and genomic instability, in human and animal cells. The acetaminophen metabolite *p*-benzoquinone has been demonstrated to inhibit human topoisomerase IIα in human cells *in vitro*, resulting in double-strand breaks and genomic instability, and in

an acellular system. The acetaminophen metabolite NAPQI has also been shown to inhibit topoisomerase IIα in human cells *in vitro* and in an acellular system.

Induces oxidative stress

ROS can be formed during the metabolism of acetaminophen either via redox cycling or oxidative reactions involving various metabolic intermediates (see Section 3.3.1). Compelling evidence for acetaminophen-induced oxidative stress comes from *in vivo* and *in vitro* studies conducted in both humans and animals with data showing, for example, increased formation of 8-oxodG or increased generation of hydrogen peroxide. Most of the studies were conducted at high doses; however, markers of oxidative stress have been reported in adults and children taking therapeutic doses (Kozer et al. 2003; Nuttall et al. 2003), in rats administered 150 mg/kg (Marotta et al. 2009), in mice administered 200 mg/kg (Pu et al. 2016), and in *in vitro* studies conducted at low doses (e.g., Jamil et al. (1999) or Vad et al. (2009)) (Section 3.3.8). In addition, omics studies discussed in Section 3.3.9 have reported that acetaminophen can regulate genes in biological pathways related to oxidative stress, including in a study in humans exposed to therapeutic doses (Jetten et al. 2012) and in a study in mice exposed to 151 mg/kg acetaminophen (Williams et al. 2004).

5. REVIEWS BY OTHER AGENCIES

IARC has classified acetaminophen in Group 3 (not classifiable as to its carcinogenicity) (IARC 1999a). The US EPA, the National Institute for Occupational Safety and Health, the NTP Report on Carcinogens, and the US FDA have not classified acetaminophen as to its potential carcinogenicity.

IARC (1990) initially reviewed the carcinogenicity of acetaminophen in 1990 and classified it in Group 3 (not classifiable as to its carcinogenicity to humans), based on *inadequate data* in humans, and *limited evidence* of carcinogenicity in experimental animals (IARC 1990). In 1999, IARC re-evaluated acetaminophen and the chemical was again classified in Group 3. In its 1999 review, IARC concluded there was *inadequate evidence* of carcinogenicity in humans and *inadequate evidence* of carcinogenicity in experimental animals.

Acetaminophen has been approved by the US FDA since the 1960s as a prescription and OTC drug to treat fever and pain.

6. SUMMARY OF EVIDENCE

Epidemiological studies

A number of human epidemiological studies have investigated the relationship between acetaminophen and cancer for various cancer sites.

Kidney cancer

The association between the use of acetaminophen and kidney cancer was assessed in four cohort studies, two nested case-control studies, 12 publications from case-control studies, and two meta-analyses. Twelve case-control studies reported on RCC, and four assessed cancer of the renal pelvis, a rare subtype of kidney cancer.

A Danish cohort study that assessed acetaminophen use through prescription records found a non-statistically signficant increased risk of RCC, but was not able to account for important potential confounders (Friis et al. 2002). An American cohort study that collected acetaminophen use prospectively through self-report observed a statistically significant increased risk of RCC with regular and less than 10 years of use, but not with 10 or more years of use, after adjustment for BMI, smoking, and other potential confounders (Karami et al. 2016). A cohort study from Washington state (Walter et al. 2011a) showed no significant increase in risk, and a study combining data from two large American prospective studies showed positive, but not statistically significant,

increases in RCC in men and women associated with regular acetaminophen use (Cho et al. 2011).

The two most informative studies were large case-control studies nested in health databases in the US and UK. Acetaminophen prescriptions were recorded prospectively, and therefore the exposure assessment method was less subject to information bias as compared to self-report (Derby and Jick 1996; Kaye et al. 2001). Both of these studies reported a statistically significant increasing risk of RCC with an increasing number of acetaminophen prescriptions filled. Kaye et al. (2001) adjusted for smoking and BMI.

The case-control studies generally reported small or modest but non-statistically significant increases in risk for both RCC (Cho et al. 2011; Gago-Dominguez et al. 1999; Karami et al. 2016; McCredie et al. 1988; McCredie and Stewart 1988; McCredie et al. 1995; McLaughlin et al. 1985; Rosenberg et al. 1998) and cancer of the renal pelvis (McCredie and Stewart 1988; McCredie et al. 1993; McLaughlin et al. 1985; Pommer et al. 1999). Several of the individual studies were not informative on their own because of limited statistical power due to the rarity of kidney cancer, small numbers of exposed cases and/or crude exposure assessments. The exceptions were the two largest case-control studies (Gago-Dominguez et al. 1999; Karami et al. 2016) and a large pooled analysis of case-control studies (McCredie et al. 1995), which reported roughly two-fold increased risk of RCC (either borderline significant or statistically significant) or positive dose response relationships.

Two meta-analyses also found a statistically significant increased risk between acetaminophen use and kidney cancer (Choueiri et al. 2014; Karami et al. 2016) and RCC specifically (Karami et al. 2016), although both analyses had some limitations.

Urinary bladder cancer

Three cohort studies, two nested case-control studies and six case-control studies examined the association of acetaminophen with bladder cancer. The three most informative studies assessed acetaminophen use prospectively through medical records; two reported a non-significant increased risk of bladder cancer with low to moderate use and no dose-response trend (Friis et al. 2002; Kaye et al. 2001), and the third reported a non-statistically significant increase with high use (Derby and Jick 1996). Of the two cohort studies that assessed acetaminophen use through self-report, one reported non-significant increased risks with use (Walter et al. 2011a).

The case-control studies had a mix of positive and null findings. A case-control study from Spain (Fortuny et al. 2006) observed no cancer association with acetaminophen overall, but found an increased risk of bladder cancer among acetaminophen users with a genotype (*GSTP1* Val/Val) encoding for a decrease in GSTP1 function and hence

reduced capacity for glutathione conjugation with acetaminophen metabolites, such as NAPQI. In those with the *GSTP1* Val/Val genotype, bladder cancer risk was increased roughly two-fold in acetaminophen users and in those who used acetaminophen regularly for more than four years (though not statistically significant), suggesting that this *GST* genotype may be important to consider in studies assessing the carcinogenicity of acetaminophen. Three of the case-control studies observed no association between bladder cancer and various metrics of acetaminophen intake (Fortuny et al. 2007; McCredie and Stewart 1988; Pommer et al. 1999), three reported non-statistically significant elevated risks of bladder cancer (Castelao et al. 2000; Fortuny et al. 2006; Piper et al. 1985), and one reported a statistically significant increase in risk of bladder cancer (Baris et al. 2013).

Urinary tract cancers

In addition to kidney and bladder cancer, there were two cohort and six case-control studies that assessed the association between acetaminophen use and cancer of other sites in the urinary tract or combined several sites of the urinary tract (three studies of renal pelvis and ureter, two studies of transitional cell cancers that included multiple sites within the urinary tract, and one study of ureter cancer).

Significant increases were not observed in the cohort studies of urinary tract cancer (Friis et al. 2002; Walter et al. 2011a). The population-based case-control study of transitional cell cancer had a statistically significant increase in risk with any acetaminophen use (Steineck et al. 1995), whereas the findings from the hospital-based case-control study were not statistically significant (Rosenberg et al. 1998). Both studies had significant limitations. The only study specifically of ureter cancer reported a statistically significant two-fold increased risk for greater than 0.1 kg of lifetime acetaminophen intake (McCredie and Stewart 1988).

Lymphohematopoietic system cancers

The association between acetaminophen use and several types of LH cancers has been studied in a number of cohort and case-control studies. For all LH cancers combined, significantly increased risks of LH cancer-related deaths were found in the one cohort study in Danish North Jutland County (Lipworth et al. 2003) but not in another in the same geographic area for incidence of LH cancer (Friis et al. 2002). A US cohort study reporting on “hematologic malignancies” found statistically significant increases in women that were high users through self-report on a questionnaire (Walter et al. 2011b).

For myeloid leukemia, statistically significant increases were reported for one cohort in men and women combined (Walter et al. 2011b) and for women but not men in one case-control study (Ross et al. 2011), with significant trends observed in terms of

duration of use and tablets per week. A second case-control study reported a non-significant elevation in risk (Friedman 1982).

AML was assessed in two case-control studies: a borderline significant increase was reported in one study (Weiss et al. 2006) and a statistically significant increase in women with a dose-response trend was reported in the second study (Ross et al. 2011). CML was assessed in one case-control study that reported non-significant elevations in risk in men and women (Ross et al. 2011).

For lymphoma, a statistically significant increase was reported in one case-control study (Becker et al. 2009). A statistically significant increase in risk of two types of B-cell lymphoma was observed in a cohort study (Walter et al. 2011b), with a dose-response trend in one type. For total NHL, a non-significant elevation in risk was observed in one cohort (Friis et al. 2002). Elevations were observed in two case-control studies, which were statistically significant in women but not men in one study (Baker et al. 2005) and not significant in women the other study (Kato et al. 2002). There were increases in the risk of three cell types of NHL, but not one cell type.

Two studies reported an increased risk of multiple myeloma, which was not significant in the cohort study (Friis et al. 2002). The case-control study reported a significantly increased risk with several metrics of exposure (regular use, times per week, years of use), with a significant dose-response trend (Moysich et al. 2007).

Two studies reported on the risk in Hodgkin's lymphoma. A cohort study reported a non-significant elevation but contained only one case (Friis et al. 2002). A case-control study found significantly increased risks with significant trends by several metrics of exposure (Chang et al. 2004).

Potential confounding by smoking was possible for some of these LH cancers since it was adjusted for in only three studies (Chang et al. 2004; Moysich et al. 2007; Walter et al. 2011b).

Liver cancer

The association between acetaminophen use and liver cancer was examined in two large independent cohorts that assessed acetaminophen use through prescription records databases, one from Denmark (Friis et al. 2002; Lipworth et al. 2003) and the other from the UK (McGlynn et al. 2015; Yang et al. 2016). In the Danish cohort, elevations in mortality and incidence risk were observed that were statistically significant for mortality from liver cancer (Lipworth et al. 2003) but not incidence of liver cancer (Friis et al. 2002). This cohort could not control for potential confounders such as smoking and alcohol use. In the UK database, statistically significant elevations in the risk of liver cancer were reported, after adjusting for several covariates including smoking status, alcohol-related disorders, hepatitis B or C virus infection, and use of

non-steroidal anti-inflammatory drugs (Yang et al. 2016). Dose-response analyses showed increasing risk with increasing acetaminophen prescriptions overall and when restricted to individuals without liver disease.

Other cancers

For cancers of the breast, ovary, uterine endometrium, prostate, skin, and colorectum, the association with acetaminophen use was either decreased, null, or inconsistent. The data from cohort and case-control studies from a number of other cancer sites were too sparse to evaluate thoroughly, namely the brain, respiratory tract, gastrointestinal tract (stomach, esophagus, oral/pharyngeal cancer), pancreas, cervix, and all cancers combined.

Animal studies

Long-term carcinogenicity studies of acetaminophen have been conducted in mice and rats. Significant tumor findings were observed in three of ten studies in mice and in three of seven studies in rats. The positive findings are as follows:

Liver tumors

- In the female B6C3F1 mice exposed to acetaminophen in feed for up to 134 weeks (Amo and Matsuyama 1985), the incidence of hepatocellular adenoma or carcinoma combined was significantly increased in the high-dose group by pairwise comparison with controls, with a significant dose-related trend.
- In the male as well as the female IF mice exposed to acetaminophen in feed for up to 18 months (Flaks and Flaks 1983), the incidences of hepatocellular adenoma, and adenoma or carcinoma combined were significantly increased in the high-dose groups by pairwise comparison with controls, with significant dose-related trends. Carcinoma was also similarly increased in male mice.
- In the male as well as the female Leeds rats exposed to acetaminophen in feed for up to 18 months (Flaks et al. 1985), the incidences of hepatocellular adenomas were significantly increased in the high-dose groups by pairwise comparison with controls, with significant dose-related trends.

Pituitary gland

- In the female B6C3F1 mice exposed to acetaminophen in feed for up to 134 weeks (Amo and Matsuyama 1985), the incidence of pituitary gland adenoma was significantly increased in the high-dose group by pairwise comparison with controls, with a significant dose-related trend.

Urinary bladder

- In the male Leeds rats exposed to acetaminophen in feed for up to 18 months (Flaks et al. 1985), the incidences of rare urinary bladder papilloma and papilloma or carcinoma combined were significantly increased in the high-dose group by pairwise comparison with controls, with significant dose-related trends. In the female Leeds rats similarly exposed, the incidence of urinary bladder papilloma or carcinoma combined was significantly increased in the low-dose group by pairwise comparison with controls.

Mononuclear cell leukemia (MNCL)

- In the female F344/N rats exposed to acetaminophen in feed for up to 103 weeks (NTP 1993), the incidence of MNCL was significantly increased in the high-dose group by pairwise comparison with controls, with a significant dose-related trend.

Significant tumor findings were not observed in long-term carcinogenicity studies of acetaminophen in male and female B6C3F1 mice exposed for 103 weeks (NTP 1993), male B6C3F1 mice exposed for either 134 weeks (Amo and Matsuyama 1985) or 70 weeks (Hagiwara and Ward 1986), male and female Swiss mice exposed for 11 months (Weisburger et al. 1973), female ABC-A mice exposed for life (mean survival ≤ 40 weeks) (Wright 1967), male F344 rats exposed for 103 weeks (NTP 1993), male and female F344/DuCrj rats exposed for 104 weeks (Hiraga and Fujii 1985), or male Sprague-Dawley rats exposed for 117 weeks (Johansson 1981).

Pharmacokinetic and Mechanistic Data

Pharmacokinetics

Metabolism of acetaminophen leads to the formation of electrophilic and genotoxic metabolites, including NAPQI, NAPSQI, PAP, *p*-benzoquinone imine, *p*-benzoquinone, and the *N*-acetyl-*p*-aminophenoxy and *p*-aminophenoxy free radicals. ROS can be formed during the metabolism of acetaminophen via either redox cycling or oxidative reactions involving various metabolic intermediates. The generation of electrophilic and genotoxic metabolites of acetaminophen can vary among individuals, due to variability in genetic factors, such as *GSTP1*, and non-genetic factors, such as nutritional status.

Genotoxicity data

Acetaminophen was not mutagenic in bacteria. Acetaminophen has tested positive for a number of other genotoxicity endpoints:

- Mutations in rodent cells *in vitro*
- DNA strand breaks in mouse liver, and in mussels *in vivo*; in human cells *in vitro*
- DNA adducts in mouse liver and kidney *in vivo*; in human granulocytes *in vitro*; in acellular systems

- DNA oxidation in mouse *in vivo*, and in rat cells *in vitro*
- Impairment of DNA repair in rat and mouse *in vivo*, and in human and rodent cells *in vitro*
- Aneuploidy in rat embryos *in vivo*
- MN formation in human lymphocytes, rat, mouse bone marrow, mouse peripheral blood and mussels *in vivo*; in human and rodent cells *in vitro*
- CAs in human lymphocytes and mouse bone marrow *in vivo*; in human and rodent cells *in vitro*
- SCEs in human lymphocytes and mouse bone marrow *in vivo*; in human and rodent cells *in vitro*

Genotoxicity of acetaminophen metabolites

There is also evidence for the genotoxicity of three metabolites of acetaminophen:

- NAPQI, which tests positive for DNA strand breaks in human and rodent cells *in vitro* and in acellular systems, and which is observed to form DNA adducts in multiple acellular systems.
- PAP, which tests positive for mutations in mice sperm *in vivo*, rodent cells *in vitro*, and in *E. coli* and *Drosophila*. PAP also forms DNA strand breaks in human and rodent cells *in vitro* and DNA adducts in human granulocytes *in vitro*. It also tests positive for chromosomal effects - MN in mouse *in vivo*; CAs in human and rodent cells *in vitro*, mouse and plants *in vivo*; SCEs in human and rodent cells *in vitro*; and intra-chromosomal recombination in yeast.
- *p*-Benzoquinone, which tests positive for mutations in *Salmonella*, and in rodent cells *in vitro*, DNA strand breaks in human and rodent cells *in vitro*, and DNA adducts in human cells and in acellular systems. Chromosomal effects include MN in human and rodent cells *in vitro*, and in mouse bone marrow and liver cells *in vivo*; CAs in mouse bone marrow *in vivo*; SCEs in human lymphocytes *in vivo*, and in rodent cells *in vitro*. *p*-Benzoquinone also inhibits topoisomerase IIα in human cells *in vitro*, and in an acellular system. It also induced effects associated with genomic instability in mouse and human cells, and decreased *Ogg1* expression in rodent cells *in vitro*.

Structure activity comparisons

The biological activity of acetaminophen was compared to five structurally related compounds: phenacetin, aniline, PAP, 2,4-diaminophenol dihydrochloride, and 3-amino-4-ethoxyacetanilide. Of these five comparison chemicals, two (phenacetin and aniline) are listed as Proposition 65 carcinogens.

Common target tumor types observed between acetaminophen and some of the comparison chemicals are urinary bladder tumors (observed for phenacetin), and

pituitary tumors (observed for 3-amino-4-ethoyacetanilide). All five structurally related comparison chemicals have genotoxic activity.

Key characteristics

The key characteristics of carcinogens (IARC 2019a; Smith et al. 2016) were used to organize the data relevant to carcinogenicity from mechanistic studies of acetaminophen. These studies provide evidence on four of the 10 key characteristics of carcinogens enumerated below.

- Is electrophilic or can be metabolically activated

  Acetaminophen can be metabolized by CYP enzymes, PGES, and other peroxidases to form electrophilic compounds. Electrophilic metabolites of acetaminophen include NAPQI, NAPSQI, *p*-benzoquinone imine, *p*-benzoquinone and the *N*-acetyl-*p*-aminophenoxy and *p*-aminophenoxy free radicals.

- Is genotoxic

  There is evidence on the genotoxicity of acetaminophen and its metabolites, NAPQI, PAP, and *p*-benzoquinone, as outlined above.

- Alters DNA repair or causes genomic instability

  There is evidence that acetaminophen alters DNA repair and causes genomic instability. The evidence includes (i) inhibition of ribonucleotide reductase (which results in impairment of nucleotide excision repair) by acetaminophen; (ii) decreased protein expression and activity of the DNA repair enzyme Ogg1 by acetaminophen and decreased gene expression of Ogg1 by *p*-benzoquinone; (iii) increased levels of γ-H2AX (indication of DNA double-strand breaks) by acetaminophen and *p*-benzoquinone; and (iv) inhibition of human topoisomerase IIα (leading to DNA double-strand breaks) by NAPQI and *p*-benzoquinone.

- Induces oxidative stress

  Evidence for induction of oxidative stress comes from *in vivo* and *in vitro* studies of acetaminophen conducted in both humans and animals, and includes observations of markers of oxidative stress in adults and children taking therapeutic doses, and in rats and mice administered doses of 150 mg/kg and 200 mg/kg, respectively. Transcriptomic studies have reported that acetaminophen can regulate genes in biological pathways related to oxidative stress, including in a study in humans exposed to therapeutic doses and a study in mice exposed to 151 mg/kg acetaminophen.

7. REFERENCES

Abdul Hamid Z, Chow P, Mathialagan R, Rajab N, Shuib S, Abdul Razak S. 2019. Mechanism of 1,4-benzoquinone-induced genotoxicity in haematopoietic stem/progenitor cells: linking benzene to haematological disorders. In: International Conference on Drug Discovery and Translational Medicine 2018 (ICDDTM '18) “Seizing Opportunities and Addressing Challenges of Precision Medicine”. Putrajaya, Malaysia.

Acetaminophen Awareness Coalition. 2019. KnowYourDose.org. Available: https://www.knowyourdose.org/ [accessed on February 12].

ACS. 2016. Basal and Squamous Cell Skin Cancer Risk Factors. Available: https://www.cancer.org/cancer/basal-and-squamous-cell-skin-cancer/causes-risks-prevention/risk-factors.html [accessed on August 15, 2019].

ACS. 2017. Risk Factors for Kidney Cancer. Available: https://www.cancer.org/cancer/kidney-cancer/causes-risks-prevention/risk-factors.html [accessed on July 18, 2019].

ACS. 2018a. Colorectal Cancer Risk Factors. Available: https://www.cancer.org/cancer/colon-rectal-cancer/causes-risks-prevention/risk-factors.html [accessed on August 15, 2019].

ACS. 2018b. American Cancer Society, Key Statistics About Kidney Cancer. Available: https://www.cancer.org/cancer/kidney-cancer/about/key-statistics.html [accessed on July 18, 2019].

ACS. 2018c. American Cancer Society, What is Ovarian Cancer? Available: https://www.cancer.org/cancer/ovarian-cancer/about/what-is-ovarian-cancer.html [accessed on August 15, 2019].

ACS. 2019a. Key Statistics for Bladder Cancer. Available: https://www.cancer.org/cancer/bladder-cancer/about/key-statistics.html [accessed on August 15, 2019].

ACS. 2019b. American Cancer Society, Liver Cancer Risk Factors. Available: https://www.cancer.org/cancer/liver-cancer/causes-risks-prevention/risk-factors.html [accessed on June 3, 2019].

ACS. 2019c. American Cancer Society, How Common is Breast Cancer? Available: https://www.cancer.org/cancer/breast-cancer/about/how-common-is-breast-cancer.html [accessed on August 15, 2019].

ACS. 2019d. American Cancer Society, Cancer A-Z. Available: https://www.cancer.org/cancer.html [accessed on June 17, 2019].

Albano E, Rundgren M, Harvison PJ, Nelson SD, Moldeus P. 1985. Mechanisms of N-acetyl-p-benzoquinone imine cytotoxicity. Molecular pharmacology 28:306-311.

Allegaert K, de Hoon J, Verbesselt R, Vanhole C, Devlieger H, Tibboel D. 2005. Intra- and interindividual variability of glucuronidation of paracetamol during repeated administration of propacetamol in neonates. Acta paediatrica (Oslo, Norway : 1992) 94:1273-1279.

Amacher DE, Turner GN. 1982. Mutagenic evaluation of carcinogens and non-carcinogens in the L5178Y/TK assay utilizing postmitochondrial fractions (S9) from normal rat liver. Mutation research 97:49-65.

Ammundsen HB, Faber MT, Jensen A, Høgdall E, Blaakær J, Høgdall C, et al. 2012. Use of analgesic drugs and risk of ovarian cancer: Results from a Danish case-control study. Acta obstetricia et gynecologica Scandinavica 91:1094-1102.

Amo H, Matsuyama M. 1985. Subchronic and chronic effects of feeding of large amounts of acetaminophen in B6C3F1 mice. Nihon eiseigaku zasshi Japanese journal of hygiene 40:567-574.

Anders MW, Lash L, Dekant W, Elfarra AA, Dohn DR. 1988. Biosynthesis and biotransformation of glutathione S-conjugates to toxic metabolites. Critical reviews in toxicology 18:311-341.

Anderson D, Yu TW, Schmezer P. 1995. An investigation of the DNA-damaging ability of benzene and its metabolites in human lymphocytes, using the comet assay. Environmental and molecular mutagenesis 26:305-314.

Anderson LA, Johnston BT, Watson RG, Murphy SJ, Ferguson HR, Comber H, et al. 2006. Nonsteroidal anti-inflammatory drugs and the esophageal inflammation-metaplasia-adenocarcinoma sequence. Cancer research 66:4975-4982.

Andersson B, Nordenskjold M, Rahimtula A, Moldeus P. 1982. Prostaglandin synthetase-catalyzed activation of phenacetin metabolism to genotoxic products. Molecular pharmacology 22:479-485.

Andreoli C, Leopardi P, Crebelli R. 1997. Detection of DNA damage in human lymphocytes by alkaline single cell gel electrophoresis after exposure to benzene or benzene metabolites. Mutation research 377:95-104.

Andrews RS, Bond CC, Burnett J, Saunders A, Watson K. 1976. Isolation and identification of paracetamol metabolites. The Journal of international medical research 4:34-39.

Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. 2016. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 127:2391-2405.

Arfe A, Corrao G. 2016. The lag-time approach improved drug-outcome association estimates in presence of protopathic bias. Journal of clinical epidemiology 78:101-107.

Ashok V, Dash C, Rohan TE, Sprafka JM, Terry PD. 2011. Selective cyclooxygenase-2 (COX-2) inhibitors and breast cancer risk. Breast (Edinburgh, Scotland) 20:66-70.

Baandrup L, Friis S, Dehlendorff C, Andersen KK, Olsen JH, Kjaer SK. 2014. Prescription use of paracetamol and risk for ovarian cancer in Denmark. Journal of the National Cancer Institute 106:dju111.

Backryd E. 2018. Gender differences in dispensed analgesics in Sweden during 2006-2015 - an observational, nationwide, whole-population study. International journal of women's health 10:55-64.

Bairam AF, Rasool MI, Alherz FA, Abunnaja MS, El Daibani AA, Kurogi K, et al. 2018. Effects of human SULT1A3/SULT1A4 genetic polymorphisms on the sulfation of acetaminophen and opioid drugs by the cytosolic sulfotransferase SULT1A3. Archives of biochemistry and biophysics 648:44-52.

Baker JA, Weiss JR, Czuczman MS, Menezes RJ, Ambrosone CB, Moysich KB. 2005. Regular use of aspirin or acetaminophen and risk of non-Hodgkin lymphoma. Cancer causes & control : CCC 16:301-308.

Baker RK, Kurz EU, Pyatt DW, Irons RD, Kroll DJ. 2001. Benzene metabolites antagonize etoposide-stabilized cleavable complexes of DNA topoisomerase IIalpha. Blood 98:830-833.

Banda PW, Quart BD. 1982. The effect of mild alcohol consumption on the metabolism of acetaminophen in man. Res Commun Chem Pathol Pharmacol 38:57-70.

Bandi S, Viswanathan P, Gupta S. 2014. Evaluation of cytotoxicity and DNA damage response with analysis of intracellular ATM signaling pathways. Assay Drug Dev Technol 12:272-281.

Banerjee S, Melnyk SB, Krager KJ, Aykin-Burns N, McCullough SS, James LP, et al. 2017. Trifluoperazine inhibits acetaminophen-induced hepatotoxicity and hepatic reactive nitrogen formation in mice and in freshly isolated hepatocytes. Toxicology reports 4:134-142.

Bannwarth B, Netter P, Lapicque F, Gillet P, Pere P, Boccard E, et al. 1992. Plasma and cerebrospinal fluid concentrations of paracetamol after a single intravenous dose of propacetamol. British journal of clinical pharmacology 34:79-81.

Baris D, Karagas MR, Koutros S, Colt JS, Johnson A, Schwenn M, et al. 2013. Nonsteroidal anti-inflammatory drugs and other analgesic use and bladder cancer in northern New England. International journal of cancer 132:162-173.

Barnard ME, Poole EM, Curhan GC, Eliassen AH, Rosner BA, Terry KL, et al. 2018. Association of Analgesic Use With Risk of Ovarian Cancer in the Nurses' Health Studies. JAMA oncology 4:1675-1682.

Bean TG, Rattner BA, Lazarus RS, Day DD, Burket SR, Brooks BW, et al. 2018. Pharmaceuticals in water, fish and osprey nestlings in Delaware River and Bay. Environmental pollution (Barking, Essex : 1987) 232:533-545.

Becker N, Fortuny J, Alvaro T, Nieters A, Maynadie M, Foretova L, et al. 2009. Medical history and risk of lymphoma: results of a European case-control study (EPILYMPH). Journal of cancer research and clinical oncology 135:1099-1107.

Beger RD, Bhattacharyya S, Yang X, Gill PS, Schnackenberg LK, Sun J, et al. 2015. Translational biomarkers of acetaminophen-induced acute liver injury. Archives of toxicology 89:1497-1522.

Bender RP, Lindsey RH, Jr., Burden DA, Osheroff N. 2004. N-acetyl-p-benzoquinone imine, the toxic metabolite of acetaminophen, is a topoisomerase II poison. Biochemistry 43:3731-3739.

Benning V, Brault D, Duvinage C, Thybaud V, Melcion C. 1994. Validation of the in vivo CD1 mouse splenocyte micronucleus test. Mutagenesis 9:199-204.

Berg KJ, Djoseland O, Gjellan A, Hundal O, Knudsen ER, Rugstad HE, et al. 1990. Acute effects of paracetamol on prostaglandin synthesis and renal function in normal man and in patients with renal failure. Clin Nephrol 34:255-262.

Bergman K, Müller L, Teigen SW. 1996. The genotoxicity and carcinogenicity of paracetamol: A regulatory (re)view. Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis 349:263-288.

Berlin CM, Jr., Yaffe SJ, Ragni M. 1980. Disposition of acetaminophen in milk, saliva, and plasma of lactating women. Pediatric pharmacology (New York, NY) 1:135-141.

Bessems JG, Vermeulen NP. 2001. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Critical reviews in toxicology 31:55-138.

Binkova B, Topinka J, Sram RJ. 1990. The effect of paracetamol on oxidative damage in human peripheral lymphocytes. Mutation research 244:227-231.

Bittoni MA, Carbone DP, Harris RE. 2017. Ibuprofen and fatal lung cancer: A brief report of the prospective results from the Third National Health and Nutrition Examination Survey (NHANES III). Molecular and clinical oncology 6:917-920.

Bitzen PO, Gustafsson B, Jostell KG, Melander A, Wahlin-Boll E. 1981. Excretion of paracetamol in human breast milk. European journal of clinical pharmacology 20:123-125.

Blieden M, Paramore LC, Shah D, Ben-Joseph R. 2014. A perspective on the epidemiology of acetaminophen exposure and toxicity in the United States. Expert review of clinical pharmacology 7:341-348.

Bodell WJ, Levay G, Pongracz K. 1993. Investigation of benzene-DNA adducts and their detection in human bone marrow. Environmental health perspectives 99:241-244.

Bodelon C, Doherty JA, Chen C, Rossing MA, Weiss NS. 2009. Use of nonsteroidal antiinflammatory drugs and risk of endometrial cancer. American journal of epidemiology 170:1512-1517.

Bolt HM, Thier R. 2006. Relevance of the deletion polymorphisms of the glutathione S-transferases GSTT1 and GSTM1 in pharmacology and toxicology. Current drug metabolism 7:613-628.

Bomhard EM, Herbold BA. 2005. Genotoxic activities of aniline and its metabolites and their relationship to the carcinogenicity of aniline in the spleen of rats. Critical reviews in toxicology 35:783-835.

Bond GR. 2009. Acetaminophen protein adducts: a review. Clinical toxicology (Philadelphia, Pa) 47:2-7.

Bonovas S, Filioussi K, Sitaras NM. 2006. Paracetamol use and risk of ovarian cancer: a meta-analysis. British journal of clinical pharmacology 62:113-121.

Bosco JL, Palmer JR, Boggs DA, Hatch EE, Rosenberg L. 2011. Regular aspirin use and breast cancer risk in US Black women. Cancer causes & control : CCC 22:1553-1561.

Brasky TM, Bonner MR, Moysich KB, Ambrosone CB, Nie J, Tao MH, et al. 2010. Non-steroidal anti-inflammatory drug (NSAID) use and breast cancer risk in the Western New York Exposures and Breast Cancer (WEB) Study. Cancer causes & control : CCC 21:1503-1512.

Brasky TM, Liu J, White E, Peters U, Potter JD, Walter RB, et al. 2014. Non-steroidal anti-inflammatory drugs and cancer risk in women: results from the Women's Health Initiative. International journal of cancer 135:1869-1883.

Brennan RJ, Schiestl RH. 1997. Aniline and its metabolites generate free radicals in yeast. Mutagenesis 12:215-220.

Browne TR, Szabo GK, Ajami A, Browne DG. 1998. Performance of human mass balance studies with stable isotope-labeled drug and continuous flow-isotope ratio mass spectrometry: a progress report. J Clin Pharmacol 38:309-314.

Bruderer R, Bernhardt OM, Gandhi T, Miladinovic SM, Cheng LY, Messner S, et al. 2015. Extending the limits of quantitative proteome profiling with data-independent

acquisition and application to acetaminophen-treated three-dimensional liver microtissues. Molecular & cellular proteomics : MCP 14:1400-1410.

Brumovsky M, Becanova J, Kohoutek J, Borghini M, Nizzetto L. 2017. Contaminants of emerging concern in the open sea waters of the Western Mediterranean. Environmental pollution (Barking, Essex : 1987) 229:976-983.

Brunborg G, Holme JA, Hongslo JK. 1995. Inhibitory effects of paracetamol on DNA repair in mammalian cells. Mutation research 342:157-170.

Buchard A, Eefsen M, Semb S, Andersen SE, Morling N, Bendtsen F, et al. 2012. The role of the glutathione S-transferase genes GSTT1, GSTM1, and GSTP1 in acetaminophen-poisoned patients. Clinical toxicology (Philadelphia, Pa) 50:27-33.

Budnitz DS, Lovegrove MC, Crosby AE. 2011. Emergency department visits for overdoses of acetaminophen-containing products. American journal of preventive medicine 40:585-592.

Buniva G, Sassella D, Beretta E. 1977. Plasma levels and urinary elimination of diftalone, paracetamol, and their metabolites after single oral administration in man. International journal of clinical pharmacology and biopharmacy 15:460-467.

Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, et al. 2013. Epidemiology and risk factors of urothelial bladder cancer. European urology 63:234-241.

Burke DA, Wedd DJ, Herriott D, Bayliss MK, Spalding DJ, Wilcox P. 1994. Evaluation of pyrazole and ethanol induced S9 fraction in bacterial mutagenicity testing. Mutagenesis 9:23-29.

Burnett CM, Re TA, Rodriguez S, Loehr RF, Dressler WE. 1989. The toxicity of p-aminophenol in the Sprague-Dawley rat: effects on growth, reproduction and foetal development. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 27:691-698.

Cahoon EK, Rajaraman P, Alexander BH, Doody MM, Linet MS, Freedman DM. 2012. Use of nonsteroidal anti-inflammatory drugs and risk of basal cell carcinoma in the United States radiologic technologists study. International journal of cancer 130:2939-2948.

Calder IC, Yong AC, Woods RA, Crowe CA, Ham KN, Tange JD. 1979. The nephrotoxicity of p-aminophenol. II. The effect of metabolic inhibitors and inducers. Chemico-biological interactions 27:245-254.

Camus AM, Friesen M, Croisy A, Bartsch H. 1982. Species-specific activation of phenacetin into bacterial mutagens by hamster liver enzymes and identification of N-hydroxyphenacetin O-glucuronide as a promutagen in the urine. Cancer research 42:3201-3208.

Canadian Cancer Society. 2019. Risk factors for non-melanoma skin cancer. Available: https://www.cancer.ca/en/cancer-information/cancer-type/skin-non-melanoma/risks/?region=on.

Caparrotta TM, Antoine DJ, Dear JW. 2018. Are some people at increased risk of paracetamol-induced liver injury? A critical review of the literature. European journal of clinical pharmacology 74:147-160.

Carmona E, Andreu V, Pico Y. 2017. Multi-residue determination of 47 organic compounds in water, soil, sediment and fish-Turia River as case study. J Pharm Biomed Anal 146:117-125.

Castelao JE, Yuan JM, Gago-Dominguez M, Yu MC, Ross RK. 2000. Non-steroidal anti-inflammatory drugs and bladder cancer prevention. British journal of cancer 82:1364-1369.

Cea Soriano L, Soriano-Gabarro M, Garcia Rodriguez LA. 2016a. Validation of low-dose aspirin prescription data in The Health Improvement Network: how much misclassification due to over-the-counter use? Pharmacoepidemiology and drug safety 25:392-398.

Cea Soriano L, Soriano-Gabarro M, Garcia Rodriguez LA. 2016b. The Protective Effect of Low-Dose Aspirin against Colorectal Cancer Is Unlikely Explained by Selection Bias: Results from Three Different Study Designs in Clinical Practice. PloS one 11:e0159179.

Chang ET, Zheng T, Weir EG, Borowitz M, Mann RB, Spiegelman D, et al. 2004. Aspirin and the risk of Hodgkin's lymphoma in a population-based case-control study. Journal of the National Cancer Institute 96:305-315.

Chen C, Krausz KW, Idle JR, Gonzalez FJ. 2008. Identification of novel toxicity-associated metabolites by metabolomics and mass isotopomer analysis of acetaminophen metabolism in wild-type and Cyp2e1-null mice. The Journal of biological chemistry 283:4543-4559.

Chen C, Krausz KW, Shah YM, Idle JR, Gonzalez FJ. 2009. Serum metabolomics reveals irreversible inhibition of fatty acid beta-oxidation through the suppression of PPARalpha activation as a contributing mechanism of acetaminophen-induced hepatotoxicity. Chemical research in toxicology 22:699-707.

Chen W, Koenigs LL, Thompson SJ, Peter RM, Rettie AE, Trager WF, et al. 1998. Oxidation of acetaminophen to its toxic quinone imine and nontoxic catechol metabolites by baculovirus-expressed and purified human cytochromes P450 2E1 and 2A6. Chemical research in toxicology 11:295-301.

Chen W, Shockcor JP, Tonge R, Hunter A, Gartner C, Nelson SD. 1999. Protein and nonprotein cysteinyl thiol modification by N-acetyl-p-benzoquinone imine via a novel ipso adduct. Biochemistry 38:8159-8166.

Cheung C, Yu AM, Ward JM, Krausz KW, Akiyama TE, Feigenbaum L, et al. 2005. The cyp2e1-humanized transgenic mouse: role of cyp2e1 in acetaminophen hepatotoxicity. Drug metabolism and disposition: the biological fate of chemicals 33:449-457.

Cho E, Curhan G, Hankinson SE, Kantoff P, Atkins MB, Stampfer M, et al. 2011. Prospective evaluation of analgesic use and risk of renal cell cancer. Archives of internal medicine 171:1487-1493.

Choueiri TK, Je Y, Cho E. 2014. Analgesic use and the risk of kidney cancer: a meta-analysis of epidemiologic studies. International journal of cancer 134:384-396.

Chow WH, McLaughlin JK, Linet MS, Niwa S, Mandel JS. 1994. Use of analgesics and risk of renal cell cancer. International journal of cancer 59:467-470.

CIIT. 1982. 104-week chronic toxicitys study in rats aniline hydrochloride finaly report volume I of II. Chemical Industry Institute of Toxicology.

Ciranni R, Barale R, Marrazzini A, Loprieno N. 1988a. Benzene and the genotoxicity of its metabolites. I. Transplacental activity in mouse fetuses and in their dams. Mutation research 208:61-67.

Ciranni R, Barale R, Ghelardini G, Loprieno N. 1988b. Benzene and the genotoxicity of its metabolites. II. The effect of the route of administration on the micronuclei and bone marrow depression in mouse bone marrow cells. Mutation research 209:23-28.

Clark PM, Clark JD, Wheatley T. 1986. Urine discoloration after acetaminophen overdose. Clinical chemistry 32:1777-1778.

Clarke CA, Canchola AJ, Moy LM, Neuhausen SL, Chung NT, Lacey JV, Jr., et al. 2017. Regular and low-dose aspirin, other non-steroidal anti-inflammatory medications and prospective risk of HER2-defined breast cancer: the California Teachers Study. Breast cancer research : BCR 19:52.

Clements JA, Heading RC, Nimmo WS, Prescott LF. 1978. Kinetics of acetaminophen absorption and gastric emptying in man. Clinical pharmacology and therapeutics 24:420-431.

Cliet I, Fournier E, Melcion C, Cordier A. 1989. In vivo micronucleus test using mouse hepatocytes. Mutation research 216:321-326.

Coen M. 2015. Metabolic phenotyping applied to pre-clinical and clinical studies of acetaminophen metabolism and hepatotoxicity. Drug metabolism reviews 47:29-44.

Coles B, Wilson I, Wardman P, Hinson JA, Nelson SD, Ketterer B. 1988. The spontaneous and enzymatic reaction of N-acetyl-p-benzoquinonimine with glutathione: a stopped-flow kinetic study. Archives of biochemistry and biophysics 264:253-260.

Conzelmann A, Reif A, Jacob C, Weyers P, Lesch KP, Lutz B, et al. 2012. A polymorphism in the gene of the endocannabinoid-degrading enzyme FAAH (FAAH C385A) is associated with emotional-motivational reactivity. Psychopharmacology 224:573-579.

Corbett MD, Corbett BR, Hannothiaux MH, Quintana SJ. 1989. Metabolic activation and nucleic acid binding of acetaminophen and related arylamine substrates by the respiratory burst of human granulocytes. Chemical research in toxicology 2:260-266.

Court MH, Duan SX, von Moltke LL, Greenblatt DJ, Patten CJ, Miners JO, et al. 2001. Interindividual variability in acetaminophen glucuronidation by human liver microsomes: identification of relevant acetaminophen UDP-glucuronosyltransferase isoforms. The Journal of pharmacology and experimental therapeutics 299:998-1006.

Court MH, Freytsis M, Wang X, Peter I, Guillemette C, Hazarika S, et al. 2013. The UDP-glucuronosyltransferase (UGT) 1A polymorphism c.2042C>G (rs8330) is associated with increased human liver acetaminophen glucuronidation, increased UGT1A exon 5a/5b splice variant mRNA ratio, and decreased risk of unintentional acetaminophen-induced acute liver failure. The Journal of pharmacology and experimental therapeutics 345:297-307.

Court MH, Peter I, Hazarika S, Vasiadi M, Greenblatt DJ, Lee WM. 2014. Candidate gene polymorphisms in patients with acetaminophen-induced acute liver failure. Drug metabolism and disposition: the biological fate of chemicals 42:28-32.

Court MH, Zhu Z, Masse G, Duan SX, James LP, Harmatz JS, et al. 2017. Race, Gender, and Genetic Polymorphism Contribute to Variability in Acetaminophen Pharmacokinetics, Metabolism, and Protein-Adduct Concentrations in Healthy African-American and European-American Volunteers. The Journal of pharmacology and experimental therapeutics 362:431-440.

Couto AC, Ferreira JD, Pombo-de-Oliveira MS, Koifman S. 2015. Pregnancy, maternal exposure to analgesic medicines, and leukemia in Brazilian children below 2 years of age. European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP) 24:245-252.

Cramer DW, Harlow BL, Titus-Ernstoff L, Bohlke K, Welch WR, Greenberg ER. 1998. Over-the-counter analgesics and risk of ovarian cancer. Lancet (London, England) 351:104-107.

Creton S, Aardema MJ, Carmichael PL, Harvey JS, Martin FL, Newbold RF, et al. 2012. Cell transformation assays for prediction of carcinogenic potential: state of the science and future research needs. Mutagenesis 27:93-101.

Crowe CA, Yong AC, Calder IC, Ham KN, Tange JD. 1979. The nephrotoxicity of p-aminophenol. I. The effect on microsomal cytochromes, glutathione and covalent binding in kidney and liver. Chemico-biological interactions 27:235-243.

Curhan GC, Bullock AJ, Hankinson SE, Willett WC, Speizer FE, Stampfer MJ. 2002. Frequency of use of acetaminophen, nonsteroidal anti-inflammatory drugs, and aspirin in US women. Pharmacoepidemiology and drug safety 11:687-693.

Dahlin DC, Miwa GT, Lu AY, Nelson SD. 1984. N-acetyl-p-benzoquinone imine: a cytochrome P-450-mediated oxidation product of acetaminophen. Proceedings of the National Academy of Sciences of the United States of America 81:1327-1331.

Dale O, Borchgrevink PC, Fredheim OM, Mahic M, Romundstad P, Skurtveit S. 2015. Prevalence of use of non-prescription analgesics in the Norwegian HUNT3 population: Impact of gender, age, exercise and prescription of opioids. BMC public health 15:461.

Dawson PA, Markovich D. 2007. Genetic Polymorphisms of Human Sulfate Transporters. Current Pharmacogenomics 5:262-274.

Dawson PA, Russell CS, Lee S, McLeay SC, van Dongen JM, Cowley DM, et al. 2010. Urolithiasis and hepatotoxicity are linked to the anion transporter Sat1 in mice. The Journal of clinical investigation 120:706-712.

Dawson PA, Sim P, Mudge DW, Cowley D. 2013. Human SLC26A1 gene variants: a pilot study. ScientificWorldJournal 2013:541710.

De Flora S, Camoirano A, Zanacchi P, Bennicelli C. 1984. Mutagenicity testing with TA97 and TA102 of 30 DNA-damaging compounds, negative with other Salmonella strains. Mutation research 134:159-165.

de Morais SM, Wells PG. 1989. Enhanced acetaminophen toxicity in rats with bilirubin glucuronyl transferase deficiency. Hepatology (Baltimore, Md) 10:163-167.

de Morais SM, Uetrecht JP, Wells PG. 1992. Decreased glucuronidation and increased bioactivation of acetaminophen in Gilbert's syndrome. Gastroenterology 102:577-586.

de Pedro M, Baeza S, Escudero MT, Dierssen-Sotos T, Gomez-Acebo I, Pollan M, et al. 2015. Effect of COX-2 inhibitors and other non-steroidal inflammatory drugs on breast cancer risk: a meta-analysis. Breast cancer research and treatment 149:525-536.

Degawa M, Shoji Y, Masuko K, Hashimoto Y. 1979. Mutagenicity of metabolites of carcinogenic aminoazo dyes. Cancer letters 8:71-76.

den Braver-Sewradj SP, den Braver MW, van Dijk M, Zhang Y, Dekker SJ, Wijaya L, et al. 2018. Inter-individual Variability in Activity of the Major Drug Metabolizing Enzymes in Liver Homogenates of 20 Individuals. Current drug metabolism 19:370-381.

Derby LE, Jick H. 1996. Acetaminophen and renal and bladder cancer. Epidemiology (Cambridge, Mass) 7:358-362.

Ding YY, Yao P, Verma S, Han ZK, Hong T, Zhu YQ, et al. 2017. Use of acetaminophen and risk of endometrial cancer: evidence from observational studies. Oncotarget 8:34643-34651.

Divoll M, Greenblatt DJ, Ameer B, Abernethy DR. 1982. Effect of food on acetaminophen absorption in young and elderly subjects. J Clin Pharmacol 22:571-576.

Dix DJ, Houck KA, Martin MT, Richard AM, Setzer RW, Kavlock RJ. 2007. The ToxCast program for prioritizing toxicity testing of environmental chemicals. Toxicological sciences : an official journal of the Society of Toxicology 95:5-12.

Dragovic S, Venkataraman H, Begheijn S, Vermeulen NP, Commandeur JN. 2014. Effect of human glutathione S-transferase hGSTP1-1 polymorphism on the detoxification of reactive metabolites of clozapine, diclofenac and acetaminophen. Toxicology letters 224:272-281.

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, et al. 1985. Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in Salmonella typhimurium and Escherichia coli. Environ Mutagen 7 Suppl 5:1-248.

Dunn TL, Gardiner RA, Seymour GJ, Lavin MF. 1987. Genotoxicity of analgesic compounds assessed by an in vitro micronucleus assay. Mutation research 189:299-306.

Dybing E, Holme JA, Gordon WP, Soderlund EJ, Dahlin DC, Nelson SD. 1984. Genotoxicity studies with paracetamol. Mutation research 138:21-32.

Easterbrook J, Lu C, Sakai Y, Li AP. 2001. Effects of organic solvents on the activities of cytochrome P450 isoforms, UDP-dependent glucuronyl transferase, and phenol sulfotransferase in human hepatocytes. Drug metabolism and disposition: the biological fate of chemicals 29:141-144.

Eastmond DA. 1993. Induction of micronuclei and aneuploidy by the quinone-forming agents benzene and o-phenylphenol. Toxicology letters 67:105-118.

Egan KM, Nabors LB, Thompson ZJ, Rozmeski CM, Anic GA, Olson JJ, et al. 2016. Analgesic use and the risk of primary adult brain tumor. European journal of epidemiology 31:917-925.

Eiche A, Bexell G, Sandelin K. 1990. Genotoxicity of p-aminophenol in somatic and germ line cells of Drosophila melanogaster. Mutation research 240:87-92.

El-Hage AN, Herman EH, Ferrans VJ. 1983. Examination of the protective effect of ICRF-187 and dimethyl sulfoxide against acetaminophen-induced hepatotoxicity in Syrian golden hamsters. Toxicology 28:295-303.

Eliassen AH, Chen WY, Spiegelman D, Willett WC, Hunter DJ, Hankinson SE. 2009. Use of aspirin, other nonsteroidal anti-inflammatory drugs, and acetaminophen and risk of breast cancer among premenopausal women in the Nurses' Health Study II. Archives of internal medicine 169:115-121; discussion 121.

Emeigh Hart SG, Birge RB, Cartun RW, Tyson C, Dabbs JE, Nishanian EV, et al. 1991a. In vivo and in vitro evidence for in situ activation and selective covalent binding of acetaminophen (APAP) in mouse kidney. Adv Exp Med Biol 283:711-716.

Emeigh Hart SG, Beierschmitt WP, Bartolone JB, Wyand DS, Khairallah EA, Cohen SD. 1991b. Evidence against deacetylation and for cytochrome P450-mediated activation in acetaminophen-induced nephrotoxicity in the CD-1 mouse. Toxicology and applied pharmacology 107:1-15.

Emeigh Hart SG, Beierschmitt WP, Wyand DS, Khairallah EA, Cohen SD. 1994. Acetaminophen nephrotoxicity in CD-1 mice. I. Evidence of a role for in situ activation in selective covalent binding and toxicity. Toxicology and applied pharmacology 126:267-275.

Emeigh Hart SG, Wyand DS, Khairallah EA, Cohen SD. 1996. Acetaminophen nephrotoxicity in the CD-1 mouse. II. Protection by probenecid and AT-125 without diminution of renal covalent binding. Toxicology and applied pharmacology 136:161-169.

Epplein M, Nomura AMY, Wilkens LR, Henderson BE, Kolonel LN. 2009. Nonsteroidal antiinflammatory drugs and risk of gastric adenocarcinoma. American journal of epidemiology 170:507-514.

Erexson GL, Wilmer JL, Kligerman AD. 1985. Sister chromatid exchange induction in human lymphocytes exposed to benzene and its metabolites in vitro. Cancer research 45:2471-2477.

Erickson P, Gardner LD, Loffredo CA, St. George DM, Bowman ED, Deepak J, et al. 2018. Racial and ethnic differences in the relationship between aspirin use and non–small cell lung cancer risk and survival. Cancer Epidemiology Biomarkers and Prevention 27:1518-1526.

Ersboll AS, Hedegaard M, Damm P, Johansen M, Tabor A, Hegaard HK. 2015. Changes in the pattern of paracetamol use in the periconception period in a Danish cohort. Acta obstetricia et gynecologica Scandinavica 94:898-903.

EU-SCCS. 2011. SCCS (Scientific Committee on Consumer Safety), Opinion on p-aminophenol. Brussels:European Union. Available: https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_078.pdf.

Fairfield KM, Hunter DJ, Fuchs CS, Colditz GA, Hankinson SE. 2002. Aspirin, other NSAIDs, and ovarian cancer risk (United States). Cancer causes & control : CCC 13:535-542.

Fannin RD, Russo M, O'Connell TM, Gerrish K, Winnike JH, Macdonald J, et al. 2010. Acetaminophen dosing of humans results in blood transcriptome and metabolome changes consistent with impaired oxidative phosphorylation. Hepatology (Baltimore, Md) 51:227-236.

Fannin RD, Gerrish K, Sieber SO, Bushel PR, Watkins PB, Paules RS. 2016. Blood transcript immune signatures distinguish a subset of people with elevated serum ALT from others given acetaminophen. Clinical pharmacology and therapeutics 99:432-441.

Finn L, Sproat L, Heckman MG, Jiang L, Diehl NN, Ketterling R, et al. 2015. Epidemiology of adult acute myeloid leukemia: Impact of exposures on clinical phenotypes and outcomes after therapy. Cancer epidemiology 39:1084-1092.

Fischer V, West PR, Nelson SD, Harvison PJ, Mason RP. 1985. Formation of 4-aminophenoxyl free radical from the acetaminophen metabolite N-acetyl-p-benzoquinone imine. The Journal of biological chemistry 260:11446-11450.

Fisher MB, Vandenbranden M, Findlay K, Burchell B, Thummel KE, Hall SD, et al. 2000. Tissue distribution and interindividual variation in human UDP-glucuronosyltransferase activity: relationship between UGT1A1 promoter genotype and variability in a liver bank. Pharmacogenetics 10:727-739.

Flaks A. 1968. The susceptibility of various strains of neonatal mice to the carcinogenic effects of 9,10-dimethyl-1,2-benzanthracene. European Journal of Cancer (1965) 4:579-585.

Flaks A, Flaks B. 1982. 3-Methylcholanthrene-inhibition of hepatocarcinogenesis in the rat due to 3′ -methyl-4-dimethylaminoazobenzene or 2-acetylaminofluorene: a comparative study. Carcinogenesis 3:981-991.

Flaks A, Flaks B. 1983. Induction of liver cell tumours in IF mice by paracetamol. Carcinogenesis 4:363-368.

Flaks B. 1978. Effects of chronic oral dosing with quinine sulphate in the rat. Pathol Res Pract 163:373-377.

Flaks B, Flaks A, Shaw AP. 1985. Induction by paracetamol of bladder and liver tumours in the rat. Effects on hepatocyte fine structure. Acta Pathol Microbiol Immunol Scand A 93:367-377.

Foreman BD, Tarloff JB. 2008. Contribution of reactive oxygen species to para-aminophenol toxicity in LLC-PK1 cells. Toxicology and applied pharmacology 230:144-149.

Forrest JA, Clements JA, Prescott LF. 1982. Clinical pharmacokinetics of paracetamol. Clinical pharmacokinetics 7:93-107.

Forte AJ, Wilson JM, Slattery JT, Nelson SD. 1984. The formation and toxicity of catechol metabolites of acetaminophen in mice. Drug metabolism and disposition: the biological fate of chemicals 12:484-491.

Fortuny J, Kogevinas M, Garcia-Closas M, Real FX, Tardon A, Garcia-Closas R, et al. 2006. Use of analgesics and nonsteroidal anti-inflammatory drugs, genetic predisposition, and bladder cancer risk in Spain. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 15:1696-1702.

Fortuny J, Kogevinas M, Zens MS, Schned A, Andrew AS, Heaney J, et al. 2007. Analgesic and anti-inflammatory drug use and risk of bladder cancer: a population based case control study. BMC urology 7:13.

Fowler LM, Moore RB, Foster JR, Lock EA. 1991. Nephrotoxicity of 4-aminophenol glutathione conjugate. Human & experimental toxicology 10:451-459.

Fowler LM, Foster JR, Lock EA. 1993. Effect of ascorbic acid, acivicin and probenecid on the nephrotoxicity of 4-aminophenol in the Fischer 344 rat. Archives of toxicology 67:613-621.

Fowler LM, Foster JR, Lock EA. 1994. Nephrotoxicity of 4-amino-3-S-glutathionylphenol and its modulation by metabolism or transport inhibitors. Archives of toxicology 68:15-23.

Fram MS, Belitz K. 2011. Occurrence and concentrations of pharmaceutical compounds in groundwater used for public drinking-water supply in California. The Science of the total environment 409:3409-3417.

Friedman GD. 1982. Phenylbutazone, musculoskeletal disease, and leukemia. Journal of chronic diseases 35:233-243.

Friel G, Liu CS, Kolomeyevskaya NV, Hampras SS, Kruszka B, Schmitt K, et al. 2015. Aspirin and Acetaminophen Use and the Risk of Cervical Cancer. Journal of lower genital tract disease 19:189-193.

Friis S, Nielsen GL, Mellemkjaer L, McLaughlin JK, Thulstrup AM, Blot WJ, et al. 2002. Cancer risk in persons receiving prescriptions for paracetamol: a Danish cohort study. International journal of cancer 97:96-101.

Friis S, Thomassen L, Sorensen HT, Tjonneland A, Overvad K, Cronin-Fenton DP, et al. 2008. Nonsteroidal anti-inflammatory drug use and breast cancer risk: a Danish cohort study. European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP) 17:88-96.

Friis S, Poulsen AH, Sørensen HT, Tjønneland A, Overvad K, Vogel U, et al. 2009. Aspirin and other non-steroidal anti-inflammatory drugs and risk of colorectal cancer: A Danish cohort study. Cancer Causes and Control 20:731-740.

Frith CH, Ward JM, Turusov VS. 1994. Tumours of the liver. In: IARC Scientific Publications No 111 Pathology of tumours in laboratory animals Volume II - Tumours of the mouse, (Turusov VS, Mohr U, eds). Lyon, France, 223-269.

Frith CH, Eighmy JJ, Fukushima S, Cohen SM, Squire RA, Chandra M. 1995. Proliferative lesions in the urinary tract in rats. In: Guides for toxicologic pathology. Washington, D.C.:STP/ARP/AFIP, 1-13.

Gago-Dominguez M, Yuan JM, Castelao JE, Ross RK, Yu MC. 1999. Regular use of analgesics is a risk factor for renal cell carcinoma. British journal of cancer 81:542-548.

Gaillard ET. 1999. Ureter, urinary bladder, and urethra. In: Pathology of the mouse Reference and atlas, (Maronpot RR, Boorman GA, Gaul BW, eds):Cache River Press, 235-258.

Gallicchio L, McSorley MA, Newschaffer CJ, Thuita LW, Huang H-Y, Hoffman SC, et al. 2006. Nonsteroidal antiinflammatory drugs, cyclooxygenase polymorphisms, and the risk of developing breast carcinoma among women with benign breast disease. Cancer (Hoboken, NJ, U S) 106:1443-1452.

Gallicchio L, Visvanathan K, Burke A, Hoffman SC, Helzlsouer KJ. 2007. Nonsteroidal anti-inflammatory drugs and the risk of developing breast cancer in a population-based prospective cohort study in Washington County, MD. International journal of cancer 121:211-215.

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, et al. 1987. Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals. Environmental and molecular mutagenesis 10 Suppl 10:1-175.

Gamba CA, Swetter SM, Stefanick ML, Kubo J, Desai M, Spaunhurst KM, et al. 2013. Aspirin is associated with lower melanoma risk among postmenopausal Caucasian women: the Women's Health Initiative. Cancer 119:1562-1569.

Garberg P, Akerblom EL, Bolcsfoldi G. 1988. Evaluation of a genotoxicity test measuring DNA-strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution. Mutation research 203:155-176.

Garcia Rodriguez LA, Perez Gutthann S. 1998. Use of the UK General Practice Research Database for pharmacoepidemiology. British journal of clinical pharmacology 45:419-425.

Garcia Rodriguez LA, Huerta-Alvarez C. 2001. Reduced risk of colorectal cancer among long-term users of aspirin and nonaspirin nonsteroidal antiinflammatory drugs. Epidemiology (Cambridge, Mass) 12:88-93.

Garcia Rodriguez LA, Gonzalez-Perez A. 2004a. Risk of breast cancer among users of aspirin and other anti-inflammatory drugs. British journal of cancer 91:525-529.

Garcia Rodriguez LA, Gonzalez-Perez A. 2004b. Inverse association between nonsteroidal anti-inflammatory drugs and prostate cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 13:649-653.

Garner RC, Nutman CA. 1977. Testing of some azo dyes and their reduction products for mutagenicity using Salmonella typhimurium TA 1538. Mutation research 44:9-19.

Gartland KP, Bonner FW, Timbrell JA, Nicholson JK. 1989. Biochemical characterisation of para-aminophenol-induced nephrotoxic lesions in the F344 rat. Archives of toxicology 63:97-106.

Gartland KP, Eason CT, Bonner FW, Nicholson JK. 1990. Effects of biliary cannulation and buthionine sulphoximine pretreatment on the nephrotoxicity of para-aminophenol in the Fischer 344 rat. Archives of toxicology 64:14-25.

Gaskell M, McLuckie KI, Farmer PB. 2005. Genotoxicity of the benzene metabolites para-benzoquinone and hydroquinone. Chemico-biological interactions 153-154:267-270.

Gelotte CK, Auiler JF, Lynch JM, Temple AR, Slattery JT. 2007. Disposition of acetaminophen at 4, 6, and 8 g/day for 3 days in healthy young adults. Clinical pharmacology and therapeutics 81:840-848.

Gemborys MW, Mudge GH. 1981. Formation and disposition of the minor metabolites of acetaminophen in the hamster. Drug metabolism and disposition: the biological fate of chemicals 9:340-351.

Genkinger JM, De Vivo I, Stampfer MJ, Giovannucci E, Michaud DS. 2007. Nonsteroidal antiinflammatory drug use and risk of bladder cancer in the health professionals follow-up study. International journal of cancer 120:2221-2225.

Ghanem CI, Ruiz ML, Villanueva SS, Luquita MG, Catania VA, Jones B, et al. 2005. Shift from biliary to urinary elimination of acetaminophen-glucuronide in acetaminophen-pretreated rats. The Journal of pharmacology and experimental therapeutics 315:987-995.

Ghosh J, Das J, Manna P, Sil PC. 2010. Acetaminophen induced renal injury via oxidative stress and TNF-α production: Therapeutic potential of arjunolic acid. Toxicology 268:8-18.

Gill JK, Maskarinec G, Wilkens LR, Pike MC, Henderson BE, Kolonel LN. 2007. Nonsteroidal antiinflammatory drugs and breast cancer risk: the multiethnic cohort. American journal of epidemiology 166:1150-1158.

Giri AK, Sivam SS, Khan KA. 1992. Sister-chromatid exchange and chromosome aberrations induced by paracetamol in vivo in bone-marrow cells of mice. Mutation research 278:253-258.

Glatt H, Gemperlein I, Setiabudi F, Platt KL, Oesch F. 1990. Expression of xenobiotic-metabolizing enzymes in propagatable cell cultures and induction of micronuclei by 13 compounds. Mutagenesis 5:241-249.

Godfrey E, Woessner WW, Benotti MJ. 2007. Pharmaceuticals in on-site sewage effluent and ground water, Western Montana. Ground water 45:263-271.

Goedert JJ, Sampson JN, Moore SC, Xiao Q, Xiong X, Hayes RB, et al. 2014. Fecal metabolomics: assay performance and association with colorectal cancer. Carcinogenesis 35:2089-2096.

Gold LS, Slone TH, Manley NB, Garfinkel GB, Rohrbach L, Ames BN. 1997. Chapter 1. The Carcinogenic Potency Database. In: Handbook of carcinogenic potency and genotoxicity databases, (Gold LS, Zeiger E, eds):CRC Press.

Gonzalez FJ. 2007. The 2006 Bernard B. Brodie Award Lecture. Cyp2e1. Drug metabolism and disposition: the biological fate of chemicals 35:1-8.

Grafstrom R, Ormstad K, Moldeus P, Orrenius S. 1979. Paracetamol metabolism in the isolated perfused rat liver with further metabolism of a biliary paracetamol conjugate by the small intestine. Biochemical pharmacology 28:3573-3579.

Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF. 2013. The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. Inflammopharmacology 21:201-232.

Greenland S, Rothman KJ. 2008. Introduction to stratified analysis. In: Modern Epidemiology, (Rothman KJ, Greenland S, Lash TL, eds):Lippincott Williams & Williams.

Gregoire N, Hovsepian L, Gualano V, Evene E, Dufour G, Gendron A. 2007. Safety and pharmacokinetics of paracetamol following intravenous administration of 5 g during the first 24 h with a 2-g starting dose. Clinical pharmacology and therapeutics 81:401-405.

Gregus Z, Madhu C, Klaassen CD. 1988. Species variation in toxication and detoxication of acetaminophen in vivo: a comparative study of biliary and urinary excretion of acetaminophen metabolites. The Journal of pharmacology and experimental therapeutics 244:91-99.

Guengerich FP, Avadhani NG. 2018. Roles of Cytochrome P450 in Metabolism of Ethanol and Carcinogens. Advances in experimental medicine and biology 1032:15-35.

Hagiwara A, Ward JM. 1986. The chronic hepatotoxic, tumor-promoting, and carcinogenic effects of acetaminophen in male B6C3F1 mice. Fundam Appl Toxicol 7:376-386.

Hanigan MH. 2014. Gamma-glutamyl transpeptidase: redox regulation and drug resistance. Advances in cancer research 122:103-141.

Hannibal CG, Rossing MA, Wicklund KG, Cushing-Haugen KL. 2008. Analgesic drug use and risk of epithelial ovarian cancer. American journal of epidemiology 167:1430-1437.

Hannibal CG, Dehlendorff C, Kjaer SK. 2018. Use of paracetamol, low-dose aspirin, or non-aspirin non-steroidal anti-inflammatory drugs and risk of ovarian borderline tumors in Denmark. Gynecologic oncology 151:513-518.

Hansen TG, O'Brien K, Morton NS, Rasmussen SN. 1999. Plasma paracetamol concentrations and pharmacokinetics following rectal administration in neonates and young infants. Acta anaesthesiologica Scandinavica 43:855-859.

Hantson P, de Saint-Georges L, Mahieu P, Leonard ED, Crutzen-Fayt MC, Leonard A. 1996. Evaluation of the ability of paracetamol to produce chromosome aberrations in man. Mutation research 368:293-300.

Harada T, Enomoto A, Boorman GA, Maronpot RR. 1999. Liver and gallbladder. In: Pathology of the mouse Reference and atlas, (Maronpot RR, Boorman GA, Gaul BW, eds):Cache River Press, 119-183.

Hardell L, Fredrikson M, Axelson O. 1996. Case-control study on colon cancer regarding previous diseases and drug intake. International journal of oncology 8:439-444.

Harris RE, Kasbari S, Farrar WB. 1999. Prospective study of nonsteroidal anti-inflammatory drugs and breast cancer. Oncology reports 6:71-73.

Harris RE, Chlebowski RT, Jackson RD, Frid DJ, Ascenseo JL, Anderson G, et al. 2003. Breast cancer and nonsteroidal anti-inflammatory drugs: prospective results from the Women's Health Initiative. Cancer research 63:6096-6101.

Harris RE, Beebe-Donk J, Alshafie GA. 2006. Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors. BMC cancer 6:27.

Harris RE, Beebe-Donk J, Alshafie GA. 2007. Reduced risk of human lung cancer by selective cyclooxygenase 2 (COX-2) blockade: results of a case control study. International journal of biological sciences 3:328-334.

Harris RE, Beebe-Donk J, Alshafie GA. 2008. Similar reductions in the risk of human colon cancer by selective and nonselective cyclooxygenase-2 (COX-2) inhibitors. BMC cancer 8:237.

Harvison PJ, Guengerich FP, Rashed MS, Nelson SD. 1988. Cytochrome P-450 isozyme selectivity in the oxidation of acetaminophen. Chemical research in toxicology 1:47-52.

Hasegawa R, Furukawa F, Toyoda K, Jang JJ, Yamashita K, Sato S, et al. 1988. Study for tumor-initiating effect of acetaminophen in two-stage liver carcinogenesis of male F344 rats. Carcinogenesis 9:755-759.

Haworth S, Lawlor T, Mortelmans K, Speck W, Zeiger E. 1983. Salmonella mutagenicity test results for 250 chemicals. Environ Mutagen 5 Suppl 1:1-142.

Hayashi S, Watanabe J, Kawajiri K. 1991. Genetic polymorphisms in the 5'-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. J Biochem 110:559-565.

Hayes JD, Strange RC. 2000. Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology 61:154-166.

Hayward NK, Lavin MF, Craswell PW. 1982. Inhibition of DNA synthesis and alteration to DNA structure by the phenacetin analog p-aminophenol. Biochemical pharmacology 31:1425-1429.

Hazai E, Vereczkey L, Monostory K. 2002. Reduction of toxic metabolite formation of acetaminophen. Biochemical and biophysical research communications 291:1089-1094.

Heading RC, Nimmo J, Prescott LF, Tothill P. 1973. The dependence of paracetamol absorption on the rate of gastric emptying. British journal of pharmacology 47:415-421.

Hellmer L, Bolcsfoldi G. 1992. An evaluation of the E. coli K-12 uvrB/recA DNA repair host-mediated assay. I. In vitro sensitivity of the bacteria to 61 compounds. Mutation research 272:145-160.

Hickman D, Wang JP, Wang Y, Unadkat JD. 1998. Evaluation of the selectivity of In vitro probes and suitability of organic solvents for the measurement of human cytochrome P450 monooxygenase activities. Drug metabolism and disposition: the biological fate of chemicals 26:207-215.

Higgins JP, Thompson SG, Deeks JJ, Altman DG. 2003. Measuring inconsistency in meta-analyses. BMJ (Clinical research ed) 327:557-560.

Hinson JA, Pohl LR, Monks TJ, Gillette JR, Guengerich FP. 1980. 3-Hydroxyacetaminophen: a microsomal metabolite of acetaminophen. Evidence against an epoxide as the reactive metabolite of acetaminophen. Drug metabolism and disposition: the biological fate of chemicals 8:289-294.

Hinson JA, Monks TJ, Hong M, Highet RJ, Pohl LR. 1982. 3-(glutathion-S-yl)acetaminophen: a biliary metabolite of acetaminophen. Drug metabolism and disposition: the biological fate of chemicals 10:47-50.

Hinson JA. 1983. Reactive metabolites of phenacetin and acetaminophen: a review. Environmental health perspectives 49:71-79.

Hinson JA, Reid AB, McCullough SS, James LP. 2004. Acetaminophen-induced hepatotoxicity: role of metabolic activation, reactive oxygen/nitrogen species, and mitochondrial permeability transition. Drug metabolism reviews 36:805-822.

Hiraga K, Fujii T. 1985. Carcinogenicity testing of acetaminophen in F344 rats. Japanese journal of cancer research : Gann 76:79-85.

Hjelle JJ, Klaassen CD. 1984. Glucuronidation and biliary excretion of acetaminophen in rats. The Journal of pharmacology and experimental therapeutics 228:407-413.

Hoffmann KJ, Axworthy DB, Baillie TA. 1990. Mechanistic studies on the metabolic activation of acetaminophen in vivo. Chemical research in toxicology 3:204-211.

Hogestatt ED, Jonsson BA, Ermund A, Andersson DA, Bjork H, Alexander JP, et al. 2005. Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. The Journal of biological chemistry 280:31405-31412.

Hoivik DJ, Manautou JE, Tveit A, Hart SG, Khairallah EA, Cohen SD. 1995. Gender-related differences in susceptibility to acetaminophen-induced protein arylation and nephrotoxicity in the CD-1 mouse. Toxicology and applied pharmacology 130:257-271.

Holme JA, Soderlund E. 1986. Species differences in cytotoxic and genotoxic effects of phenacetin and paracetamol in primary monolayer cultures of hepatocytes. Mutation research 164:167-175.

Holme JA, Hongslo JK, Bjornstad C, Harvison PJ, Nelson SD. 1988. Toxic effects of paracetamol and related structures in V79 Chinese hamster cells. Mutagenesis 3:51-56.

Holme JA, Hongslo JK, Bjorge C, Nelson SD. 1991. Comparative cytotoxic effects of acetaminophen (N-acetyl-p-aminophenol), a non-hepatotoxic regioisomer acetyl-m-aminophenol and their postulated reactive hydroquinone and quinone metabolites in monolayer cultures of mouse hepatocytes. Biochemical pharmacology 42:1137-1142.

Holmer Pettersson P, Owall A, Jakobsson J. 2004. Early bioavailability of paracetamol after oral or intravenous administration. Acta anaesthesiologica Scandinavica 48:867-870.

Hongslo JK, Christensen T, Brunborg G, Bjornstad C, Holme JA. 1988. Genotoxic effects of paracetamol in V79 Chinese hamster cells. Mutation research 204:333-341.

Hongslo JK, Bjorge C, Schwarze PE, Brogger A, Mann G, Thelander L, et al. 1990. Paracetamol inhibits replicative DNA synthesis and induces sister chromatid exchange and chromosomal aberrations by inhibition of ribonucleotide reductase. Mutagenesis 5:475-480.

Hongslo JK, Brogger A, Bjorge C, Holme JA. 1991. Increased frequency of sister-chromatid exchange and chromatid breaks in lymphocytes after treatment of human volunteers with therapeutic doses of paracetamol. Mutation research 261:1-8.

Hongslo JK, Brunborg G, Steffensen IL, Holme JA. 1993. Paracetamol inhibits UV-induced DNA repair in resting human mononuclear blood cells in vitro. Mutagenesis 8:423-429.

Hongslo JK, Holme JA. 1994. Genotoxic effects of paracetamol. TIDSSKR NOR LAEGEFOREN 114:1204-1206.

Hongslo JK, Smith CV, Brunborg G, Soderlund EJ, Holme JA. 1994. Genotoxicity of paracetamol in mice and rats. Mutagenesis 9:93-100.

Hossack DJ, Richardson JC. 1977. Examination of the potential mutagenicity of hair dye constituents using the micronucleus test. Experientia 33:377-378.

HSDB. 2014. Dimethyl Sulfoxide. CASRN: 67-68-5. Hazardous Substances Data Bank. Reviewed by SRP on 9/9/2014. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@DOCNO+80.

Hu DG, Mackenzie PI, McKinnon RA, Meech R. 2016. Genetic polymorphisms of human UDP-glucuronosyltransferase (UGT) genes and cancer risk. Drug metabolism reviews 48:47-69.

Hu JJ, Lee MJ, Vapiwala M, Reuhl K, Thomas PE, Yang CS. 1993. Sex-related differences in mouse renal metabolism and toxicity of acetaminophen. Toxicology and applied pharmacology 122:16-26.

Hustert E, Haberl M, Burk O, Wolbold R, He YQ, Klein K, et al. 2001. The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics 11:773-779.

IARC. 1980. Some pharmaceutical drugs. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 24). Lyon, France. Available: http://publications.iarc.fr/42.

IARC. 1987. Aniline. IARC Monographs Supplement 7. (Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1–42). Lyon, France. Available: https://publications.iarc.fr/_publications/media/download/3304/3026469d63eef51733ef5d008a9434552aacee02.pdf.

IARC. 1990. Paracetamol (acetaminophen). Pharmaceutical Drugs. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 50). Available: http://publications.iarc.fr/68.

IARC. 1999a. Paracetamol. Some Chemicals that Cause Tumours of the Kidney or Urinary Bladder in Rodents and Some Other Substances. (IARC monographs on the evaluation of carcinogenic risks to humans). Available: https://publications.iarc.fr/91.

IARC. 1999b. 1,4-Benzoquinone (para-quinone). Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide (Part 1, Part 2, Part 3). (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 71). Available: https://publications.iarc.fr/89.

IARC. 2012. Phenacetin. Pharmaceuticals. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 100A). Available: http://monographs.iarc.fr/ENG/Monographs/vol100A/mono100A-24.pdf.

IARC. 2017. IARC Monographs: Instructions to Authors. Originally posted on December 4, 2014. Updated on March 10, 2017. Lyon, France: International Agency for Research on Cancer. Available: http://monographs.iarc.fr/ENG/Preamble/previous/Instructions_to_Authors_S4.pdf.

IARC. 2018. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 120). Available: http://publications.iarc.fr/576.

IARC. 2019a. The IARC Monographs: Updated procedures for modern and transparent evidence synthesis in cancer hazard identification. Journal of the National Cancer Institute.

IARC. 2019b. List of Classifications by cancer sites with sufficient or limited evidence in humans, Volumes 1 to 124. Lyon, France: International Agency for Research on Cancer. Available: https://monographs.iarc.fr/wp-content/uploads/2019/07/Classifications_by_cancer_site.pdf.

Ibanez Y, Rodriguez JM, Lujan M, Grattan TJ, Martin AJ, Burnett I. 2006. A pharmacokinetic study investigating the rate of absorption of a 500 mg dose of a rapidly absorbed paracetamol tablet and a standard paracetamol tablet. Current medical research and opinion 22:1893-1897.

Ibrulj S, Rahmanovic A, Haveric S, Haveric A, Pasic AD. 2007. Cytogenetic evaluation of paracetamol effects in human lymphocytes culture. Drug and chemical toxicology 30:133-143.

ICD9Data. ICD9Data.com. Available: http://www.icd9data.com/2012/Volume1/140-239/179-189/default.htm.

Idkaidek N, Arafat T. 2014. Saliva vs. plasma bioequivalence of paracetamol in humans: validation of class I drugs of the salivary excretion classification system. Drug research 64:559-562.

Imamura A, Kurumi Y, Danzuka T, Kodama M, Kawachi T, Nagao M. 1983. Classification of compounds by cluster analysis of Ames test data. Gan 74:196-204.

Ishidate M, Jr H, M., Sawada M, Matsuoka A, Yoshikwa K, Ono M, et al. 1978. Cyotoxicity test on medical drugs-chromosome aberrtion tests with Chinese hamster cells in vitro. Bull natl Inst Hyg Sci (Tokyo) 96:55-61.

Ishidate M, Jr., Harnois MC, Sofuni T. 1988. A comparative analysis of data on the clastogenicity of 951 chemical substances tested in mammalian cell cultures. Mutation research 195:151-213.

Ishihama M, Toyooka T, Ibuki Y. 2008. Generation of phosphorylated histone H2AX by benzene metabolites. Toxicology in vitro : an international journal published in association with BIBRA 22:1861-1868.

Ishmael J, Dugard PH. 2006. A review of perchloroethylene and rat mononuclear cell leukemia. Regul Toxicol Pharmacol 45:178-184.

Islami F, Miller KD, Siegel RL, Fedewa SA, Ward EM, Jemal A. 2017. Disparities in liver cancer occurrence in the United States by race/ethnicity and state. CA: a cancer journal for clinicians 67:273-289.

Jackson CH, MacDonald NC, Cornett JW. 1984. Acetaminophen: a practical pharmacologic overview. Canadian Medical Association journal 131:25-32, 37.

Jacobs EJ, Newton CC, Stevens VL, Gapstur SM. 2011. A large cohort study of long-term acetaminophen use and prostate cancer incidence. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 20:1322-1328.

Jaeschke H, Cover C, Bajt ML. 2006. Role of caspases in acetaminophen-induced liver injury. Life sciences 78:1670-1676.

Jaeschke H, McGill MR, Ramachandran A. 2012. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. Drug metabolism reviews 44:88-106.

Jamil I, Symonds A, Lynch S, Alalami O, Smyth M, Martin J. 1999. Divergent effects of paracetamol on reactive oxygen intermediate and reactive nitrogen intermediate production by U937 cells. International journal of molecular medicine 4:309-312.

Jannuzzi AT, Kara M, Alpertunga B. 2018. Celastrol ameliorates acetaminophen-induced oxidative stress and cytotoxicity in HepG2 cells. Hum Exp Toxicol 37:742-751.

Jasiewicz ML, Richardson JC. 1987. Absence of mutagenic activity of benorylate, paracetamol and aspirin in the Salmonella/mammalian microsome test. Mutation research 190:95-100.

Jawahar R, Yang S, Eaton CB, McAlindon T, Lapane KL. 2012. Gender-specific correlates of complementary and alternative medicine use for knee osteoarthritis. Journal of women's health (2002) 21:1091-1099.

Jayasinghe KS, Roberts CJ, Read AE. 1986. Is biliary excretion of paracetamol significant in man? British journal of clinical pharmacology 22:363-366.

Jeffery EH, Haschek WM. 1988. Protection by dimethylsulfoxide against acetaminophen-induced hepatic, but not respiratory toxicity in the mouse. Toxicology and applied pharmacology 93:452-461.

Jeter JM, Bonner JD, Johnson TM, Gruber SB. 2011. Nonsteroidal anti-inflammatory drugs and risk of melanoma. Journal of Skin Cancer.

Jeter JM, Han J, Martinez ME, Alberts DS, Qureshi AA, Feskanich D. 2012. Non-steroidal anti-inflammatory drugs, acetaminophen, and risk of skin cancer in the Nurses' Health Study. Cancer causes & control : CCC 23:1451-1461.

Jetten MJ, Gaj S, Ruiz-Aracama A, de Kok TM, van Delft JH, Lommen A, et al. 2012. 'Omics analysis of low dose acetaminophen intake demonstrates novel response pathways in humans. Toxicology and applied pharmacology 259:320-328.

Jetten MJ, Claessen SM, Dejong CH, Lahoz A, Castell JV, van Delft JH, et al. 2014. Interindividual variation in response to xenobiotic exposure established in precision-cut human liver slices. Toxicology 323:61-69.

Jick H. 1997. A database worth saving. Lancet (London, England) 350:1045-1046.

Johansson SL. 1981. Carcinogenicity of analgesics: long-term treatment of Sprague-Dawley rats with phenacetin, phenazone, caffeine and paracetamol (acetamidophen). International journal of cancer 27:521-529.

Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR, Brodie BB. 1973. Acetaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo. The Journal of pharmacology and experimental therapeutics 187:195-202.

Joseph KS, Mehrabadi A, Lisonkova S. 2014. Confounding by indication and related concepts. Curr Epidemiol Rep 1:1-8.

Josephy PD, Eling TE, Mason RP. 1983. Oxidation of p-aminophenol catalyzed by horseradish peroxidase and prostaglandin synthase. Molecular pharmacology 23:461-466.

Josephy PD. 2005. The molecular toxicology of acetaminophen. Drug metabolism reviews 37:581-594.

Judson RS, Houck KA, Kavlock RJ, Knudsen TB, Martin MT, Mortensen HM, et al. 2010. In vitro screening of environmental chemicals for targeted testing prioritization: the ToxCast project. Environmental health perspectives 118:485-492.

Kamali F, Fry JR, Bell GD. 1987. Temporal variations in paracetamol absorption and metabolism in man. Xenobiotica; the fate of foreign compounds in biological systems 17:635-641.

Kanaya N. 1990. Induction of chromosome damage by aniline and its C-hydroxylated metabolites in the root cells of Vicia faba. Mutation research 245:311-315.

Kandemir FM, Kucukler S, Eldutar E, Caglayan C, Gulcin I. 2017. Chrysin Protects Rat Kidney from Paracetamol-Induced Oxidative Stress, Inflammation, Apoptosis, and Autophagy: A Multi-Biomarker Approach. Scientia pharmaceutica 85.

Kanki K, Nishikawa A, Masumura K, Umemura T, Imazawa T, Kitamura Y, et al. 2005. In vivo mutational analysis of liver DNA in gpt delta transgenic rats treated with the hepatocarcinogens N-nitrosopyrrolidine, 2-amino-3-methylimidazo[4,5-f]quinoline, and di(2-ethylhexyl)phthalate. Molecular carcinogenesis 42:9-17.

Karami S, Daughtery SE, Schwartz K, Davis FG, Ruterbusch JJ, Wacholder S, et al. 2016. Analgesic use and risk of renal cell carcinoma: A case-control, cohort and meta-analytic assessment. International journal of cancer 139:584-592.

Kato I, Koenig KL, Shore RE, Baptiste MS, Lillquist PP, Frizzera G, et al. 2002. Use of anti-inflammatory and non-narcotic analgesic drugs and risk of non-Hodgkin's lymphoma (NHL) (United States). Cancer causes & control : CCC 13:965-974.

Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N, et al. 2012. Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. Chemical research in toxicology 25:1287-1302.

Kaye JA, Myers MW, Jick H. 2001. Acetaminophen and the risk of renal and bladder cancer in the general practice research database. Epidemiology (Cambridge, Mass) 12:690-694.

Kehm RD, Hopper JL, John EM, Phillips KA, MacInnis RJ, Dite GS, et al. 2019. Regular use of aspirin and other non-steroidal anti-inflammatory drugs and breast cancer risk for women at familial or genetic risk: a cohort study. Breast cancer research : BCR 21:52.

Kennedy JM, van Rij AM. 2006. Drug absorption from the small intestine in immediate postoperative patients. British journal of anaesthesia 97:171-180.

Kho PF, Fawcett J, Fritschi L, Risch H, Webb PM, Whiteman DC, et al. 2016. Nonsteroidal anti-inflammatory drugs, statins, and pancreatic cancer risk: a population-based case-control study. Cancer causes & control : CCC 27:1457-1464.

King MT, Beikirch H, Eckhardt K, Gocke E, Wild D. 1979. Mutagenicity studies with x-ray-contrast media, analgesics, antipyretics, antirheumatics and some other pharmaceutical drugs in bacterial, Drosophila and mammalian test systems. Mutation research 66:33-43.

Kirchner G, Bayer U. 1982. Genotoxic activity of the aminophenols as evidenced by the induction of sister chromatid exchanges. Human toxicology 1:387-392.

Kirkland DJ, Dresp JH, Marshall RR, Baumeister M, Gerloff C, Gocke E. 1992. Normal chromosomal aberration frequencies in peripheral lymphocytes of healthy human volunteers exposed to a maximum daily dose of paracetamol in a double blind trial. Mutation research 279:181-194.

Klopcic I, Poberznik M, Mavri J, Dolenc MS. 2015. A quantum chemical study of the reactivity of acetaminophen (paracetamol) toxic metabolite N-acetyl-p-benzoquinone imine with deoxyguanosine and glutathione. Chemico-biological interactions 242:407-414.

Klos C, Koob M, Kramer C, Dekant W. 1992. p-aminophenol nephrotoxicity: biosynthesis of toxic glutathione conjugates. Toxicology and applied pharmacology 115:98-106.

Klutch A, Levin W, Chang RL, Vane F, Conney AH. 1978. Formation of a thiomethyl metabolite of phenacetin and acetaminophen in dogs and man. Clinical pharmacology and therapeutics 24:287-293.

Knox JH, Jurand J. 1977. Determination of paracetamol and its metabolites in urine by high-performance liquid chromatography using reversed-phase bonded supports. Journal of chromatography 142:651-670.

Kocisova J, Rossner P, Binkova B, Bavorova H, Sram RJ. 1988. Mutagenicity studies on paracetamol in human volunteers. I. Cytogenetic analysis of peripheral lymphocytes and lipid peroxidation in plasma. Mutation research 209:161-165.

Kocisova J, Sram RJ. 1990. Mutagenicity studies on paracetamol in human volunteers. III. Cytokinesis block micronucleus method. Mutation research 244:27-30.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, et al. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. Environmental science & technology 36:1202-1211.

Koonrungsesomboon N, Khatsri R, Wongchompoo P, Teekachunhatean S. 2018. The impact of genetic polymorphisms on CYP1A2 activity in humans: a systematic review and meta-analysis. The pharmacogenomics journal 18:760-768.

Koop DR, Morgan ET, Tarr GE, Coon MJ. 1982. Purification and characterization of a unique isozyme of cytochrome P-450 from liver microsomes of ethanol-treated rabbits. The Journal of biological chemistry 257:8472-8480.

Kozer E, Evans S, Barr J, Greenberg R, Soriano I, Bulkowstein M, et al. 2003. Glutathione, glutathione-dependent enzymes and antioxidant status in erythrocytes from children treated with high-dose paracetamol. British journal of clinical pharmacology 55:234-240.

Kreiger N, Marrett LD, Dodds L, Hilditch S, Darlington GA. 1993. Risk factors for renal cell carcinoma: results of a population-based case-control study. Cancer causes & control : CCC 4:101-110.

Kunze E, Chowaniec J. 1990. Tumours of the urinary bladder. In: IARC Scientific Publicaitons No 99 Pathology of tumours in laboratory animals Volume 1 - Tumours of the rat, (Turusov VS, Mohr U, eds). Lyon, France, 345-397.

Kwan ML, Habel LA, Slattery ML, Caan B. 2007. NSAIDs and breast cancer recurrence in a prospective cohort study. Cancer causes & control : CCC 18:613-620.

Kyriacou DN, Lewis RJ. 2016. Confounding by Indication in Clinical Research. Jama 316:1818-1819.

Lacey Jr JV, Sherman ME, Hartge P, Schatzkin A, Schairer C. 2004. Medication use and risk of ovarian carcinoma: A prospective study. International journal of cancer 108:281-286.

Ladds G, Wilson K, Burnett D. 1987. Automated liquid chromatographic method for the determination of paracetamol and six metabolites in human urine. Journal of chromatography 414:355-364.

Lahouel M, Boulkour S, Segueni N, Fillastre JP. 2004. [The flavonoids effect against vinblastine, cyclophosphamide and paracetamol toxicity by inhibition of lipid-peroxydation and increasing liver glutathione concentration]. Pathologie-biologie 52:314-322.

Laine JE, Auriola S, Pasanen M, Juvonen RO. 2009. Acetaminophen bioactivation by human cytochrome P450 enzymes and animal microsomes. Xenobiotica; the fate of foreign compounds in biological systems 39:11-21.

Lamba JK, Lin YS, Schuetz EG, Thummel KE. 2002. Genetic contribution to variable human CYP3A-mediated metabolism. Adv Drug Deliv Rev 54:1271-1294.

Last JM. 2001. A Dictionary of Epidemiology. Fourth Edition., (Spasoff RA, Harris SS, Thuriaux MC, eds). New York, NY:Oxford University Press.

LaVoie E, Tulley L, Fow E, Hoffmann D. 1979. Mutagenicity of aminophenyl and nitrophenyl ethers, sulfides, and disulfides. Mutation research 67:123-131.

Lee S, Dawson PA, Hewavitharana AK, Shaw PN, Markovich D. 2006. Disruption of NaS1 sulfate transport function in mice leads to enhanced acetaminophen-induced hepatotoxicity. Hepatology (Baltimore, Md) 43:1241-1247.

Lee SS, Buters JT, Pineau T, Fernandez-Salguero P, Gonzalez FJ. 1996. Role of CYP2E1 in the hepatotoxicity of acetaminophen. The Journal of biological chemistry 271:12063-12067.

Levay G, Pongracz K, Bodell WJ. 1991. Detection of DNA adducts in HL-60 cells treated with hydroquinone and p-benzoquinone by 32P-postlabeling. Carcinogenesis 12:1181-1186.

Levy G. 1981. Comparative pharmacokinetics of aspirin and acetaminophen. Archives of internal medicine 141:279-281.

Li N, Kong M, Zeng S, Xu Z, Li M, Hong W, et al. 2018. The chromatin remodeling protein BRG1 regulates APAP-induced liver injury by modulating CYP3A11 transcription in hepatocyte. Biochimica et biophysica acta Molecular basis of disease 1864:3487-3495.

Lieber CS, DeCarli LM. 1970. Hepatic microsomal ethanol-oxidizing system. In vitro characteristics and adaptive properties in vivo. The Journal of biological chemistry 245:2505-2512.

Lim WY, Chuah KL, Eng P, Leong SS, Lim E, Lim TK, et al. 2012. Aspirin and non-aspirin non-steroidal anti-inflammatory drug use and risk of lung cancer. Lung cancer (Amsterdam, Netherlands) 77:246-251.

Lindsey RH, Bender RP, Osheroff N. 2005. Stimulation of topoisomerase II-mediated DNA cleavage by benzene metabolites. Chemico-biological interactions 153-154:197-205.

Lindsey RH, Jr., Bromberg KD, Felix CA, Osheroff N. 2004. 1,4-Benzoquinone is a topoisomerase II poison. Biochemistry 43:7563-7574.

Linet MS, Chow WH, McLaughlin JK, Wacholder S, Yu MC, Schoenberg JB, et al. 1995. Analgesics and cancers of the renal pelvis and ureter. International journal of cancer 62:15-18.

Lipworth L, Friis S, Mellemkjaer L, Signorello LB, Johnsen SP, Nielsen GL, et al. 2003. A population-based cohort study of mortality among adults prescribed paracetamol in Denmark. Journal of clinical epidemiology 56:796-801.

Lo-Ciganic WH, Zgibor JC, Bunker CH, Moysich KB, Edwards RP, Ness RB. 2012. Aspirin, nonaspirin nonsteroidal anti-inflammatory drugs, or acetaminophen and risk of ovarian cancer. Epidemiology (Cambridge, Mass) 23:311-319.

Logan RF, Little J, Hawtin PG, Hardcastle JD. 1993. Effect of aspirin and non-steroidal anti-inflammatory drugs on colorectal adenomas: case-control study of subjects participating in the Nottingham faecal occult blood screening programme. BMJ (Clinical research ed) 307:285-289.

Ludewig G, Dogra S, Glatt H. 1989. Genotoxicity of 1,4-benzoquinone and 1,4-naphthoquinone in relation to effects on glutathione and NAD(P)H levels in V79 cells. Environmental health perspectives 82:223-228.

Madle S, Dean SW, Andrae U, Brambilla G, Burlinson B, Doolittle DJ, et al. 1994. Recommendations for the performance of UDS tests in vitro and in vivo. Mutation research 312:263-285.

Mahler JF, Elwell MR. 1999. Pituitary gland. In: Pathology of the mouse Reference and atlas, (Maronpot RR, Boorman GA, Gaul BW, eds):Cache River Press, 491-507.

Majeska JB, Holden HE. 1995. Genotoxic effects of p-aminophenol in Chinese hamster ovary and mouse lymphoma cells: results of a multiple endpoint test. Environmental and molecular mutagenesis 26:163-170.

Mamber SW, Bryson V, Katz SE. 1983. The Escherichia coli WP2/WP100 rec assay for detection of potential chemical carcinogens. Mutation research 119:135-144.

Markovic D, Katic J, Stojkovic R, Borovic S, Zarkovic N, Fucic A. 2013. Lipid peroxidation, detoxification capacity, and genome damage in mice after transplacental exposure to pharmaceutical drugs. Braz J Med Biol Res 46:1014-1020.

Markovich D, Forgo J, Stange G, Biber J, Murer H. 1993. Expression cloning of rat renal Na+/SO4(2-) cotransport. Proceedings of the National Academy of Sciences of the United States of America 90:8073-8077.

Markovich D. 2001. Physiological roles and regulation of mammalian sulfate transporters. Physiol Rev 81:1499-1533.

Markovich D. 2012. Slc13a1 and Slc26a1 KO models reveal physiological roles of anion transporters. Physiology (Bethesda) 27:7-14.

Marotta F, Yadav H, Gumaste U, Helmy A, Jain S, Minelli E. 2009. Protective effect of a phytocompound on oxidative stress and DNA fragmentation against paracetamol-induced liver damage. Annals of hepatology 8:50-56.

Marshall PJ, Kulmacz RJ. 1988. Prostaglandin H synthase: distinct binding sites for cyclooxygenase and peroxidase substrates. Archives of biochemistry and biophysics 266:162-170.

Marshall SF, Bernstein L, Anton-Culver H, Deapen D, Horn-Ross PL, Mohrenweiser H, et al. 2005. Nonsteroidal anti-inflammatory drug use and breast cancer risk by stage and hormone receptor status. Journal of the National Cancer Institute 97:805-812.

Martinez A, Urios A, Blanco M. 2000. Mutagenicity of 80 chemicals in Escherichia coli tester strains IC203, deficient in OxyR, and its oxyR(+) parent WP2 uvrA/pKM101: detection of 31 oxidative mutagens. Mutation research 467:41-53.

Mason RP, Fischer V. 1986. Free radicals of acetaminophen: their subsequent reactions and toxicological significance. Federation proceedings 45:2493-2499.

Matsushima T, Hayashi M, Matsuoka A, Ishidate M, Jr., Miura KF, Shimizu H, et al. 1999. Validation study of the in vitro micronucleus test in a Chinese hamster lung cell line (CHL/IU). Mutagenesis 14:569-580.

Matsushita K, Kijima A, Ishii Y, Takasu S, Jin M, Kuroda K, et al. 2013. Development of a Medium-term Animal Model Using gpt Delta Rats to Evaluate Chemical Carcinogenicity and Genotoxicity. Journal of toxicologic pathology 26:19-27.

Matsuyama M, Amo H. 1983. Promoting effects of long-term administration of barbital on spontaneous hepatic tumorigenesis in B6C3F1 mice. Nihon eiseigaku zasshi Japanese journal of hygiene 37:892-896.

Mazaleuskaya LL, Sangkuhl K, Thorn CF, FitzGerald GA, Altman RB, Klein TE. 2015. PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. Pharmacogenetics and genomics 25:416-426.

Mazer M, Perrone J. 2008. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. Journal of medical toxicology : official journal of the American College of Medical Toxicology 4:2-6.

McCarthy M. 2014. FDA acts to limit exposure to acetaminophen in combination products. BMJ (Clinical research ed) 348:g356.

McCarver DG, Byun R, Hines RN, Hichme M, Wegenek W. 1998. A genetic polymorphism in the regulatory sequences of human CYP2E1: association with increased chlorzoxazone hydroxylation in the presence of obesity and ethanol intake. Toxicology and applied pharmacology 152:276-281.

McConnell EE, Solleveld HA, Swenberg JA, Boorman GA. 1986. Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. Journal of the National Cancer Institute 76:283-289.

McCredie M, Ford JM, Stewart JH. 1988. Risk factors for cancer of the renal parenchyma. International journal of cancer 42:13-16.

McCredie M, Stewart JH. 1988. Does paracetamol cause urothelial cancer or renal papillary necrosis? Nephron 49:296-300.

McCredie M, Stewart JH, Day NE. 1993. Different roles for phenacetin and paracetamol in cancer of the kidney and renal pelvis. International journal of cancer 53:245-249.

McCredie M, Pommer W, McLaughlin JK, Stewart JH, Lindblad P, Mandel JS, et al. 1995. International renal-cell cancer study. II. Analgesics. International journal of cancer 60:345-349.

McDonald TA, Waidyanatha S, Rappaport SM. 1993. Production of benzoquinone adducts with hemoglobin and bone-marrow proteins following administration of [13C6]benzene to rats. Carcinogenesis 14:1921-1925.

McGill MR, Jaeschke H. 2013. Metabolism and disposition of acetaminophen: recent advances in relation to hepatotoxicity and diagnosis. Pharmaceutical research 30:2174-2187.

McGilveray IJ, Mattok GL. 1972. Some factors affecting the absorption of paracetamol. The Journal of pharmacy and pharmacology 24:615-619.

McGlynn KA, Hagberg K, Chen J, Graubard BI, London WT, Jick S, et al. 2015. Statin use and risk of primary liver cancer in the Clinical Practice Research Datalink. Journal of the National Cancer Institute 107.

McLaughlin JK, Blot WJ, Mehl ES, Fraumeni JF, Jr. 1985. Relation of analgesic use to renal cancer: population-based findings. National Cancer Institute monograph 69:217-222.

McMurtry RJ, Snodgrass WR, Mitchell JR. 1978. Renal necrosis, glutathione depletion, and covalent binding after acetaminophen. Toxicology and applied pharmacology 46:87-100.

Meier CR, Schmitz S, Jick H. 2002. Association between acetaminophen or nonsteroidal antiinflammatory drugs and risk of developing ovarian, breast, or colon cancer. Pharmacotherapy 22:303-309.

Mellemgaard A, Niwa S, Mehl ES, Engholm G, McLaughlin JK, Olsen JH. 1994. Risk factors for renal cell carcinoma in Denmark: role of medication and medical history. International journal of epidemiology 23:923-930.

Merritt MA, Rice MS, Barnard ME, Hankinson SE, Matulonis UA, Poole EM, et al. 2018. Pre-diagnosis and post-diagnosis use of common analgesics and ovarian cancer prognosis (NHS/NHSII): a cohort study. The Lancet Oncology 19:1107-1116.

Milam KM, Byard JL. 1985. Acetaminophen metabolism, cytotoxicity, and genotoxicity in rat primary hepatocyte cultures. Toxicology and applied pharmacology 79:342-347.

Miner DJ, Kissinger PT. 1979. Evidence for the involvement of N-acetyl-p- quinoneimine in acetaminophen metabolism. Biochemical pharmacology 28:3285-3290.

Mirkovic B, Sosic I, Gobec S, Kos J. 2011. Redox-based inactivation of cysteine cathepsins by compounds containing the 4-aminophenol moiety. PloS one 6:e27197.

Mitchell JR, Jollow DJ, Potter WZ, Davis DC, Gillette JR, Brodie BB. 1973. Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. The Journal of pharmacology and experimental therapeutics 187:185-194.

Mitchell JR, Thorgeirsson SS, Potter WZ, Jollow DJ, Keiser H. 1974. Acetaminophen-induced hepatic injury: Protective role of glutathione in man and rationale for therapy. Clinical pharmacology and therapeutics 16:676-684.

Mitra AB, Manna GK. 1971. Effect of some phenolic compounds on chromosomes of bone marrow cells of mice. The Indian journal of medical research 59:1442-1447.

Miyakawa K, Albee R, Letzig LG, Lehner AF, Scott MA, Buchweitz JP, et al. 2015. A Cytochrome P450-Independent Mechanism of Acetaminophen-Induced Injury in Cultured Mouse Hepatocytes. The Journal of pharmacology and experimental therapeutics 354:230-237.

Mladenovic D, Radosavljevic T, Ninkovic M, Vucevic D, Jesic-Vukicevic R, Todorovic V. 2009. Liver antioxidant capacity in the early phase of acute paracetamol-induced liver injury in mice. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 47:866-870.

Modick H, Weiss T, Dierkes G, Bruning T, Koch HM. 2014. Ubiquitous presence of paracetamol in human urine: sources and implications. Reproduction 147:R105-117.

Moldeus P. 1978. Paracetamol metabolism and toxicity in isolated hepatocytes from rat and mouse. Biochemical pharmacology 27:2859-2863.

Moldeus P, Andersson B, Rahimtula A, Berggren M. 1982. Prostaglandin synthetase catalyzed activation of paracetamol. Biochemical pharmacology 31:1363-1368.

Möller-Hartmann W, Siegers CP. 1991. Nephrotoxicity of paracetamol in the rat--mechanistic and therapeutic aspects. Journal of applied toxicology : JAT 11:141-146.

Morris ME, Levy G. 1984. Renal clearance and serum protein binding of acetaminophen and its major conjugates in humans. Journal of pharmaceutical sciences 73:1038-1041.

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B, Zeiger E. 1986. Salmonella mutagenicity tests: II. Results from the testing of 270 chemicals. Environ Mutagen 8 Suppl 7:1-119.

Moysich KB, Mettlin C, Piver MS, Natarajan N, Menezes RJ, Swede H. 2001. Regular use of analgesic drugs and ovarian cancer risk. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 10:903-906.

Moysich KB, Baker JA, Rodabaugh KJ, Villella JA. 2005. Regular analgesic use and risk of endometrial cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 14:2923-2928.

Moysich KB, Bonner MR, Beehler GP, Marshall JR, Menezes RJ, Baker JA, et al. 2007. Regular analgesic use and risk of multiple myeloma. Leukemia research 31:547-551.

Mrochek JE, Katz S, Christie WH, Dinsmore SR. 1974. Acetaminophen metabolism in man, as determined by high-resolution liquid chromatography. Clinical chemistry 20:1086-1096.

Mudge GH, Gemborys MW, Duggin GG. 1978. Covalent binding of metabolites of acetaminophen to kidney protein and depletion of renal glutathione. The Journal of pharmacology and experimental therapeutics 206:218-226.

Mugford CA, Tarloff JB. 1995. Contribution of oxidation and deacetylation to the bioactivation of acetaminophen in vitro in liver and kidney from male and female Sprague-Dawley rats. Drug metabolism and disposition: the biological fate of chemicals 23:290-294.

Mugford CA TJ. 1997. The contribution of oxidation and deacetylation to acetaminophen nephrotoxicity in female Sprague-Dawley rats. Toxicol Lett 2014 93:15-22.

Muller-Tegethoff K, Kasper P, Muller L. 1995. Evaluation studies on the in vitro rat hepatocyte micronucleus assay. Mutation research 335:293-307.

Muller L, Kasper P, Madle S. 1991. Further investigations on the clastogenicity of paracetamol and acetylsalicylic acid in vitro. Mutation research 263:83-92.

Muller L, Kasper P. 1995. OTC pharmaceuticals and genotoxicity testing: the paracetamol, anthraquinone, and griseofulvin cases. Archives of toxicology Supplement = Archiv fur Toxikologie Supplement 17:312-325.

Murad AS, Down L, Davey Smith G, Donovan JL, Athene Lane J, Hamdy FC, et al. 2011. Associations of aspirin, nonsteroidal anti-inflammatory drug and paracetamol use with PSA-detected prostate cancer: findings from a large, population-based, case-control study (the ProtecT study). International journal of cancer 128:1442-1448.

Muscat JE, Stellman SD, Wynder EL. 1994. Nonsteroidal antiinflammatory drugs and colorectal cancer. Cancer 74:1847-1854.

Mutlib AE, Goosen TC, Bauman JN, Williams JA, Kulkarni S, Kostrubsky S. 2006. Kinetics of acetaminophen glucuronidation by UDP-glucuronosyltransferases 1A1, 1A6, 1A9 and 2B15. Potential implications in acetaminophen-induced hepatotoxicity. Chemical research in toxicology 19:701-709.

Nagar S, Walther S, Blanchard RL. 2006. Sulfotransferase (SULT) 1A1 polymorphic variants *1, *2, and *3 are associated with altered enzymatic activity, cellular phenotype, and protein degradation. Mol Pharmacol 69:2084-2092.

Nagle CM, Ibiebele TI, DeFazio A, Protani MM, Webb PM. 2015. Aspirin, nonaspirin nonsteroidal anti-inflammatory drugs, acetaminophen and ovarian cancer survival. Cancer epidemiology 39:196-199.

Napier MD, Poole C, Stewart JR, Weber DJ, Glassmeyer ST, Kolpin DW, et al. 2018. Exposure to Human-Associated Chemical Markers of Fecal Contamination and Self-Reported Illness among Swimmers at Recreational Beaches. Environmental science & technology 52:7513-7523.

Nazar MA, Rapson WH, Brook MA, May S, Tarhanen J. 1981. Mutagenic reaction products of aqueous chlorination of catechol. Mutation research 89:45-55.

NCI. 1978a. Bioassay of 3-amino-4-ethoxyacetanilide for possible carcinogenicity CAS No. 17026-81-2. (National Cancer Institute Carcinogenesis Techical Report Series No 112).

NCI. 1978b. Bioassay of aniline hydrochloride for possible carcinogenicity. CAS No. 142-04-1. (National Cancer Institute Carcinogenesis Techical Report Series No 130).

NCI. 2019a. SEER Cancer Stat Facts: Ovarian cancer. Available: https://seer.cancer.gov/statfacts/html/ovary.html [accessed on August 15].

NCI. 2019b. PDQ Ovarian Epithelial, Fallopian Tube, and Primary Peritoneal Cancer Treatment. Available: https://www.cancer.gov/types/ovarian/hp/ovarian-epithelial-treatment-pdq [accessed on August 15].

NCI. 2019c. Cancer Stat Facts: Kidney and Renal Pelvis Cancer. Available: https://seer.cancer.gov/statfacts/html/kidrp.html [accessed on July 18].

NCI. 2019d. Skin Cancer (Including Melanoma)—Patient Version. Available: https://www.cancer.gov/types/skin [accessed on August 15].

Neill AS, Nagle CM, Protani MM, Obermair A, Spurdle AB, Webb PM. 2013. Aspirin, nonsteroidal anti-inflammatory drugs, paracetamol and risk of endometrial cancer: a case-control study, systematic review and meta-analysis. International journal of cancer 132:1146-1155.

Nelson JE, Harris RE. 2000. Inverse association of prostate cancer and non-steroidal anti-inflammatory drugs (NSAIDs): results of a case-control study. Oncology reports 7:169-170.

Newton JF, Kuo CH, Gemborys MW, Mudge GH, Hook JB. 1982a. Nephrotoxicity of p-aminophenol, a metabolite of acetaminophen, in the fischer 344 rat. Toxicology and applied pharmacology 65:336-344.

Newton JF, Braselton WE, Jr., Kuo CH, Kluwe WM, Gemborys MW, Mudge GH, et al. 1982b. Metabolism of acetaminophen by the isolated perfused kidney. The Journal of pharmacology and experimental therapeutics 221:76-79.

Newton JF, Bailie MB, Hook JB. 1983. Acetaminophen nephrotoxicity in the rat. Renal metabolic activation in vitro. Toxicology and applied pharmacology 70:433-444.

Newton JF, Pasino DA, Hook JB. 1985. Acetaminophen nephrotoxicity in the rat: quantitation of renal metabolic activation in vivo. Toxicology and applied pharmacology 78:39-46.

Newton JF, Hoefle D, Gemborys MW, Mudge GH, Hook JB. 1986. Metabolism and excretion of a glutathione conjugate of acetaminophen in the isolated perfused rat kidney. The Journal of pharmacology and experimental therapeutics 237:519-524.

NHS. 2016. Nurses' Health Study. Available: https://www.nurseshealthstudy.org/about-nhs [accessed on September 12, 2019].

Nielsen GL, Sorensen HT, Zhou W, Steffensen FH, Olsen J. 1997. The Pharmacoepidemiologic Prescription Database of North Jutland - a valid tool in pharmacoepidemiological research. The International journal of risk & safety in medicine 10:203-205.

Ning B, Nowell S, Sweeney C, Ambrosone CB, Williams S, Miao X, et al. 2005. Common genetic polymorphisms in the 5'-flanking region of the SULT1A1 gene: haplotypes and their association with platelet enzymatic activity. Pharmacogenetics and genomics 15:465-473.

Nishimura M, Ueda N, Naito S. 2003. Effects of dimethyl sulfoxide on the gene induction of cytochrome P450 isoforms, UGT-dependent glucuronosyl transferase isoforms, and ABCB1 in primary culture of human hepatocytes. Biological & pharmaceutical bulletin 26:1052-1056.

Nitsche JF, Patil AS, Langman LJ, Penn HJ, Derleth D, Watson WJ, et al. 2017. Transplacental Passage of Acetaminophen in Term Pregnancy. American journal of perinatology 34:541-543.

Notarianni LJ, Oldham HG, Bennett PN. 1987. Passage of paracetamol into breast milk and its subsequent metabolism by the neonate. British journal of clinical pharmacology 24:63-67.

Nowell S, Falany CN. 2006. Pharmacogenetics of human cytosolic sulfotransferases. Oncogene 25:1673-1678.

NTP. 1992. Toxicology and Carcinogenesis Studies of 2,4-Diaminophenol Dihydrochloride (CAS NO. 137-09-7) in F344/N Rats and B6C3F1 Mice (Gavage Studies). National Toxicology Program technical report series 401:1-232.

NTP. 1993. Toxicology and Carcinogenesis Studies of Acetaminophen (CAS No. 103-90-2) in F344 Rats and B6C3F1 Mice (Feed Studies). National Toxicology Program technical report series 394:1-274.

NTP. 1999. NTP Historical Controls Database. 1999/Chronic, Mice/B6C3F1, Gavage/Corn Oil. Available at: https://ntp.niehs.nih.gov/results/dbsearch/historical/index.html [accessed on July 25, 2019].

NTP. 2016a. NTP Historical Controls Report. All Routes and Vehicles. Wistar-Han RATS

NTP. 2016b. Report on Carcinogens, Fourteenth Edition. Phenacetin. Research Triangle Park, NC: U.S. Department of Health and Human Services, Public Health Service. Available: https://ntp.niehs.nih.gov/go/roc1.

NTP. 2017. NTP Historical Controls Report. All Routes and Vehicles. Harlan Sprague-Dawley RATS.

Nuttall SL, Khan JN, Thorpe GH, Langford N, Kendall MJ. 2003. The impact of therapeutic doses of paracetamol on serum total antioxidant capacity. Journal of clinical pharmacy and therapeutics 28:289-294.

O'Connell TM, Watkins PB. 2010. The application of metabonomics to predict drug-induced liver injury. Clinical pharmacology and therapeutics 88:394-399.

Oberly TJ, Bewsey BJ, Probst GS. 1984. An evaluation of the L5178Y TK+/- mouse lymphoma forward mutation assay using 42 chemicals. Mutation research 125:291-306.

Oberly TJ, Michaelis KC, Rexroat MA, Bewsey BJ, Garriott ML. 1993. A comparison of the CHO/HGPRT+ and the L5178Y/TK+/- mutation assays using suspension treatment and soft agar cloning: results for 10 chemicals. Cell biology and toxicology 9:243-257.

Ognjanovic S, Blair C, Spector LG, Robison LL, Roesler M, Ross JA. 2011. Analgesic use during pregnancy and risk of infant leukaemia: a Children's Oncology Group study. British journal of cancer 104:532-536.

Oldham JW, Preston RF, Paulson JD. 1986. Mutagenicity testing of selected analgesics in Ames Salmonella strains. Journal of applied toxicology : JAT 6:237-243.

Olsen JH, Friis S, Poulsen AH, Fryzek J, Harving H, Tjonneland A, et al. 2008. Use of NSAIDs, smoking and lung cancer risk. British journal of cancer 98:232-237.

O'Neil MJ. 2006. The Merck index: an encyclopedia of chemicals, drugs, and biologicals. Whitehouse Station, N.J.:Merck.

Oshida K, Iwanaga E, Miyamoto-Kuramitsu K, Miyamoto Y. 2008. An in vivo comet assay of multiple organs (liver, kidney and bone marrow) in mice treated with methyl methanesulfonate and acetaminophen accompanied by hematology and/or blood chemistry. The Journal of toxicological sciences 33:515-524.

Pandeya N, Olsen CM, Thompson BS, Dusingize JC, Neale RE, Green AC, et al. 2019. Aspirin and nonsteroidal anti-inflammatory drug use and keratinocyte cancers: a large population-based cohort study of skin cancer in Australia. The British journal of dermatology.

Park BK, Laverty H, Srivastava A, Antoine DJ, Naisbitt D, Williams DP. 2011. Drug bioactivation and protein adduct formation in the pathogenesis of drug-induced toxicity. Chemico-biological interactions 192:30-36.

Parolini M, Binelli A, Cogni D, Provini A. 2010. Multi-biomarker approach for the evaluation of the cyto-genotoxicity of paracetamol on the zebra mussel (Dreissena polymorpha). Chemosphere 79:489-498.

Pascoe GA, Calleman CJ, Baille TA. 1988. Identification of S-(2,5-dihydroxyphenyl)-cysteine and S-(2,5-dihydroxyphenyl)-N-acetyl-cysteine as urinary metabolites of acetaminophen in the mouse. Evidence for p-benzoquinone as a reactive intermediate in acetaminophen metabolism. Chemico-biological interactions 68:85-98.

Patierno SR, Lehman NL, Henderson BE, Landolph JR. 1989. Study of the ability of phenacetin, acetaminophen, and aspirin to induce cytotoxicity, mutation, and morphological transformation in C3H/10T1/2 clone 8 mouse embryo cells. Cancer research 49:1038-1044.

Patten CJ, Thomas PE, Guy RL, Lee M, Gonzalez FJ, Guengerich FP, et al. 1993. Cytochrome P450 enzymes involved in acetaminophen activation by rat and human liver microsomes and their kinetics. Chemical research in toxicology 6:511-518.

Paulose-Ram R, Hirsch R, Dillon C, Gu Q. 2005. Frequent monthly use of selected non-prescription and prescription non-narcotic analgesics among U.S. adults. Pharmacoepidemiology and drug safety 14:257-266.

Peleg II, Lubin MF, Cotsonis GA, Clark WS, Wilcox CM. 1996. Long-term use of nonsteroidal antiinflammatory drugs and other chemopreventors and risk of subsequent colorectal neoplasia. Digestive diseases and sciences 41:1319-1326.

Pellack-Walker P, Blumer JL. 1986. DNA damage in L5178YS cells following exposure to benzene metabolites. Molecular pharmacology 30:42-47.

Pelucchi C, Bosetti C, Negri E, Malvezzi M, La Vecchia C. 2006. Mechanisms of disease: The epidemiology of bladder cancer. Nature clinical practice Urology 3:327-340.

Peres LC, Camacho F, Abbott SE, Alberg AJ, Bandera EV, Barnholtz-Sloan J, et al. 2016. Analgesic medication use and risk of epithelial ovarian cancer in African American women. British journal of cancer 114:819-825.

Persson I, Johansson I, Bergling H, Dahl ML, Seidegard J, Rylander R, et al. 1993. Genetic polymorphism of cytochrome P4502E1 in a Swedish population. Relationship to incidence of lung cancer. FEBS Lett 319:207-211.

Philbrook NA, Winn LM. 2016. Benzoquinone toxicity is not prevented by sulforaphane in CD-1 mouse fetal liver cells. Journal of applied toxicology : JAT 36:1015-1024.

Pinheiro SP, Tworoger SS, Cramer DW, Rosner BA, Hankinson SE. 2009. Use of nonsteroidal antiinflammatory agents and incidence of ovarian cancer in 2 large prospective cohorts. American journal of epidemiology 169:1378-1387.

Pinheiro SP, Gates MA, Devivo I, Rosner BA, Tworoger SS, Titus-Ernstoff L, et al. 2010. Interaction between use of non-steroidal anti-inflammatory drugs and selected genetic polymorphisms in ovarian cancer risk. International Journal of Molecular Epidemiology and Genetics 1:320-331.

Piper JM, Tonascia J, Matanoski GM. 1985. Heavy phenacetin use and bladder cancer in women aged 20 to 49 years. The New England journal of medicine 313:292-295.

Plattner S, Erb R, Pitterl F, Brouwer HJ, Oberacher H. 2012. Formation and characterization of covalent guanosine adducts with electrochemistry-liquid chromatography-mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci 883-884:198-204.

Platz EA, Rohrmann S, Pearson JD, Corrada MM, Watson DJ, De Marzo AM, et al. 2005. Nonsteroidal anti-inflammatory drugs and risk of prostate cancer in the Baltimore Longitudinal Study of Aging. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 14:390-396.

Pommer W, Bronder E, Klimpel A, Helmert U, Greiser E, Molzahn M. 1999. Urothelial cancer at different tumour sites: role of smoking and habitual intake of analgesics and laxatives. Results of the Berlin Urothelial Cancer Study. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association 14:2892-2897.

Porta Me. 2014. Dictionary of Epidemiology. Oxford:Oxford University Press, Incorporated.

Potter DW, Hinson JA. 1987b. The 1- and 2-electron oxidation of acetaminophen catalyzed by prostaglandin H synthase. The Journal of biological chemistry 262:974-980.

Potter DW, Hinson JA. 1987a. Mechanisms of acetaminophen oxidation to N-acetyl-P-benzoquinone imine by horseradish peroxidase and cytochrome P-450. The Journal of biological chemistry 262:966-973.

Potter DW, Hinson JA. 1989. Acetaminophen peroxidation reactions. Drug metabolism reviews 20:341-358.

Potter WZ, Davis DC, Mitchell JR, Jollow DJ, Gillette JR, Brodie BB. 1973. Acetaminophen-induced hepatic necrosis. 3. Cytochrome P-450-mediated covalent binding in vitro. The Journal of pharmacology and experimental therapeutics 187:203-210.

Prejean JD, Peckham JC, Casey AE, Griswold DP, Weisburger EK, Weisburger JH. 1973. Spontaneous Tumors in Sprague-Dawley Rats and Swiss Mice. Cancer research 33:2768-2773.

Prescott LF. 1980. Kinetics and metabolism of paracetamol and phenacetin. British journal of clinical pharmacology 10 Suppl 2:291s-298s.

Probst GS, McMahon RE, Hill LE, Thompson CZ, Epp JK, Neal SB. 1981. Chemically-induced unscheduled DNA synthesis in primary rat hepatocyte cultures: a comparison with bacterial mutagenicity using 218 compounds. Environ Mutagen 3:11-32.

Pu S, Ren L, Liu Q, Kuang J, Shen J, Cheng S, et al. 2016. Loss of 5-lipoxygenase activity protects mice against paracetamol-induced liver toxicity. British journal of pharmacology 173:66-76.

Raffa RB, Pergolizzi JV, Jr., Taylor R, Jr., Decker JF, Patrick JT. 2014. Acetaminophen (paracetamol) oral absorption and clinical influences. Pain practice : the official journal of World Institute of Pain 14:668-677.

Rahme E, Ghosn J, Dasgupta K, Rajan R, Hudson M. 2005. Association between frequent use of nonsteroidal anti-inflammatory drugs and breast cancer. BMC cancer 5.

Ramachandran A, Jaeschke H. 2017. Mechanisms of acetaminophen hepatotoxicity and their translation to the human pathophysiology. Journal of clinical and translational research 3:157-169.

Ramachandran A, Jaeschke H. 2018. Acetaminophen Toxicity: Novel Insights Into Mechanisms and Future Perspectives. Gene expression 18:19-30.

Ramachandran A, Jaeschke H. 2019. Acetaminophen Hepatotoxicity. Seminars in liver disease 39:221-234.

Rannug U, Holme JA, Hongslo JK, Sram R. 1995. International Commission for Protection against Environmental Mutagens and Carcinogens. An evaluation of the genetic toxicity of paracetamol. Mutation research 327:179-200.

Raucy JL, Lasker JM, Lieber CS, Black M. 1989. Acetaminophen activation by human liver cytochromes P450IIE1 and P450IA2. Archives of biochemistry and biophysics 271:270-283.

Rawlins MD, Henderson DB, Hijab AR. 1977. Pharmacokinetics of paracetamol (acetaminophen) after intravenous and oral administration. European journal of clinical pharmacology 11:283-286.

Rebelatto MC. 2018. Chapter 24 - Spleen, Lymph Nodes, and Thymus. In: Boorman's Pathology of the Rat (Second Edition), (Suttie AW, ed). Boston:Academic Press, 469-491.

Reddy G. 1984. Effects of aracetamol on chromosomes of mouse bone marrow. Caryologia 37:127-132.

Reddy G, Subramanyam S. 1985. Cytogenetic response of meiocytes of Swiss Albino mice to paracetamol. Caryologia 38:347-355.

Reddy GA, Subramanyam S. 1981. Respnse of mitotic cells of Allium cepa to paracetamol. . In: Perspectives in Cytology and Genetics, Vol. 3, (Manna GKS, U., eds, ed). Delhi:Hindasi Publishers, 571-576.

Reissig JL. 1963. Induction of forward mutants in the pyr-3 region of Neurospora. Journal of general microbiology 30:317-325.

Retaco P, González M, Pizzorno M, Volonté M. 1996. Bioavailability study of paracetamol tablets in saliva and urine. Eur J Drug Metab Pharmacokinet 21:295-300.

Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. 2010. Oxidative stress, inflammation, and cancer: how are they linked? Free radical biology & medicine 49:1603-1616.

Reznikoff CA, Brankow DW, Heidelberger C. 1973. Establishment and characterization of a cloned line of C3H mouse embryo cells sensitive to postconfluence inhibition of division. Cancer research 33:3231-3238.

Rinaldi R, Eliasson E, Swedmark S, Morgenstern R. 2002. Reactive intermediates and the dynamics of glutathione transferases. Drug metabolism and disposition: the biological fate of chemicals 30:1053-1058.

Riordan SM, Williams R. 2002. Alcohol exposure and paracetamol-induced hepatotoxicity. Addiction biology 7:191-206.

Rodriguez C, Henley SJ, Calle EE, Thun MJ. 1998. Paracetamol and risk of ovarian cancer mortality in a prospective study of women in the USA. Lancet (London, England) 352:1354-1355.

Rogers LK, Moorthy B, Smith CV. 1997. Acetaminophen binds to mouse hepatic and renal DNA at human therapeutic doses. Chemical research in toxicology 10:470-476.

Rohrborn G, Vogel F. 1967. [Chemically induced mutations in mammals and humans. 2. Genetic studies on the mouse]. Deutsche medizinische Wochenschrift (1946) 92:2315-2321.

Ronquist G, Rodriguez LA, Ruigomez A, Johansson S, Wallander MA, Frithz G, et al. 2004. Association between captopril, other antihypertensive drugs and risk of prostate cancer. The Prostate 58:50-56.

Rosen GM, Singletary WV, Jr., Rauckman EJ, Killenberg PG. 1983. Acetaminophen hepatotoxicity. An alternative mechanism. Biochemical pharmacology 32:2053-2059.

Rosenberg L, Rao RS, Palmer JR, Strom BL, Zauber A, Warshauer ME, et al. 1998. Transitional cell cancer of the urinary tract and renal cell cancer in relation to acetaminophen use (United States). Cancer causes & control : CCC 9:83-88.

Rosenberg L, Palmer JR, Rao RS, Coogan PF, Strom BL, Zauber AG, et al. 2000. A case-control study of analgesic use and ovarian cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 9:933-937.

Ross JA, Blair CK, Cerhan JR, Soler JT, Hirsch BA, Roesler MA, et al. 2011. Nonsteroidal anti-inflammatory drug and acetaminophen use and risk of adult myeloid leukemia. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 20:1741-1750.

Ross RK, Paganini-Hill A, Landolph J, Gerkins V, Henderson BE. 1989. Analgesics, cigarette smoking, and other risk factors for cancer of the renal pelvis and ureter. Cancer research 49:1045-1048.

Rothman KJ, Greenland S, Lash TL. 2008. Validity in epidemiologic studies. In: Modern Epidemiology, Part 3rd Edition (Rothman KJ, Greenland S, Lash TL, eds). Philadelphia:Wolters Kluwer Health/Lippincott Williams & Wilkins, 128-147.

Ruepp SU, Tonge RP, Shaw J, Wallis N, Pognan F. 2002. Genomics and proteomics analysis of acetaminophen toxicity in mouse liver. Toxicological sciences : an official journal of the Society of Toxicology 65:135-150.

Rumack BH. 2002. Acetaminophen hepatotoxicity: the first 35 years. Journal of toxicology Clinical toxicology 40:3-20.

Sadeghi S, Bain CJ, Pandeya N, Webb PM, Green AC, Whiteman DC. 2008. Aspirin, nonsteroidal anti-inflammatory drugs, and the risks of cancers of the esophagus. Cancer epidemiology, biomarkers & prevention : a publication of the American

Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 17:1169-1178.

Salas M, Hofman A, Stricker BH. 1999. Confounding by indication: an example of variation in the use of epidemiologic terminology. American journal of epidemiology 149:981-983.

Salinas CA, Kwon EM, FitzGerald LM, Feng Z, Nelson PS, Ostrander EA, et al. 2010. Use of aspirin and other nonsteroidal antiinflammatory medications in relation to prostate cancer risk. American journal of epidemiology 172:578-590.

Samuelsen PJ, Slordal L, Mathisen UD, Eggen AE. 2015. Analgesic use in a Norwegian general population: change over time and high-risk use--The Tromso Study. BMC pharmacology & toxicology 16:16.

Sandler DP, Smith JC, Weinberg CR, Buckalew VM, Jr., Dennis VW, Blythe WB, et al. 1989. Analgesic use and chronic renal disease. The New England journal of medicine 320:1238-1243.

Sarganas G, Buttery AK, Zhuang W, Wolf IK, Grams D, Rosario AS, et al. 2015. Prevalence, trends, patterns and associations of analgesic use in Germany. BMC pharmacology & toxicology 16:28.

Sasaki M, Sugiura K, Yoshida MA, Ab S. 1980. Cytogenetic effecs of 60 chemicals on cultured human and Chinese hamster cells. Kromosomo II:574-584.

Sasaki M, Yoshida S, Hiraga K. 1983. Additional effect of acetaminophen on the mutagenicity and clastogenicity of N-methyl-N'-nitro-N-nitrosoguanidine in cultured Chinese hamster CHO-KI cells. Mutation research 122:367-372.

Sasaki M. 1986. Enhancing effect of acetaminophen on mutagenesis. Progress in clinical and biological research 209a:365-372.

Schildkraut JM, Moorman PG, Halabi S, Calingaert B, Marks JR, Berchuck A. 2006. Analgesic drug use and risk of ovarian cancer. Epidemiology (Cambridge, Mass) 17:104-107.

Schiodt FV, Lee WM, Bondesen S, Ott P, Christensen E. 2002. Influence of acute and chronic alcohol intake on the clinical course and outcome in acetaminophen overdose. Alimentary pharmacology & therapeutics 16:707-715.

Schneeweiss S, Suissa S. 2013. Advanced approaches to controlling confounding in pharmacoepidemiologic studies. In: Textbook of Pharmacoepidemiology, Part Second (Strom BL, Kimmel SE, Hennessy S, eds):John Wiley & Sons, Ltd.

SEER. 2019. Cancer Stat Facts: Bladder Cancer. Available: https://seer.cancer.gov/statfacts/html/urinb.html [accessed on August 13].

Setiawan VW, Matsuno RK, Lurie G, Wilkens LR, Carney ME, Henderson BE, et al. 2012. Use of nonsteroidal anti-inflammatory drugs and risk of ovarian and endometrial cancer: the Multiethnic Cohort. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 21:1441-1449.

Severin E, Beleuta A. 1995. Induction of chromosome aberrations in vivo in bone-marrow cells of mice by paracetamol. Romanian journal of morphology and embryology = Revue roumaine de morphologie et embryologie 41:117-120.

Shimane Y. 1985. [Mutagenicity of acetaminophen on Chinese hamster V79 cells]. Shigaku = Odontology; journal of Nihon Dental College 72:1175-1187.

Sicardi SM, Martiarena JL, Iglesias MT. 1991. Mutagenic and analgesic activities of aniline derivatives. Journal of pharmaceutical sciences 80:761-764.

Siegers CP. 1978. Antidotal effects of dimethyl sulphoxide against paracetamol-, bromobenzene-, and thioacetamide-induced hepatotoxicity. The Journal of pharmacy and pharmacology 30:375-377.

Siegers CP, Rozman K, Klaassen CD. 1983. Biliary excretion and enterohepatic circulation of paracetamol in the rat. Xenobiotica; the fate of foreign compounds in biological systems 13:591-596.

Siegers CP, Loeser W, Gieselmann J, Oltmanns D. 1984. Biliary and renal excretion of paracetamol in man. Pharmacology 29:301-303.

Signorello LB, McLaughlin JK, Lipworth L, Friis S, Sorensen HT, Blot WJ. 2002. Confounding by indication in epidemiologic studies of commonly used analgesics. American journal of therapeutics 9:199-205.

Simko M, Kriehuber R, Lange S. 1998. Micronucleus formation in human amnion cells after exposure to 50 Hz MF applied horizontally and vertically. Mutation research 418:101-111.

Sipe JC, Chiang K, Gerber AL, Beutler E, Cravatt BF. 2002. A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proceedings of the National Academy of Sciences of the United States of America 99:8394-8399.

Sivak-Sears NR, Schwartzbaum JA, Miike R, Moghadassi M, Wrensch M. 2004. Case-control study of use of nonsteroidal antiinflammatory drugs and glioblastoma multiforme. American journal of epidemiology 159:1131-1139.

Smarr MM, Kannan K, Chen Z, Kim S, Buck Louis GM. 2017. Male urinary paracetamol and semen quality. Andrology 5:1082-1088.

Smith GE, Griffiths LA. 1974. Metabolism of N-acylated and O-alkylated drugs by the intestinal microflora during anaerobic incubation in vitro. Xenobiotica; the fate of foreign compounds in biological systems 4:477-487.

Smith GE, Griffiths LA. 1976. Comparative metabolic studies of phenacetin and structurally-related compounds in the rat. Xenobiotica; the fate of foreign compounds in biological systems 6:217-236.

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I, et al. 2016. Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis. Environmental health perspectives 124:713-721.

Smith SA, Colley HE, Sharma P, Slowik KM, Sison-Young R, Sneddon A, et al. 2018. Expression and enzyme activity of cytochrome P450 enzymes CYP3A4 and CYP3A5 in human skin and tissue-engineered skin equivalents. Exp Dermatol 27:473-475.

Smith WL, Wilkin GP. 1977. Immunochemistry of prostaglandin endoperoxide-forming cyclooxygenases: the detection of the cyclooxygenases in rat, rabbit, and guinea pig kidneys by immunofluorescence. Prostaglandins 13:873-892.

Song Y, Buettner GR. 2010. Thermodynamic and kinetic considerations for the reaction of semiquinone radicals to form superoxide and hydrogen peroxide. Free radical biology & medicine 49:919-962.

Sorensen HT, Larsen BO. 1994. A population-based Danish data resource with possible high validity in pharmacoepidemiological research. Journal of medical systems 18:33-38.

Spagnolo PA, Ramchandani VA, Schwandt ML, Kwako LE, George DT, Mayo LM, et al. 2016. FAAH Gene Variation Moderates Stress Response and Symptom Severity in Patients with Posttraumatic Stress Disorder and Comorbid Alcohol Dependence. Alcoholism, clinical and experimental research 40:2426-2434.

Stålberg K, Haglund B, Strömberg B, Kieler H. 2010. Prenatal exposure to medicines and the risk of childhood brain tumor. Cancer epidemiology 34:400-404.

Steineck G, Wiholm BE, Gerhardsson de Verdier M. 1995. Acetaminophen, some other drugs, some diseases and the risk of transitional cell carcinoma. A population-based case-control study. Acta oncologica (Stockholm, Sweden) 34:741-748.

Stern ST, Bruno MK, Hennig GE, Horton RA, Roberts JC, Cohen SD. 2005. Contribution of acetaminophen-cysteine to acetaminophen nephrotoxicity in CD-1 mice: I. Enhancement of acetaminophen nephrotoxicity by acetaminophen-cysteine. Toxicology and applied pharmacology 202:151-159.

Strom BL. 2013. Study designs available for pharmacoepidemiologic studies. In: Textbook of Pharmacoepidemiology, Part 2nd (Strom BL, Kimmel SE, Hennessy S, eds):John Wiley & Sons, Ltd.

Stromberg PC, Vogtsberger LM. 1983. Pathology of the mononuclear cell leukemia of Fischer rats. I. Morphologic studies. Vet Pathol 20:698-708.

Stromberg PC. 1985. Large granular lymphocyte leukemia in F344 rats. Model for human T gamma lymphoma, malignant histiocytosis, and T-cell chronic lymphocytic leukemia. The American journal of pathology 119:517-519.

Suzuki Y, Goto K, Nakayama Y, Saratani M, Takata T, Okamoto T, et al. 2016. Evaluation of a single-dose PIGRET assay for acetaminophen in rats compared with the RBC Pig-a assay. Mutation research 811:16-20.

Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. 2016. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood 127:2375-2390.

Takehisa S, Kanaya N. 1982. SCE induction in human lymphocytes by combined treatment with aniline and norharman. Mutation research 101:165-172.

Tan XL, Reid Lombardo KM, Bamlet WR, Oberg AL, Robinson DP, Anderson KE, et al. 2011. Aspirin, nonsteroidal anti-inflammatory drugs, acetaminophen, and pancreatic cancer risk: a clinic-based case-control study. Cancer prevention research (Philadelphia, Pa) 4:1835-1841.

Terry MB, Gammon MD, Zhang FF, Tawfik H, Teitelbaum SL, Britton JA, et al. 2004. Association of frequency and duration of aspirin use and hormone receptor status with breast cancer risk. Jama 291:2433-2440.

Thomas J, Haseman JK, Goodman JI, Ward JM, Loughran TP, Jr., Spencer PJ. 2007. A review of large granular lymphocytic leukemia in Fischer 344 rats as an initial step toward evaluating the implication of the endpoint to human cancer risk assessment. Toxicological sciences : an official journal of the Society of Toxicology 99:3-19.

Thompson CZ, Hill LE, Epp JK, Probst GS. 1983. The induction of bacterial mutation and hepatocyte unscheduled DNA synthesis by monosubstituted anilines. Environ Mutagen 5:803-811.

Thummel KE, Lee CA, Kunze KL, Nelson SD, Slattery JT. 1993. Oxidation of acetaminophen to N-acetyl-p-aminobenzoquinone imine by human CYP3A4. Biochemical pharmacology 45:1563-1569.

Thummel KE, Wilkinson GR. 1998. In vitro and in vivo drug interactions involving human CYP3A. Annu Rev Pharmacol Toxicol 38:389-430.

Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath CW, Jr. 1993. Aspirin use and risk of fatal cancer. Cancer research 53:1322-1327.

Tise CG, Perry JA, Anforth LE, Pavlovich MA, Backman JD, Ryan KA, et al. 2016. From Genotype to Phenotype: Nonsense Variants in SLC13A1 Are Associated with

Decreased Serum Sulfate and Increased Serum Aminotransferases. G3 (Bethesda, Md) 6:2909-2918.

Tonge RP, Kelly EJ, Bruschi SA, Kalhorn T, Eaton DL, Nebert DW, et al. 1998. Role of CYP1A2 in the hepatotoxicity of acetaminophen: investigations using Cyp1a2 null mice. Toxicology and applied pharmacology 153:102-108.

Topham JC. 1980. The detection of carcinogen-induced sperm head abnormalities in mice. Mutation research 69:149-155.

Topinka J, Sram RJ, Sirinjan G, Kocisova J, Binkova B, Fojtikova I. 1989. Mutagenicity studies on paracetamol in human volunteers. II. Unscheduled DNA synthesis and micronucleus test. Mutation research 227:147-152.

Torre LA, Islami F, Siegel RL, Ward EM, Jemal A. 2017. Global Cancer in Women: Burden and Trends. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 26:444-457.

Torti DC, Christensen BC, Storm CA, Fortuny J, Perry AE, Zens MS, et al. 2011. Analgesic and nonsteroidal anti-inflammatory use in relation to nonmelanoma skin cancer: a population-based case-control study. Journal of the American Academy of Dermatology 65:304-312.

TOXNET databases. 2019. Aniline. CAS # 62-53-3. Available at: https://toxnet.nlm.nih.gov/.

Trabert B, Ness RB, Lo-Ciganic WH, Murphy MA, Goode EL, Poole EM, et al. 2014. Aspirin, nonaspirin nonsteroidal anti-inflammatory drug, and acetaminophen use and risk of invasive epithelial ovarian cancer: a pooled analysis in the Ovarian Cancer Association Consortium. Journal of the National Cancer Institute 106:djt431.

Trabert B, Poole EM, White E, Visvanathan K, Adami HO, Anderson GL, et al. 2019. Analgesic Use and Ovarian Cancer Risk: An Analysis in the Ovarian Cancer Cohort Consortium. Journal of the National Cancer Institute.

Tsuruzaki T, Watanabe G, Yamamoto M. 1982. [The effects of aspirin and acetaminophen during pregnancy on chromosomal structure in rat fetuses]. Nihon eiseigaku zasshi Japanese journal of hygiene 37:787-796.

Tung EW, Philbrook NA, Macdonald KD, Winn LM. 2012. DNA double-strand breaks and DNA recombination in benzene metabolite-induced genotoxicity. Toxicological sciences : an official journal of the Society of Toxicology 126:569-577.

Uematsu F, Kikuchi H, Motomiya M, Abe T, Sagami I, Ohmachi T, et al. 1991. Association between restriction fragment length polymorphism of the human cytochrome P450IIE1 gene and susceptibility to lung cancer. Japanese journal of cancer research : Gann 82:254-256.

US EPA. 1988. Aniline; CASRN 62-53-3. (Integrated Risk Information System (IRIS) US Environmental Protection Agency Chemical Assessment Summary). Available: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0350_summary.pdf.

US EPA. 2005. Provisional Peer Reviewed Toxicity Values for p-Amionphenol (CASRN 123-30-8) [EPA Report]. Cincinnati, OH:U.S. Environmental Protection Agency. Available: https://hhpprtv.ornl.gov/issue_papers/Aminophenolp.pdf.

US FDA. 2009. Organ-Specific Warnings; Internal Analgesic, Antipyretic, and Antirheumatic Drug Products for Over-the-Counter Human Use; Final Monograph.

US FDA. 2014. All manufacturers of prescription combination drug products with more than 325 mg of acetaminophen have discontinued marketing. Available: https://www.fda.gov/drugs/information-drug-class/all-manufacturers-prescription-combination-drug-products-more-325-mg-acetaminophen-have-discontinued [accessed on February 12].

US FDA. 2019. Drugs@FDA: FDA Approved Drug Products. Available: https://www.accessdata.fda.gov/scripts/cder/daf/ [accessed on February 12].

Utkarsh D, Loretz C, Li AP. 2016. In vitro evaluation of hepatotoxic drugs in human hepatocytes from multiple donors: Identification of P450 activity as a potential risk factor for drug-induced liver injuries. Chemico-biological interactions 255:12-22.

Vad NM, Yount G, Moore D, Weidanz J, Moridani MY. 2009. Biochemical mechanism of acetaminophen (APAP) induced toxicity in melanoma cell lines. Journal of pharmaceutical sciences 98:1409-1425.

Valencia R, Mason JM, Woodruff RC, Zimmering S. 1985. Chemical mutagenesis testing in Drosophila. III. Results of 48 coded compounds tested for the National Toxicology Program. Environ Mutagen 7:325-348.

van Bladeren PJ. 1988. Formation of toxic metabolites from drugs and other xenobiotics by glutathione conjugation. Trends in pharmacological sciences 9:295-299.

van de Straat R, Vromans RM, Bosman P, de Vries J, Vermeulen NP. 1988. Cytochrome P-450-mediated oxidation of substrates by electron-transfer; role of oxygen radicals and of 1- and 2-electron oxidation of paracetamol. Chemico-biological interactions 64:267-280.

van der Marel CD, Anderson BJ, van Lingen RA, Holford NH, Pluim MA, Jansman FG, et al. 2003. Paracetamol and metabolite pharmacokinetics in infants. European journal of clinical pharmacology 59:243-251.

Van Dyke AL, Cote ML, Prysak G, Claeys GB, Wenzlaff AS, Schwartz AG. 2008. Regular adult aspirin use decreases the risk of non-small cell lung cancer among women. Cancer epidemiology, biomarkers & prevention : a publication of the American

Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 17:148-157.

van Rongen A, Valitalo PAJ, Peeters MYM, Boerma D, Huisman FW, van Ramshorst B, et al. 2016. Morbidly Obese Patients Exhibit Increased CYP2E1-Mediated Oxidation of Acetaminophen. Clinical pharmacokinetics 55:833-847.

Veitonmaki T, Murtola TJ, Maattanen L, Taari K, Stenman UH, Tammela TL, et al. 2014. Prostate cancer risk and nonsteroidal antiinflammatory drug use in the Finnish prostate cancer screening trial. British journal of cancer 111:1421-1431.

Viswanathan AN, Feskanich D, Schernhammer ES, Hankinson SE. 2008. Aspirin, NSAID, and acetaminophen use and the risk of endometrial cancer. Cancer research 68:2507-2513.

Walker V, Mills GA, Anderson ME, Ingle BL, Jackson JM, Moss CL, et al. 2017. The acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) inhibits glutathione synthetase in vitro; a clue to the mechanism of 5-oxoprolinuric acidosis? Xenobiotica; the fate of foreign compounds in biological systems 47:164-175.

Walley T, Mantgani A. 1997. The UK General Practice Research Database. Lancet (London, England) 350:1097-1099.

Walter RB, Brasky TM, White E. 2011a. Cancer risk associated with long-term use of acetaminophen in the prospective VITamins and lifestyle (VITAL) study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 20:2637-2641.

Walter RB, Milano F, Brasky TM, White E. 2011b. Long-term use of acetaminophen, aspirin, and other nonsteroidal anti-inflammatory drugs and risk of hematologic malignancies: results from the prospective Vitamins and Lifestyle (VITAL) study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 29:2424-2431.

Wan J, Bae MA, Song BJ. 2004. Acetoaminophen-induced accumulation of 8-oxodeoxyguanosine through reduction of Ogg1 DNA repair enzyme in C6 glioma cells. Experimental & molecular medicine 36:71-77.

Wang X, Wu Q, Liu A, Anadon A, Rodriguez JL, Martinez-Larranaga MR, et al. 2017. Paracetamol: overdose-induced oxidative stress toxicity, metabolism, and protective effects of various compounds in vivo and in vitro. Drug metabolism reviews 49:395-437.

Wang Y, Li D, Cheng N, Gao H, Xue X, Cao W, et al. 2015. Antioxidant and hepatoprotective activity of vitex honey against paracetamol induced liver damage in mice. Food & function 6:2339-2349.

Watanabe M. 1982. [Cytogenetic effects of antipyretic analgesics on human lymphocytes]. Nihon eiseigaku zasshi Japanese journal of hygiene 37:673-685.

Watanabe T, Hirayama T, Fukui S. 1989. Phenazine derivatives as the mutagenic reaction product from o- or m-phenylenediamine derivatives with hydrogen peroxide. Mutation research 227:135-145.

Watari N, Iwai M, Kaneniwa N. 1983. Pharmacokinetic study of the fate of acetaminophen and its conjugates in rats. J Pharmacokinet Biopharm 11:245-272.

Watari N, Hanawa M, Iwai M, Kaneniwa N. 1984. Pharmacokinetic study of the enterohepatic circulation of acetaminophen glucuronide in rats. Journal of pharmacobio-dynamics 7:811-819.

WCRF/AICR. 2018. Diet, nutrition, physical activity and bladder cancer. (Continuous Update Project Expert Report). Washington, DC: World Cancer Research Fund/American Institute for Cancer Research. Available: https://www.wcrf.org/dietandcancer/bladder-cancer.

Webb PM, Na R, Weiderpass E, Adami HO, Anderson KE, Bertrand KA, et al. 2018. Use of aspirin, other nonsteroidal anti-inflammatory drugs and acetaminophen and risk of endometrial cancer: the Epidemiology of Endometrial Cancer Consortium. Annals of oncology : official journal of the European Society for Medical Oncology.

Weisburger JH, Weisburger EK, Madison RM, Wenk ML, Klein DS. 1973. Effect of acetanilide and p-hydroxyacetanilide on the carcinogenicity of N-2-fluorenylacetamide and N-hydroxy-N-2-fluorenylacetamide in mice, hamsters, and female rats. Journal of the National Cancer Institute 51:235-240.

Weiss JR, Baker JA, Baer MR, Menezes RJ, Nowell S, Moysich KB. 2006. Opposing effects of aspirin and acetaminophen use on risk of adult acute leukemia. Leukemia research 30:164-169.

Weiss NS. 2016. Use of acetaminophen in relation to the occurrence of cancer: a review of epidemiologic studies. Cancer causes & control : CCC 27:1411-1418.

West PR, Harman LS, Josephy PD, Mason RP. 1984. Acetaminophen: enzymatic formation of a transient phenoxyl free radical. Biochemical pharmacology 33:2933-2936.

Westphal GA, Bunger J, Lichey N, Taeger D, Monnich A, Hallier E. 2009. The benzene metabolite para-benzoquinone is genotoxic in human, phorbol-12-acetate-13-myristate induced, peripheral blood mononuclear cells at low concentrations. Archives of toxicology 83:721-729.

Whitfield JB. 2001. Gamma glutamyl transferase. Critical reviews in clinical laboratory sciences 38:263-355.

Wild D, Eckhardt K, Gocke E, King MT. 1980. In: Short-term Test Systems for Detecting Carcinogens. In: Comparative results of short-term in vitro and in vivo mutagenicity tests obtained with selectec environmental chemicals (Norpoth KH, Garner RC, eds). Berlin:Springer-Verlag, 170–179.

Williams AJ, Grulke CM, Edwards J, McEachran AD, Mansouri K, Baker NC, et al. 2017. The CompTox Chemistry Dashboard: a community data resource for environmental chemistry. J Cheminform 9:61.

Williams DP, Garcia-Allan C, Hanton G, LeNet JL, Provost JP, Brain P, et al. 2004. Time course toxicogenomic profiles in CD-1 mice after nontoxic and nonlethal hepatotoxic paracetamol administration. Chemical research in toxicology 17:1551-1561.

Williams GM, Iatropoulos MJ, Jeffrey AM, Duan JD, Perrone CE. 2007. Inhibition by acetaminophen of neoplastic initiation elicited in rat liver by the DNA-reactive hepatocarcinogen N-acetyl-2-aminofluorene. European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP) 16:528-534.

Wilmer JW, Natarajan AT. 1981. Induction of sister-chromatid exchanges and chromosome aberrations by gamma-irradiated nucleic acid constituents in CHO cells. Mutation research 88:99-107.

Wilson JM, Slattery JT, Forte AJ, Nelson SD. 1982. Analysis of acetaminophen metabolites in urine by high-performance liquid chromatography with UV and amperometric detection. Journal of chromatography 227:453-462.

Wirth PJ, Dybing E, von Bahr C, Thorgeirsson SS. 1980. Mechanism of N-hydroxyacetylarylamine mutagenicity in the Salmonella test system: metabolic activation of N-hydroxyphenacetin by liver and kidney fractions from rat, mouse, hamster, and man. Molecular pharmacology 18:117-127.

Wood M. 1969. Factors influencing the induction of tumours of the urinary bladder and liver by 2-acetylaminofluorene in the mouse. European journal of cancer (Oxford, England : 1990) 5:41-47.

Wright HN. 1967. Chronic toxicity studies of analgesic and antipyretic drugs and congeners. Toxicology and applied pharmacology 11:280-292.

Wright JL, Chery L, Holt S, Lin DW, Luedeke M, Rinckleb AE, et al. 2016. Aspirin and NSAID use in association with molecular subtypes of prostate cancer defined by TMPRSS2:ERG fusion status. Prostate cancer and prostatic diseases 19:53-56.

Wu AH, Pearce CL, Tseng CC, Templeman C, Pike MC. 2009. Markers of inflammation and risk of ovarian cancer in Los Angeles county. International journal of cancer 124:1409-1415.

Wu S, Zhang L, Chen J. 2012. Paracetamol in the environment and its degradation by microorganisms. Applied microbiology and biotechnology 96:875-884.

Wysong A, Ally MS, Gamba CS, Desai M, Swetter SM, Seiffert-Sinha K, et al. 2014. Non-melanoma skin cancer and NSAID use in women with a history of skin cancer in the Women's Health Initiative. Preventive medicine 69:8-12.

Xie Y, McGill MR, Cook SF, Sharpe MR, Winefield RD, Wilkins DG, et al. 2015. Time course of acetaminophen-protein adducts and acetaminophen metabolites in circulation of overdose patients and in HepaRG cells. Xenobiotica; the fate of foreign compounds in biological systems 45:921-929.

Yager JW, Eastmond DA, Robertson ML, Paradisin WM, Smith MT. 1990. Characterization of micronuclei induced in human lymphocytes by benzene metabolites. Cancer research 50:393-399.

Yamamoto A, Liu MY, Kurogi K, Sakakibara Y, Saeki Y, Suiko M, et al. 2015. Sulphation of acetaminophen by the human cytosolic sulfotransferases: a systematic analysis. J Biochem 158:497-504.

Yang B, Petrick JL, Chen J, Hagberg KW, Sahasrabuddhe VV, Graubard BI, et al. 2016. Associations of NSAID and paracetamol use with risk of primary liver cancer in the Clinical Practice Research Datalink. Cancer epidemiology 43:105-111.

Yang CS, Ishizaki H, Lee MJ, Wade D, Fadel A. 1991. Deuterium isotope effect in the interaction of N-nitrosodimethylamine, ethanol, and related compounds with cytochrome P-450IIE1. Chemical research in toxicology 4:408-413.

Yang F, Zhou JH. 2010. Cytotoxicity and DNA damage induced by 1, 4-benzoquinone in v79 Chinese hamster lung cells. Journal of toxicology and environmental health Part A 73:483-489.

Yang S, Jawahar R, McAlindon TE, Eaton CB, Lapane KL. 2012. Racial differences in symptom management approaches among persons with radiographic knee osteoarthritis. BMC complementary and alternative medicine 12:86.

Yang X, Flowers RC, Weinberg HS, Singer PC. 2011. Occurrence and removal of pharmaceuticals and personal care products (PPCPs) in an advanced wastewater reclamation plant. Water research 45:5218-5228.

Yang Y, Wong SE, Lightstone FC. 2014. Understanding a substrate's product regioselectivity in a family of enzymes: a case study of acetaminophen binding in cytochrome P450s. PloS one 9:e87058.

Yaxley JP. 2016. Urinary tract cancers: An overview for general practice. Journal of family medicine and primary care 5:533-538.

Yiang GT, Yu YL, Lin KT, Chen JN, Chang WJ, Wei CW. 2015. Acetaminophen induces JNK/p38 signaling and activates the caspase-9-3-dependent cell death pathway in human mesenchymal stem cells. International journal of molecular medicine 36:485-492.

Yoon MY, Kim SJ, Lee BH, Chung JH, Kim YC. 2006. Effects of dimethylsulfoxide on metabolism and toxicity of acetaminophen in mice. Biological & pharmaceutical bulletin 29:1618-1624.

Yoshida R, Oikawa S, Ogawa Y, Miyakoshi Y, Ooida M, Asanuma K, et al. 1998. Mutagenicity of p-aminophenol in E. coli WP2uvrA/pKM101 and its relevance to oxidative DNA damage. Mutation research 415:139-150.

Zaher H, Buters JT, Ward JM, Bruno MK, Lucas AM, Stern ST, et al. 1998. Protection against acetaminophen toxicity in CYP1A2 and CYP2E1 double-null mice. Toxicology and applied pharmacology 152:193-199.

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. 1988. Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. Environmental and molecular mutagenesis 11 Suppl 12:1-157.

Zhang H, Forman HJ, Choi J. 2005. Gamma-glutamyl transpeptidase in glutathione biosynthesis. Methods in enzymology 401:468-483.

Zhang H, Jiang D, Li X. 2013. Use of nonsteroidal andti-inflammatory drugs and bladder cancer risk: a meta-analysis of epidemiologic studies. PLoS One 8(7):e70008.

Zhang X, Smith-Warner SA, Collins LC, Rosner B, Willett WC, Hankinson SE. 2012. Use of aspirin, other nonsteroidal anti-inflammatory drugs, and acetaminophen and postmenopausal breast cancer incidence. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 30:3468-3477.

Zhao L, Pickering G. 2011. Paracetamol metabolism and related genetic differences. Drug metabolism reviews 43:41-52.

Appendix A Literature Search Strategies on the Carcinogenicity of Acetaminophen

General searches of the literature on the carcinogenicity of acetaminophen were conducted by Nancy Firchow, MLS, OEHHA librarian. The goal was to identify peer-reviewed open-source and proprietary journal articles, print and digital books, reports, and gray literature that potentially reported relevant toxicological and epidemiological information on the carcinogenicity of this chemical. The search sought to identify all literature relevant to the assessment of evidence on the carcinogenicity of acetaminophen. As described below, we used a similar approach as that recommended by the National Toxicology Program (NTP) Handbook for Preparing Report on Carcinogens (RoC) Monographs (NTP 2015; https://ntp.niehs.nih.gov/ntp/roc/handbook/roc_handbook_508.pdf), with the goal of systematically identifying and reviewing all literature relevant to the assessment of evidence on acetaminophen's carcinogenicity.

Search Process

Primary Search

The steps of the strategy for conducting a primary search in PubMed are presented below:

PubMed MeSH browser (https://www.ncbi.nlm.nih.gov/mesh) was used to identify subject headings, other index terms and synonyms for acetaminophen search terms (Search Step #1):

- #1: Acetaminophen search terms

National Toxicology Program's *Standard Search Strings for Literature Database Searches: Appendix to the Draft Handbook for Preparing Report on Carcinogens Monographs* (https://ntp.niehs.nih.gov/ntp/roc/handbook/rochandbookappendix_508.pdf) was used to identify search strategies for the following search terms (Search Step #2 to #8):

- #2: cancer terms
- #3: human study terms
- #4: experimental animal terms
- #5: characteristics of carcinogens terms
- #6: genotoxicity terms
- #7: other mechanistic terms
- #8: general toxicity terms

The detailed search strategy executed in PubMed is shown in Table A-1. The result of this search (i.e., Search Step # 11) yielded 587 references.

Table A1. PubMed Search Strategy as executed November 20, 2018

Search step	Search terms	Reference No.
#1	Acetaminophen search terms: acetaminophen[mh] OR acetaminophen[tiab] OR paracetamol[tiab] OR 103-90-2[rn]	26147
#2	Cancer Terms (RoC Search Strategy)	3521240
#3	Human Studies Terms (RoC Search Strategy)	4395937
#4	Experimental Animals (RoC Search Strategy)	2298007
#5	Characteristics of Carcinogens (RoC Search Strategy)	4880014
#6	Genotoxicity Terms (RoC Search Strategy)	1041085
#7	Other mechanistic terms (RoC Search Strategy)	3082776
#8	General Toxicity terms (RoC Search Strategy)	3337249
#9	Acetaminophen + cancer + (human, animal, characteristics, genotox, other mech, general tox): #1 AND #2 AND (#3 OR #4 OR #5 OR #6 OR #7 OR #8)	935
#10	Terms to exclude: (hepato*[ti] OR pancreatitis[mh] OR pancreatitis[ti] OR nephropath*[ti] OR pain management[mh] OR arthritis[ti] OR osteoarthritis[ti] OR mammography[mh] OR mammogra*[ti] OR liver failure[mh] OR liver*[ti] OR surgery[sh] OR "surgical procedures, operative"[mh] OR surg*[ti] OR hepatic[ti] OR plants[mh])	4278697
#11	FINAL with exclude terms removed: #9 NOT #10	587

Primary searches were then conducted in the additional databases and other data sources listed below. The search strategies were tailored according to the search features unique to each database and data source. In Embase, for example, Emtree was searched to identify subject headings to replace the MeSH terms used in PubMed.

The initial search results of all databases and data sources were uploaded to EndNote. After deduplication, a total of 1444 unique references were available for review.

Supplemental Targeted Searches

Metabolism, pharmacokinetics, and exposure studies were identified by searching PubMed for review articles on those topics. The full text of reviews was evaluated by an OEHHA scientist to identify specific aspects of these topics to be explored more deeply. Searches for those specific aspects were created and results reviewed.

Supplemental targeted searches were performed in PubMed and other resources as needed to expand retrieval on specific aspects of the subject. These additional searches, combined with the primary search results, yielded a total of 2003 unique references.

Data Sources

The following is a list of the major data sources that were searched to find information on acetaminophen. The list was recommended by the NTP Handbook for Preparing Report on Carcinogens (RoC) Monographs (NTP 2015) and adapted by OEHHA.

Biomedical literature databases

- PubMed (National Library of Medicine) (https://www.ncbi.nlm.nih.gov/pubmed)
- Embase (https://www.embase.com/#search)
- Scopus (https://www.scopus.com/search/form.uri?display=basic)
- SciFinder-n (https://sso.cas.org/as/GkjnW/resume/as/authorization.ping)
- TOXNET (National Library of Medicine): Toxicology Literature Online (TOXLINE) (https://toxnet.nlm.nih.gov)

Authoritative reviews and reports

- International Agency for Research on Cancer (IARC) Monographs (https://monographs.iarc.fr/)
- NTP publications, including, but not limited to, technical reports, nominations for toxicological evaluation documents, RoC monographs, RoC background documents or monographs, and NTP Office of Health Assessment and Translation (OHAT) monographs (https://ntp.niehs.nih.gov)
- US Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) (https://www.epa.gov/iris)
- Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles (https://www.atsdr.cdc.gov/toxprofiles/index.asp)

- European Chemicals Agency Risk Assessments (https://echa.europa.eu)
- National Academy of Sciences reports and publications (https://www.nationalacademies.org/publications)
- World Health Organization (WHO)/United Nations Environment Programme (UNEP) International Programme on Chemical Safety (IPCS) INCHEM-related documents (http://www.inchem.org)

Other Databases or web resources

- TOXNET Databases (https://toxnet.nlm.nih.gov/) besides TOXLINE: Genetic Toxicology Data Bank (GENE-TOX), Carcinogenic Potency Database (CPD), Chemical Carcinogenesis Research Information System (CCRIS), Hazardous Substances Data Bank (HSDB), and more
- Comparative Toxicogenomics Database (CTD) (https://ctdbase.org/)
- Computational Toxicology (CompTox) Chemicals Dashboard, (https://www.epa.gov/chemical-research/comptox-chemicals-dashboard)
- International Uniform Chemical Information Database (https://iuclid6.echa.europa.eu)
- National Institute for Occupational Safety and Health (NIOSH) publications (https://www.cdc.gov/niosh/pubs/default.html)
- PubChem BioAssay (National Library of Medicine) (https://www.ncbi.nlm.nih.gov/pcassay)

Additional focused literature searches

In addition to the literature searches described above, additional focused literature searches were performed by OEHHA for certain topics covered in various sections of the document. The specific additional search strategies used for each of these sections are briefly described as follows.

Section 2. Introduction

A focused search was conducted for acetaminophen using the US EPA National Service Center for Environmental Publications (https://www.epa.gov/nscep/). Additional relevant literature was identified from citations in US EPA publications. The following key words were used to search relevant databases:

- (“acetaminophen” OR “paracetamol” OR “N-(4-hydroxyphenyl) acetamide” OR “4-hydroxyacetanilide” OR “4-Acetaminophenol” OR “103-90-2 [RN]”) AND (“exposure”[MeSH] OR “occurrence”[MeSH] OR “uses”[MeSH])

Section 3.1 Carcinogenicity studies in humans

A focused search was conducted for human studies using PubMed. Additionally, the reference lists of reviews and the papers marked for inclusion were screened to identify additional literature.

Section 3.2 Carcinogenicity studies in animals

Focused searches were conducted in databases including PubMed, SciFinder, Embase, and Scopus. Additional literature was identified through citations of relevant studies.

Section 3.3.1. Pharmacokinetics and Metabolism, and Section 3.3.2. Factors that modulate acetaminophen metabolism

A focused literature search was conducted for metabolism, absorption, distribution, and elimination, enzyme polymorphism or inter-individual variability of acetaminophen metabolism, using PubMed. Additional relevant literature was identified from citations of individual articles. The following key words were used to search relevant databases:

- ("acetaminophen" OR "paracetamol" OR "N-(4-hydroxyphenyl) acetamide" OR "4-hydroxyacetanilide" OR "4-Acetaminophenol" OR "103-90-2 [RN]") AND ("metabolite*"[MeSH] OR "metabolism"[MeSH] OR "pharmacokinetics"[MeSH] AND "toxicity" [MeSH] OR "CYP*"[MeSH] OR "CYP450*"[MeSH] OR "polymorphism, genetic" [MeSH])

Section 3.3.3. Genotoxicity

Focused searches were conducted for acetaminophen and its metabolites, using PubMed, TOXNET databases (including TOXLINE, GENE-TOX, HSDB, and CCRIS), and Scopus. Additional relevant literature was identified from citations in individual articles.

Section 3.3.5. Animal Tumor Pathology

Focused searches were conducted using three pathology books published by IARC [Pathology of Tumors in Laboratory Animals: Volume 1-3, 1991 (Vol. 1), 1994 (Vol. 2), 1996 (Vol. 3)], NTP's historical controls database (https://ntp.niehs.nih.gov/results/dbsearch/historical/index.html), and searching for information specific to species and tumor site/type. Additional relevant literature was identified from citations in individual books or articles.

Section 3.3.6. Structure Activity Considerations

- A search in the US EPA CompTox Dashboard for structurally similar chemicals to ACETAMINOPHEN with a similarity threshold of 0.8 or above [the Tanimoto (or Jaccard) coefficient] resulted in 112 chemicals
- Among the 112 chemicals, 18 were selected based on availability of PubMed articles, or CPDAT (Chemical and Product Categories) count, or ToxCast data

- Three additional structurally similar chemicals were selected during our literature review process

Focused searches were conducted for the five comparison chemicals using the TOXNET databases, PubMed, Scopus, IARC monographs, and the NTP Report on Carcinogens. Additional relevant literature was identified from citations in individual articles.

3.3.7 ToxCast high-throughput screening assays

Focused searches for data on acetaminophen and its metabolites were conducted using the US EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard). Chemical QC data and assay descriptions for the Tox21 assays (a subset of the ToxCast assays) were identified from the Tox21 Data Browser (https://tripod.nih.gov/tox21).

Section 3.3.8 ROS production and oxidative stress and Section 4 Key characteristics

Focused searches were conducted for acetaminophen and all 10 KCs, using PubMed, TOXNET, and Scopus. Information on KC 1, 2, 3, and 5 were relevant to the carcinogenicity of acetaminophen, and included in the document.
Additional relevant literature was identified from citations in individual articles.

Section 3.3.9 Transcriptomic, proteomic, and metabolomic (Omics) studies

Focused searches were conducted for transcriptomic, proteomic, and metabolomics studies on acetaminophen using PubMed. Additional studies were identified from the CTD database.

Use of Health Assessment Workspace Collaborative (HAWC)

HAWC (https://hawcproject.org/about/) was used as a tool in the systematic review of the literature on the carcinogenicity of acetaminophen following the guidance provided in the NTP RoC Handbook (NTP 2015). Specifically:

Citations retrieved from literature searches were uploaded to EndNote libraries, and duplicates were removed. Next, the EndNote libraries were uploaded to HAWC for multi-level screening using specific inclusion and exclusion criteria.

In Level 1 screening, the citations were screened independently by two OEHHA scientists, based solely on titles and abstracts, to eliminate studies or articles that do not contain information on acetaminophen or on any of the key topics such as exposure, cancer studies in humans and animals, toxicokinetics, metabolism, genotoxicity, or cancer-associated mechanisms. The initial screen was intended to retrieve all studies deemed to have a reasonable possibility of containing information that could be useful for the review process. A positive response by only one of the reviewers was sufficient

to pass a publication on to the next review level. The initial reviewers assigned (or tagged) the citation to one or more of the key topic(s) (see Figure 6 and 7).

In Level 2 screening, the full papers were obtained for all citations that passed the Level 1 screen. These full papers were screened independently by two OEHHA scientists, using similar inclusion/exclusion criteria as was used in the Level 1 screening. However, Level 2 reviewers could make more accurate judgments about the relevance of the citations because they were reviewing the full text of the articles, in addition to the title and abstract. Following Level 2 screening, the tagging of articles according to key topics was updated in HAWC.

Level 1 and 2 screenings were repeated and HAWC search results were updated if additional relevant studies cited in the original set of publications ("secondary citations") were identified.

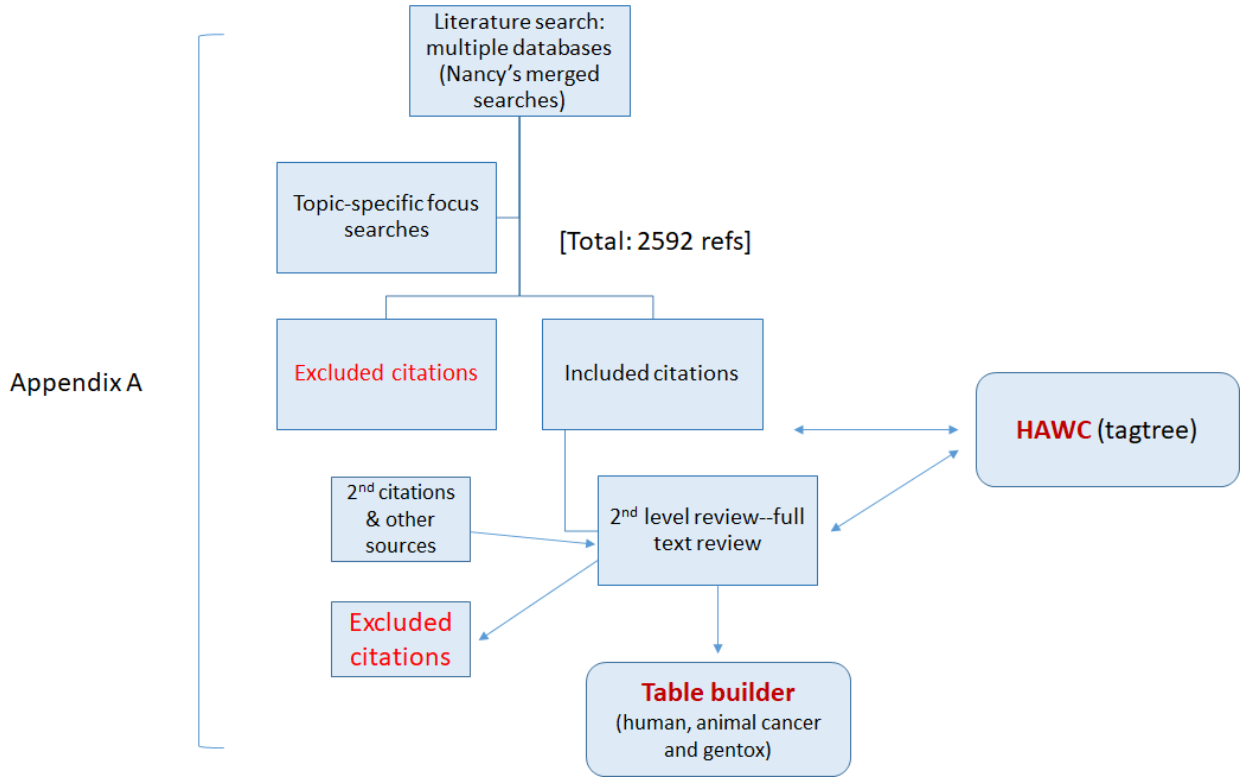


Figure 6. Literature search strategy on the carcinogenicity of acetaminophen

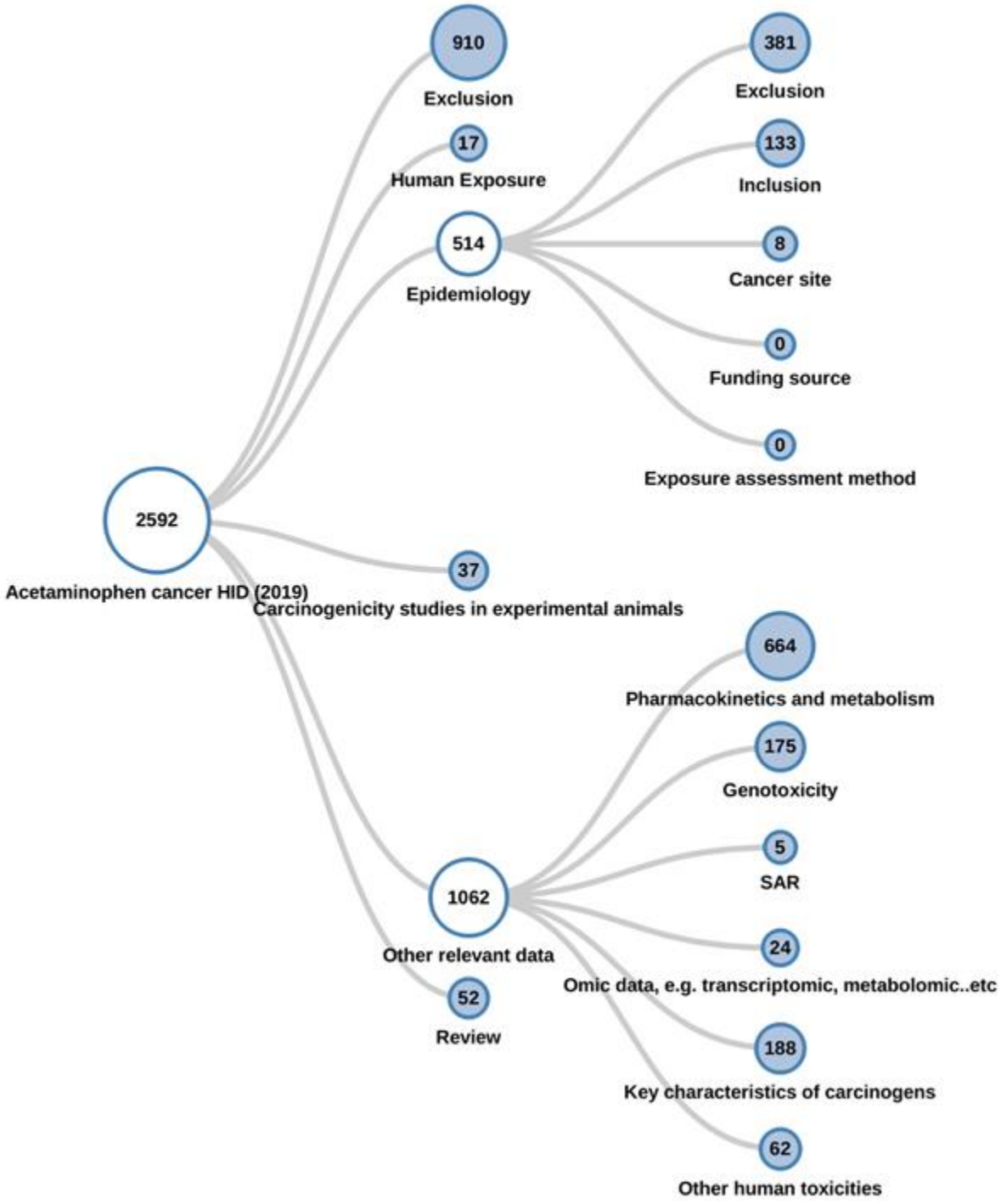


Figure 7. Overview of the systematic review of the literature on the carcinogenicity of acetaminophen

Finally, Table Builder (https://ctable.oehha.ca.gov/), a web-based application was applied to systematically extract and analyze the data that were included in Section 3.1 Carcinogenicity studies in humans, Section 3.2 Carcinogenicity studies in animals, and Section 3.3.3. Genotoxicity. Additionally, Table Builder worked as a custom-made database to generate Word tables in this document.

In summary, approximately 2592 references, including government reports and peer-reviewed journal articles, were identified through these search strategies. Among these, 641 were cited in this document.

Appendix B Human epidemiological evidence: additional tables

Table B1. Cohort and nested case-control studies of breast cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Breast (Overall) – Cohort					
Harris et al. (1999) Cohort Ohio, US **Enrollment or follow-up:** 1991-1996	**Population:** Women enrolled in the mammography screening program of the Ohio State University Comprehensive Care Center N=32,505 **Exposure assessment method:** questionnaire	**Unadjusted RR, Baseline medication use**			None
		<1 pills/week	1	240	
		1-3 pills/week	0.84 (0.55–1.28)	19	
		≥4 pills/week	0.84 (0.47–1.5)	17	
		Trend-test *p*-value: <0.35			
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	**Population:** Danish Cancer registry and prescription database. Cases excluded because of residency outside the county at the date of prescription, parent (of patient) registered as customer, error in the personal identification number, death prior to or at the date of prescription, age younger than 16 years, subjects who had a cancer diagnosis (except nonmelanoma skin cancer) prior to date of first recorded prescription, or died within the first year of follow-up N = 39,946 **Exposure assessment method:** records	**ICD-7, 170: SIR, Women prescribed acetaminophen (1989-1997)**			Age
		Prescription	0.9 (0.7–1.2)	52	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Harris et al. (2003) Cohort US **Enrollment or follow-up:** 1992-2001	**Population:** Women's Health Initiative Observational Study (WHI OS) N=80,741 **Exposure assessment method:** questionnaire	**RR, Duration of use at baseline**			Age
		0-11 mo	1	955	
		1-4 yrs	1.02 (0.75–1.37)	44	
		≥5 yrs	0.96 (0.76–1.2)	79	
		Trend-test *p*-value: 0.71			
Lipworth et al. (2003) Cohort Denmark **Enrollment or follow-up:** 1989-1996	**Population:** Individuals who filled acetaminophen prescriptions identified through the Pharmacoepidemiological Prescription Database N = 17,760 men; 32,130 women **Exposure assessment method:** records	**Mortality: SMR, Number of prescriptions**			Age, sex, calendar year
		1	2.4 (1.9–2.9)	102	
		2-4	2.8 (2.3–3.3)	108	
		5-9	3.1 (2.4–3.9)	76	
		≥10	2.2 (1.8–2.8)	76	
		Mortality: SMR, Prescribed acetaminophen (1989-1996)			
		Total	2.6 (2.3–2.8)	362	
		Men	2.4 (0.3–8.5)	2	
		Women	2.6 (2.3–2.9)	360	
		Mortality: SMR, Prescribed acetaminophen, stratified by latency period			
		<1 yr	5.6 (4.9–6.5)	198	
		1-2 yrs	2.1 (1.7–2.5)	115	
		3-4 yrs	1 (0.7–1.4)	34	
		≥5 yrs	0.9 (0.5–1.5)	15	
Marshall et al. (2005) Cohort California, US **Enrollment or follow-up:** 1995 to 2001	**Population:** Women in the California Teachers Study, aged 22-85 yrs N=114,460 **Exposure assessment method:** questionnaire	**Invasive breast cancer: RR, Duration and frequency of use at baseline**			Race, BMI, first-degree family history of breast cancer, menopausal and hormone therapy use status, smoking, alcohol intake, physical activity, mammography history, breast biopsy history, parity status before age 30, neighborhood socioeconomic status
		No regular use	1	NR	
		<5 yrs, 1-6 days/wk	1 (0.81–1.24)	NR	
		≥5 yrs, 1-6 days/wk	1.12 (0.96–1.31)	NR	
		<5 yrs daily	1.01 (0.68–1.48)	NR	
		≥5 yrs daily	0.96 (0.63–1.47)	NR	
		Trend-test *p*-value: 0.37			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
		Localized: RR, Duration and frequency of use at baseline			
		No regular use	1	NR	
		<5 yrs, 1-6 days/wk	0.99 (0.76–1.28)	NR	
		≥5 yrs, 1-6 days/wk	1.14 (0.95–1.38)	NR	
		<5 yrs daily	1.07 (0.68–1.66)	NR	
		≥5 yrs daily	0.91 (0.55–1.52)	NR	
		Trend-test *p*-value: 0.39			
		Nonlocalized: RR, Duration and frequency of use at baseline			
		No regular use	1	NR	
		<5 yrs, 1-6 days/wk	1.06 (0.73–1.55)	NR	
		≥5 yrs, 1-6 days/wk	1.08 (0.82–1.43)	NR	
		<5 yrs daily	0.88 (0.39–1.97)	NR	
		≥5 yrs daily	0.97 (0.44–2.18)	NR	
		Trend-test *p*-value: 0.77			
		Invasive: RR, Duration and frequency at baseline in ER/PR+			
		No regular use	1	NR	
		<5 yrs, 1-6 days/wk	0.99 (0.77–1.27)	NR	
		≥5 yrs, 1-6 days/wk	1.12 (0.94–1.35)	NR	
		<5 yrs daily	1.12 (0.73–1.72)	NR	
		≥5 yrs daily	0.91 (0.55–1.52)	NR	
		Trend-test *p*-value: 0.41			
		Invasive: RR, Duration and frequency at baseline in ER-/PR-			
		No regular use	1	NR	
		<5 yrs, 1-6 days/wk	1.37 (0.8–2.35)	NR	
		≥5 yrs, 1-6 days/wk	1.38 (0.92–2.08)	NR	
		<5 yrs daily	1.86 (0.77–4.54)	NR	
		≥5 yrs daily	0.8 (0.2–3.23)	NR	
		Trend-test *p*-value: 0.094			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Gallicchio et al. (2007) Cohort Maryland, US **Enrollment or follow-up:** 1989 to 2006	**Population:** Women in the CLUE II cohort N=15,651 **Exposure assessment method:** questionnaire	**Invasive breast cancer: RR, Baseline medication use (1989)**			Age
		Non-users	1	371	
		Users	0.94 (0.71–1.25)	47	
		Invasive breast cancer: RR, Use in 1996			
		Non-users	1	228	
		Users	1.11 (0.79–1.57)	69	
Gill et al. (2007) Cohort USA **Enrollment or follow-up:** 1993-2002	**Population:** Women in the Multiethnic Cohort from Hawaii and California N=98,920 **Exposure assessment method:** questionnaire	**HR, Current use**			Age, ethnicity, BMI, family history of breast cancer, education, mammography screening, alcohol intake, age at first live birth, number of children, age at menopause, use of hormone replacement therapy, all pain medication use, age at menarche, menopausal status
		Nonusers of pain medications	1	850	
		≤1 yr	0.85 (0.62–1.17)	40	
		2-5 yrs	0.99 (0.8–1.21)	100	
		≥6 yrs	0.87 (0.71–1.06)	116	
		Trend-test *p*-value: 0.23			
		HR, Past users			
		Nonusers of pain medications	1	850	
		≤1 yr	0.89 (0.72–1.11)	104	
		2-5 yrs	1.14 (0.91–1.42)	91	
		≥6 yrs	1.05 (0.83–1.33)	83	
		Trend-test *p*-value: 0.42			
Kwan et al. (2007) Cohort California and Utah, US **Enrollment or follow-up:** 1997-2006	**Population:** Women in the Life After Cancer Epidemiology Study (LACE), aged 18-79 yrs N=2,292 **Exposure assessment method:** questionnaire	**Breast cancer recurrence: RR, Current regular use**			Age at diagnosis, race, stage of breast cancer, tamoxifen treatment, BMI, chemotherapy use, post-diagnosis COX2 inhibitor use
		No use (<3 months)	1	250	
		Ever use	1.21 (0.73–2)	20	
		Post-diagnosis use	1.49 (0.76–2.93)	10	
		Pre- and post-diagnosis use	1 (0.49–2.04)	10	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Friis et al. (2008) Cohort Denmark **Enrollment or follow-up:** 1993-2003	**Population:** Women in the Danish Diet, Cancer and Health cohort N=28,695 **Exposure assessment method:** questionnaire	**RR, Frequency of use at baseline**			Age, school education, parity, number of births, use of hormone replacement therapy, history of benign breast tumor surgery
		No use (≤1 pill/month)	1	275	
		>1 pill/month	1.1 (0.91–1.33)	180	
		2-3 pills/month	1.09 (0.87–1.37)	103	
		1-6 pills/week	1.07 (0.81–1.41)	61	
		1-6 pills/day	0.76 (0.46–1.26)	16	
		RR, Use during 1-10 years of follow-up			
		No use (≤1 pill/month)	1	156	
		>1 pill/month	1.02 (0.77–1.36)	70	
Bosco et al. (2011) Cohort US **Enrollment or follow-up:** 1995-2007	**Population:** African American women in the Black Women's Health Study (BWHS), aged 21-69 yr N=53,151 **Exposure assessment method:** questionnaire	**IRR, Baseline medication use (≥3 d/wk)**			Education, BMI at age 18, vigorous activity, female hormone use, smoking, aspirin and other NSAIDs
		Nonuse in 1995	1	1037	
		Current use in 1995	0.9 (0.76–1.07)	178	
		<1 yr	1.09 (0.83–1.42)	57	
		1 to <2 yrs	0.57 (0.28–1.14)	8	
		2 to <3 yrs	0.93 (0.55–1.58)	14	
		3 to <5 yrs	0.93 (0.63–1.36)	27	
		≥5 yrs	0.87 (0.67–1.12)	66	
		Trend-test *p*-value: 0.17			
		IRR, Time-dependent current and former medication use (≥3 d/wk)			
		Nonuse	1	909	
		Former use	0.94 (0.78–1.12)	138	
		Current use	0.8 (0.65–0.98)	108	
		<1 yr	0.55 (0.24–1.23)	7	
		1 to <2 yrs	0.41 (0.06–2.94)	1	
		2 to <3 yrs	0.93 (0.68–1.27)	41	
		3 to <5 yrs	0.92 (0.6–1.39)	22	
		≥5 yrs	0.7 (0.51–0.97)	37	
		Trend-test *p*-value: 0.03			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** VITAL (VITamins And Lifestyle) study 62,841 **Exposure assessment method:** questionnaire	**HR, 10-yr use prior to baseline in women**			Age, education, race, marital status, height, BMI, physical activity, pack-years of smoking, alcohol intake at 45 yrs, fruit and vegetable intake, red meat intake, multivitamin use, self-rated health, family history of colon, lung, and hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/chronic joint pain, migraine/chronic headaches, use of NSAIDs, family history of breast cancer, mammogram in past 2 yrs, age at menarche, age at menopause, age at first birth, years of estrogen therapy, years of combined hormone therapy, hysterectomy
		No use	1	646	
		Low use (<4 d/wk or <4 yrs)	1.1 (0.91–1.33)	196	
		High use (≥4 d/wk and ≥4 yrs)	0.83 (0.59–1.15)	53	
		Trend-test *p*-value: 0.74			
Clarke et al. (2017) Cohort California **Enrollment or follow-up:** 1995-2013	**Population:** Women in the California Teachers Study N=57,164 **Exposure assessment method:** questionnaire	**HRR, Current regular use**			Age, age at menarche, parity and age at first birth, total months breastfeeding, history of benign breast biopsy, family history of breast cancer, physical activity, alcohol consumption, BMI, menopausal status, hormone therapy use, use of NSAIDs
		No use in last 3 yrs	1	1109	
		Current use, ≥3 tablets/week	1 (0.87–1.15)	264	
		HR-positive/HER2-negative subtype: HRR, Current regular use			
		No use in last 3 yrs	1	757	
		Current use, ≥3 tablets/wk	0.98 (0.83–1.16)	180	
Kehm et al. (2019) Cohort US, Canada, Australia **Enrollment or follow-up:** 2011-2017	**Population:** Combined retrospective and prospective cohort: women enrolled in the BCFR or kConFab before 30 June 2011, aged 18–79 years at baseline, who self-reported medication history by follow-up questionnaire N=5606 (prospective study); N=8233 (retrospective study)	**HR, Regular use (≥2 times/wk for ≥1 month)**			Race/ethnicity, study center, baseline age, birth cohort, familial risk profile, cigarette smoking, alcohol consumption, hormone therapy use, hormonal birth control use, other types of medication
		Non-regular use	1	1985	
		Regular use	0.98 (0.85–1.12)	318	
		HR, Regular use (≥2 times/wk for ≥1 month) by BRCA mutation status			
		Non-regular use	1	NR	
		Non-carrier	0.9 (0.78–1.05)	NR	
		BRCA1 carrier	1.02 (0.7–1.48)	NR	
		BRCA2 carrier	1.58 (0.95–2.61)	NR	
		Trend-test *p*-value: 0.21			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
	Exposure assessment method: questionnaire	**HR, Regular use (≥2 times/wk for ≥1 month) by ER status**			
		Non-regular use	1	NR	
		ER positive	0.88 (0.71–1.1)	NR	
		ER negative	1.09 (0.81–1.46)	NR	
		HR, Regular use (≥2 times/wk for ≥1 month) in prospective study			
		Non-regular use	1	116	
		Regular use	0.96 (0.55–1.65)	23	
Breast (Overall) – Nested Case-Control					
García Rodríguez and González-Pérez (2004a) Nested Case-Control UK **Enrollment or follow-up:** 1995-2001	**Population:** Women in the General Practice Research Database (GPRD), aged 30-78 yr Cases: 3708; Controls: 20,000 **Exposure assessment method:** records	**OR, Regular use**			Age, calendar year, BMI, alcohol intake, prior benign breast disease, smoking status, NSAID, steroid, and HRT use
		No use	1	1885	
		Current use	0.9 (0.82–1)	878	
		Past use	0.97 (0.89–1.07)	945	
		OR, Duration of use			
		No use	1	1885	
		0-0.9 yrs	1 (0.89–1.12)	589	
		1-1.9 yrs	0.63 (0.47–0.84)	57	
		2-3.9 yrs	0.81 (0.63–1.05)	83	
		≥4 yrs	0.77 (0.64–0.94)	149	
		OR, Dose among current long-term users			
		No use	1	1885	
		Low use (<1000 mg)	0.68 (0.55–0.84)	120	
		Medium use (1000 to <2000 mg)	0.73 (0.58–0.91)	105	
		High use (≥2000 mg)	1.06 (0.79–1.41)	64	
		OR, Duration of use (2 years lag-time analysis)			Age, calendar year, BMI, alcohol intake, prior benign breast disease, smoking status, NSAID, steroid, and HRT use
		No use	1	2244	
		0-0.9 yrs	0.92 (0.83–1.03)	499	
		1-1.9 yrs	0.92 (0.7–1.19)	70	
		2-3.9 yrs	0.72 (0.57–0.92)	87	
		≥4 yrs	0.76 (0.6–0.97)	88	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
		OR, Regular use (2 years lag-time analysis)			
		No use	1	2244	
		Current use	0.88 (0.79–0.97)	744	
		Past use	0.95 (0.86–1.04)	720	
Gallicchio et al. (2006) Nested Case-Control Maryland, US **Enrollment or follow-up:** 1989-2006	**Population:** Women in the CLUE II cohort who underwent a breast biopsy for benign breast disease Cases: 91; Controls: 1,376 **Exposure assessment method:** questionnaire	**Carcinoma: OR, Baseline medication use (1989)**			Age, other medication use
		No use	1	81	
		Regular use (≥1 in previous 48 hrs)	1.07 (0.54–2.12)	10	
		Carcinoma: OR, Follow-up medication use (1996)			
		No use	1	36	
		Regular use (≥1/week for ≥1 year)	1.05 (0.51–2.15)	10	
Ashok et al. (2011) Nested Case-Control USA **Enrollment or follow-up:** 2000 - 2006	**Population:** Cases: 18368; Controls: 73472 **Exposure assessment method:** records	**OR, Duration of acetaminophen use**			Age, time in database
		None	1	18003	
		Any	0.95 (0.85–1.06)	365	
		0-6 months	0.96 (0.85–1.08)	346	
		7-12 months	1.09 (0.61–1.92)	15	
		Trend-test *p*-value: 0.2394			
Breast (Post-Menopausal) – Cohort					
Zhang et al. (2012) Cohort US **Enrollment or follow-up:** 1980 to 2008	**Population:** Women in the Nurses' Health Study (NHS) N=84,602 **Exposure assessment method:** questionnaire	**RR, Use (1990-2008)**			Age, age at menarche, height, BMI at age 18, weight change since age 18, parity and age at first birth, history of breast cancer in parent or sibling, history of benign breast disease, alcohol consumption, physical activity, postmenopausal hormone use
		Nonuser	1	1680	
		Past	0.93 (0.86–1.01)	1216	
		Current	0.89 (0.83–0.96)	1199	
		RR, Frequency in past users			
		Nonuser	1	1680	
		<2 d/wk	0.94 (0.87–1.02)	1068	
		2-3 d/wk	0.87 (0.65–1.16)	47	
		>3 d/wk	0.97 (0.71–1.34)	40	
		Trend-test *p*-value: 0.46			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
		RR, Frequency in current users			
		Nonuser	1	1680	
		<2 d/wk	0.9 (0.82–0.99)	644	
		2-3 d/wk	0.89 (0.76–1.04)	186	
		4-5 d/wk	0.88 (0.74–1.04)	152	
		>5 d/wk	0.87 (0.72–1.04)	128	
		Trend-test *p*-value: 0.54			
		RR, Duration of use in past users			
		Nonuser	1	1680	
		≤5 yrs	0.93 (0.85–1.02)	829	
		6-10 yrs	0.94 (0.84–1.07)	337	
		>10 yrs	0.8 (0.59–1.06)	49	
		Trend-test *p*-value: 0.75			
		RR, Duration of use in current users			
		Nonuser	1	1680	
		≤5 yrs	0.92 (0.83–1.02)	570	
		6-10 yrs	0.85 (0.76–0.96)	403	
		>10 yrs	0.9 (0.78–1.04)	226	
		Trend-test *p*-value: 0.16			
		RR, Duration of use in nonregular users (<2 tablets/wk)			
		Nonuser	1	1006	
		≤5 yrs	0.84 (0.66–1.06)	78	
		6-10 yrs	0.86 (0.67–1.11)	65	
		>10 yrs	0.88 (0.68–1.15)	59	
		Trend-test *p*-value: 0.53			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
		RR, Duration of use in regular users (≥2 tablets/wk)			
		Nonuser	1	1006	
		≤5 yrs	1 (0.81–1.22)	103	
		6-10 yrs	0.76 (0.62–0.92)	114	
		>10 yrs	0.87 (0.75–1.01)	224	
		Trend-test *p*-value: 0.89			
		RR, Duration of use in higher-dose users (≥6 tablets/wk)			
		Nonuser	1	1006	
		≤10 yrs	0.87 (0.71–1.06)	104	
		>10 yrs	0.89 (0.74–1.07)	141	
		Trend-test *p*-value: 0.86			
Breast (Post-Menopausal) – Nested Case-Control					
Rahme et al. (2005) Nested Case-Control Quebec, Canada **Enrollment or follow-up:** 1998-2002	**Population:** Women in the in the RAMQ database Cases: 1090; Controls: 44990 **Exposure assessment method:** records	**ICD codes 174.0 to 174.9 and 233.0: OR, Use in yr prior to index date**			Age, breast procedure in prior 3 years, benign neoplasm of breast in prior 3 years, other breast disease in prior 3 years, estrogen replacement therapy in prior year, visit to a gynecologist in prior year, mammogram in past 2-3 years
		No use	1	375	
		Use	0.91 (0.71–1.16)	74	
		ICD codes 174.0 to 174.9 and 233.0: OR, Use in yr prior to index date and had mammography in years 2-3 prior to index date			
		No use	1	375	
		Use	0.86 (0.59–1.31)	NR	
		ICD codes 174.0 to 174.9 and 233.0: OR, Use in yr prior to index date, excluding prescriptions filled in month prior to index date			
		No use	1	375	
		Use	0.89 (0.69–1.15)	NR	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Breast (Pre-Menopausal) – Cohort					
Eliassen et al. (2009) Cohort US **Enrollment or follow-up:** 1989 to 2003	**Population:** Women in Nurses' Health Study II, aged 25-42 yrs N=112,292 **Exposure assessment method:** questionnaire	**Invasive breast cancer: RR, Regular use (≥2 times/week)**			Age at menarche, height, BMI at age 18, weight change since age 18, oral contraceptive use, parity and age at first birth, alcohol consumption, history of benign breast disease, family history of breast cancer
		Non-users	1	706	
		Past users	1.03 (0.89–1.19)	375	
		Current users	0.99 (0.84–1.16)	185	
		<5 yrs	0.97 (0.81–1.16)	142	
		≥5 yrs	1.07 (0.78–1.47)	43	
		Trend-test *p*-value: 0.91			
		Invasive breast cancer: RR, Frequency of use (follow-up 1995-2003)			
		Non-users	1	325	
		Past users	0.96 (0.81–1.14)	253	
		1 day/wk	0.93 (0.76–1.13)	152	
		2-3 days/wk	0.94 (0.72–1.22)	68	
		4-5 days/wk	1 (0.62–1.61)	18	
		≥6 days/wk	1.06 (0.64–1.76)	16	
		Trend-test *p*-value: 0.60			
		Invasive breast cancer: RR, ER+/PR+			
		Non-users	1	362	
		Past users	1 (0.83–1.2)	224	
		Current users	0.98 (0.78–1.22)	97	
		Invasive breast cancer: RR, ER-/PR-			
		Non-users	1	121	
		Past users	1 (0.7–1.43)	59	
		Current users	1 (0.67–1.47)	33	

Table B2. Case-control studies of breast cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/deaths	Co-variates controlled
Breast (Overall)					
Meier et al. (2002) Case-Control United Kingdom **Enrollment or follow-up:** 1992-1997	**Population:** Cases: 3706; Controls: 14155 **Exposure assessment method:** interview	**OR, Acetaminophen - number prescriptions filled**			Smoking status, body mass index, use of NSAIDs
		0	1	1773	
		1-9	1 (0.9–1.1)	1319	
		10-19	1 (0.8–1.2)	230	
		20-29	0.7 (0.6–0.9)	112	
		30+	0.8 (0.7–1)	272	
Terry et al. (2004) Case-Control USA **Enrollment or follow-up:** 1996-1997	**Population:** Cases: 1434; Controls: 1417 **Exposure assessment method:** interview	**OR, Ever use**			Age at diagnosis, migraine headache, aspirin and ibuprofen use, BMI
		Nonusers	1	1262	
		Ever users	1.02 (0.8–1.31)	172	
		Nonregular use	1.12 (0.8–1.57)	NR	
		Regular use (≥4 times/wk for ≥3 months)	0.95 (0.65–1.39)	NR	
Harris et al. (2006) Case-Control Ohio, US **Enrollment or follow-up:** 2003-2004	**Population:** Cases: 323; Controls: 649 **Exposure assessment method:** questionnaire	**OR, OTC use**			Age, BMI, parity, family history, smoking, alcohol intake, menopausal status
		None/infrequent use	1	262	
		Regular use	1.02 (0.39–2.2)	8	
Brasky et al. (2010) Case-Control USA **Enrollment or follow-up:** 1996 and 2001	**Population:** Cases: 1170; Controls: 2115 **Exposure assessment method:** questionnaire	**OR, Acetaminophen use, frequency and intensity**			Age, education, Age at menarche, age at menopause, parity, use of hormone replacement therapy, history of benign breast disease, family history of breast cancer, other NSAIDs, race
		Nonusers	1	449	
		Users	0.97 (0.83–1.15)	703	
		Infrequent (≤14 d/month)	0.96 (0.81–1.14)	649	
		Regular (>14 d/month)	0.98 (0.66–1.46)	54	
		Trend-test *p*-value: 0.99			
		Low (< 2 pills/day)	0.86 (0.66–1.13)	145	
		High (≥ 2 pills/day)	1.01 (0.85–1.2)	563	
		Trend-test *p*-value: 0.64			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/deaths	Co-variates controlled
Breast (Post-Menopausal)					
Terry et al. (2004) Case-Control USA **Enrollment or follow-up:** 1996-1997	**Population:** Cases: 961; Controls: 897 **Exposure assessment method:** interview	**OR, Ever use**			Age at diagnosis, migraine headache, aspirin and ibuprofen use, BMI
		Nonusers	1	864	
		Ever users	0.91 (0.67–1.25)	97	
Breast (Pre-Menopausal)					
Terry et al. (2004) Case-Control USA **Enrollment or follow-up:** 1996-1997	**Population:** Cases: 444; Controls: 461 **Exposure assessment method:** interview	**OR, Ever use**			Age at diagnosis, migraine headache, aspirin and ibuprofen use, BMI
		Nonusers	1	376	
		Ever users	1.31 (0.85–2)	68	

Table B3. Cohort studies of ovarian cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/deaths	Co-variates controlled
Ovarian (Overall)					
Fairfield et al. (2002) Cohort US **Enrollment or follow-up:** 1976-1996	**Population:** female registered nurses in the Nurses' Health Study, aged 30-55 yrs N = 76,821 **Exposure assessment method:** questionnaire	**Invasive ovarian cancer: RR, Use**			Age, BMI, duration of oral contraceptive use, parity, smoking, postmenopausal hormone use, history of tubal ligation
		Nonuser	1	41	
		1-4 days/month	0.93 (0.56–1.53)	25	
		≥5 days/month	0.81 (0.46–1.43)	17	
		Trend-test *p*-value: 0.46			
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 145: SIR, Number of acetaminophen prescriptions**			Age, calendar year
		Any prescription	0.8 (0.4–1.4)	10	
		1	0.5 (0.1–1.7)	2	
		2-4	1.5 (0.5–3.5)	5	
		5-9	0.9 (0.1–3.3)	2	
		≥10	0.3 (0–1.6)	1	
Lipworth et al. (2003) Cohort Denmark **Enrollment or follow-up:** 1989-1996	**Population:** Individuals who filled acetaminophen prescriptions identified through the Pharmacoepidemiological Prescription Database N = 17,760 men; 32,130 women **Exposure assessment method:** records	**Mortality: SMR, Number of prescriptions**			Age, calendar year
		1	2.3 (1.6–3.2)	36	
		2-4	3.3 (2.4–4.4)	45	
		5-9	2.3 (1.4–3.5)	19	
		≥10	1.1 (0.6–1.9)	13	
		Mortality: SMR, Prescribed acetaminophen (1989-1996)			
		Prescription	2.3 (1.9–2.8)	113	
		Mortality: SMR, Prescribed acetaminophen, stratified by latency period			
		<1 yr	5.4 (4.2–6.8)	68	
		1-2 yrs	1.3 (0.8–1.9)	25	
		3-4 yrs	1.3 (0.7–2.2)	15	
		≥5 yrs	0.9 (0.3–2.1)	5	
Lacey et al. (2004) Cohort US **Enrollment or follow-up:** 1979-1998	**Population:** Women in the Breast Cancer Detection Demonstration Project Follow-Up Study (conducted by the American Cancer Society and the NCI) N = 31,364 **Exposure assessment method:** questionnaire	**RR, Any regular use (weekly use for ≥1 yr)**			Attained age, ethnicity, oral contraceptive use, family history of ovarian cancer, menopausal status, duration of estrogen use
		No	1	93	
		Yes	1 (0.56–1.8)	13	
		RR, Duration of use			
		No use	1	93	
		≤5 yrs	1 (0.41–2.5)	5	
		>5 yrs	1.2 (0.5–3.1)	5	
		RR, Frequency of use			
		No use	1	93	
		≤1/day	0.83 (0.34–2.1)	5	
		>1/day	1.1 (0.45–2.7)	5	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/deaths	Co-variates controlled
Pinheiro et al. (2009) Cohort US **Enrollment or follow-up:** 1976 to 2004, 1989 to 2005	**Population:** Women in the NHS and NHS-II studies N = 197,486 **Exposure assessment method:** questionnaire	**HR, Regular use (≥2 times/week)**			Duration of oral contraceptive use, parity, history of tubal ligation, menopausal status, current postmenopausal hormone use, age, 2-year time cycle
		Never	1	295	
		Past	0.98 (0.73–1.33)	54	
		Current	1.14 (0.92–1.43)	112	
		HR, Frequency of use			
		<2 d/wk	1	360	
		2-4 d/wk	0.87 (0.58–1.3)	26	
		≥5 d/wk	1.13 (0.7–1.82)	18	
		Trend-test *p*-value: 0.70			
		HR, Duration of regular use, year(s)			
		<1	1	312	
		1-<2	1.19 (0.86–1.65)	56	
		2-<5	0.96 (0.65–1.4)	54	
		≥5	0.86 (0.55–1.37)	39	
		Trend-test *p*-value: 0.83			
Setiawan et al. (2012) Cohort Los Angeles County, CA and HI, US **Enrollment or follow-up:** 1993-2008	**Population:** Women in the Multiethnic Cohort (MEC) study N=64,828 **Exposure assessment method:** questionnaire	**RR, Acetaminophen use**			Age, age at menarche, oral contraceptive use, postmenopausal hormone use, parity, race/ethnicity
		Never use	1	192	
		Ever use	0.86 (0.67–1.12)	83	
		≤1 yr	0.59 (0.35–1)	15	
		2-5 yrs	0.96 (0.64–1.45)	27	
		≥6 yrs	1.04 (0.71–1.52)	31	
		Trend-test *p*-value: 0.89			
Trabert et al. (2019) Cohort North America and Europe **Enrollment or follow-up:**	**Population:** Women in the 13 prospective studies that were included in the Ovarian Cancer Cohort Consortium N=758,829 **Exposure assessment method:** questionnaire	**HR, Frequent use**			Baseline age, BMI, parity, duration of oral contraceptive use, duration of menopausal hormone therapy use
		Infrequent / nonuse	1	1421	
		Frequent use	1.05 (0.88–1.24)	152	
		HR, Duration of frequent use.			
		Infrequent / nonuse	1	1386	
		0.5 to <5 yrs	0.99 (0.76–1.29)	61	
		5 to <10 yrs	1.16 (0.87–1.54)	50	
		≥10 yrs	1.01 (0.73–1.41)	37	
		HR, Categories of frequent use			
		Infrequent / nonuse	1	1179	
		<Daily use	0.99 (0.7–1.39)	35	
		Daily use	1.28 (1–1.65)	71	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/deaths	Co-variates controlled
		HR, Categories of frequent use by duration			
		Infrequent / nonuse	1	1386	
		<Daily / 0.5 - <5 yrs	0.87 (0.62–1.22)	33	
		<Daily / 5 - <10 yrs	0.98 (0.66–1.46)	25	
		<Daily / ≥10 yrs	0.89 (0.58–1.36)	22	
		Daily / 0.5 - <5 yrs	1.21 (0.81–1.81)	28	
		Daily / 5 - <10 yrs	1.42 (0.94–2.13)	25	
		Daily / ≥10 yrs	1.24 (0.75–2.08)	15	
Ovary (mucinous)					
Trabert et al. (2019) Cohort North America and Europe **Enrollment or follow-up:**	**Population:** Women in the 13 prospective studies that were included in the Ovarian Cancer Cohort Consortium N=758,829 **Exposure assessment method:** questionnaire	**HR, Frequent use**			Baseline age, BMI, parity, duration of oral contraceptive use, duration of menopausal hormone therapy use
		Infrequent / nonuse	1	38	
		Frequent use	0.7 (0.16–2.99)	2	
		HR, Duration of frequent use			
		Infrequent / nonuse	1	38	
		0.5 to <5 yrs	-	0	
		5 to <10 yrs	1.68 (0.23–12.17)	1	
		≥10 yrs	1.3 (0.16–10.48)	1	
		HR, Categories of frequent use			
		Infrequent / nonuse	1	35	
		< Daily use	1.15 (0.22–6.03)	1	
		Daily use	-	0	

Table B4. Case-control studies of ovarian cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Ovary (Overall)					
Cramer et al. (1998) Case-Control Massachusetts, New Hampshire, US **Enrollment or follow-up:** 1992-1997	**Population:** Cases: 563; Controls: 523 **Exposure assessment method:** interview	**OR, OTC use**			Age, study center, education, religion, parity, use of oral contraceptives, menstrual, headache, or arthritis pain, aspirin and ibuprofen use
		No	1	537	
		Yes	0.52 (0.31–0.88)	26	
		OR, Tablets per week			Age, study center, education, religion, parity, use of oral contraceptives, menstrual, headache, or arthritis pain
		No use	1	537	
		1-6 tablets/wk	0.95 (0.39–2.33)	10	
		≥7 tablets/wk	0.39 (0.21–0.74)	16	
		OR, Years of use			
		No use	1	537	
		1-10 yrs	0.64 (0.33–1.25)	16	
		>10 yrs	0.4 (0.19–0.88)	10	
		OR, Tablet-years			
		No use	1	537	
		1-20	0.57 (0.3–1.1)	16	
		>20	0.45 (0.2–0.99)	10	
Moysich et al. (2001) Case-Control NY, US **Enrollment or follow-up:** 1982-1998	**Population:** Cases: 547; Controls: 1094 **Exposure assessment method:** questionnaire	**Epithelial: OR, Regular use (1982-1996)**			Age, age at first birth, history of tubal ligation, parity, presence of irregular menses, family history of ovarian cancer
		Nonuser	1	518	
		Regular user	0.56 (0.34–0.86)	29	
		1-6 tablets/wk	0.62 (0.37–0.98)	26	
		≥7 tablets/wk	0.32 (0.09–1.08)	3	
		0.5-10 yr of use	0.6 (0.34–1.07)	16	
		≥11 yr of use	0.51 (0.27–0.97)	13	
Meier et al. (2002) Case-Control United Kingdom **Enrollment or follow-up:** 1992-1997	**Population:** Cases: 483; Controls: 1877 **Exposure assessment method:** records	**ICD code 183.0: OR, Number of acetaminophen prescriptions**			Smoking status, NSAID prescription, BMI
		0	1	210	
		1-9	1.2 (1–1.6)	185	
		10-19	1.4 (0.9–2.2)	37	
		20-29	1.7 (1–2.9)	21	
		≥30	1 (0.6–1.5)	30	
Wu et al. (2009) Case-Control Los Angeles County, US **Enrollment or follow-up:** 1998-2002	**Population:** Cases: 609; Controls: 688 **Exposure assessment method:** questionnaire	**OR, Duration (yr) of acetaminophen use**			Age, education, race, tubal ligation, family history of breast cancer or ovarian cancer, menopausal status, use of oral contraceptives, parity, talc use, aspirin and other NSAIDs
		Never	1	491	
		1-5 yrs	0.87 (0.53–1.41)	47	
		>5 yrs	1.71 (0.94–3.09)	44	
		Trend-test *p*-value: 0.12			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
		OR, Frequency of acetaminophen use			
		Never	1	491	
		1-≤7/wk	1.04 (0.63–1.71)	48	
		>7/wk	1.36 (0.78–2.36)	43	
		Trend-test *p*-value: 0.33			
Pinheiro et al. (2010) Case-Control USA (Eastern MA & NH) **Enrollment or follow-up:** 1989-2004	**Population:** New England Case-Control Study (NECC); Nurses Health Study (NHS) Cases: 1120 (NECC); 233 (NHS); Controls: 1160 (NECC); 663 (NHS) **Exposure assessment method:** questionnaire	**OR, Regular use in NECC**			Age, study center
		No use	1	1041	
		Regular use	0.9 (0.65–1.23)	79	
		OR, Regular use in NHS			Age, menopausal status, date, time, and fasting status at blood collection, PMH use at blood collection, DNA type
		No regular use	1	180	
		Regular use	0.55 (0.26–1.13)	10	
		OR, Regular use in NECC and NHS pooled			Parity, duration of OC use, history of tubal ligation, PMH use, menopausal status
		No regular use	1	1221	
		Regular use	0.78 (0.51–1.21)	89	
Ammundsen et al. (2012) Case-Control Denmark **Enrollment or follow-up:** 1995-1999	**Population:** Cases: 756; Controls: 1564 **Exposure assessment method:** interview	**OR, Ever use and duration of use**			Age, pregnancy, number of pregnancies, oral contraceptive use, duration of oral contraceptive use
		Never	1	738	
		Ever use	0.73 (0.42–1.26)	18	
		<5 yrs	1.25 (0.61–2.57)	12	
		≥5 yrs	0.53 (0.17–1.67)	4	
		Per year used	0.97 (0.9–1.05)	NR	
Lo-Ciganic et al. (2012) Case-Control US **Enrollment or follow-up:** 2003-2008	**Population:** Cases: 902; Controls: 1802 **Exposure assessment method:** interview	**OR, Regular use**			Age at reference year, interview year, study center, race, education, breastfeeding, numbers of full-term births, duration of oral contraceptive use, BMI, PMH use, arthritis, diabetes, prior tubal ligation
		Nonuser	1	738	
		Regular user	0.98 (0.79–1.23)	164	
		Continuous user	0.98 (0.74–1.3)	98	
		Current user	0.81 (0.36–1.83)	9	
		Past user	1.02 (0.72–1.45)	57	
Trabert et al. (2014) Case-Control USA (n=9), Denmark (n=1), UK (n=1), Australia (n=1) **Enrollment or follow-up:** 1992-2009	**Population:** Ovarian Cancer Association Consortium (OCAC); 12 population-based case–control studies Cases: 7776; Controls: 11843 **Exposure assessment**	**OR, Frequency of acetaminophen use**			Age, race, oral contraceptive use, parity, Menopausal status, BMI, first-degree family history of breast cancer
		No regular use	1	3497	
		< 30 days per month	1.1 (0.96–1.26)	1439	
		Daily	0.95 (0.74–1.23)	427	
		OR, Dose of acetaminophen			
		No regular use	1	1516	
		Low dose (<500 mg)	1.15 (0.84–1.59)	68	
		High dose (>500 mg)	0.9 (0.68–1.19)	293	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
	method: records or questionnaire	**OR, Duration of acetaminophen use**			
		No regular use (<1 per wk)	1	3918	
		< 60 months	0.88 (0.72–1.08)	243	
		≥ 60 months	1.13 (0.92–1.39)	438	
Ovary (mucinous)					
Cramer et al. (1998) Case-Control Massachusetts, New Hampshire, US **Enrollment or follow-up:** 1992-1997	**Population:** Cases: 83; Controls: 523 **Exposure assessment method:** interview	**OR (95% CI) OTC use**			Age, study center, education, religion, parity, use of oral contraceptives, menstrual, headache, or arthritis pain
		Use	0.36 (0.11–1.21)	83	
Baandrup et al. (2014) Case-Control Denmark **Enrollment or follow-up:** 2000-2009	**Population:** Cases: 411; Controls: 50576 **Exposure assessment method:** records	**ICD-O-3: 84703, 84713, 84803, 84813, and 90153: OR, Regular use**			Age, parity, use of oral contraceptives, use of menopausal hormones, infertility, endometriosis, hysterectomy, tubal sterilization, diabetes mellitus, chronic obstructive pulmonary disease or asthma, education, use of statins, low-dose aspirin, and NSAIDs
		Nonuse	1	364	
		Ever use	0.83 (0.59–1.16)	47	
		Recent use	0.99 (0.68–1.45)	36	
		Former use	0.54 (0.29–1.01)	11	
Trabert et al. (2014) Case-Control USA (n=9), Denmark (n=1), UK (n=1), Australia (n=1) **Enrollment or follow-up:** 1992-2007	**Population:** OCAC; 12 population-based case–control studies Cases: 380; Controls: 10914 **Exposure assessment method:** records or questionnaire	**OR, Regular use**			Age, race, oral contraceptive use, parity, Menopausal status, BMI, first-degree family history of female or male breast cancer or ovarian cancer
		No regular use (<1 per wk)	1	314	
		Use	0.9 (0.66–1.23)	66	
Hannibal et al. (2018) Case-Control Denmark **Enrollment or follow-up:** 1997-2015	**Population:** Cases: 1380; Controls: 20700 **Exposure assessment method:** records	**Mucinous borderline tumors[a]: OR, Overall use**			Age, educational status, income group, parity, oral contraceptive use, HRT use, infertility, endometriosis, pelvic inflammatory disease, hysterectomy, tubal sterilization, salpingectomy, diabetes mellitus, COPD or asthma, use of statins
		Non-use	1	1239	
		Ever use	0.87 (0.71–1.06)	141	
		Recent use	0.77 (0.6–0.98)	86	
		Former use	1 (0.75–1.33)	55	
		Defined daily dose (DDD/day (per 1 DDD/day increase)	0.85 (0.49–1.5)	NR	
		Duration of use (per 1 year increase)	1.01 (0.97–1.05)	NR	
		Time since last redeemed prescription (per 1 year increase)	1.04 (0.98–1.1)	NR	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Ovary (serous)					
Cramer et al. (1998) Case-Control MA, NH, US **Enrollment or follow-up:** 1992-1997	**Population:** Cases: 315; Controls: 523 **Exposure assessment method:** interview	**OR, OTC use**			Age, study center, education, religion, parity, use of oral contraceptives, menstrual, headache, or arthritis pain
		Use	0.53 (0.28–0.98)	315	
Ammundsen et al. (2012) Case-Control Denmark **Enrollment or follow-up:** 1995-1999	**Population:** Cases: 447; Controls: 1564 **Exposure assessment method:** interview	**OR, Regular use**			Age, pregnancy, number of pregnancies, oral contraceptive use
		Never	1	431	
		Ever	1.09 (0.61–1.96)	16	
Baandrup et al. (2014) Case-Control Denmark **Enrollment or follow-up:** 2000-2009	**Population:** Cases: 2262; Controls: 50576 **Exposure assessment method:** records	**ICD-O-3: 84413, 84603, 84613, and 90143: OR, Overall use**			Age, parity, use of oral contraceptives, use of menopausal hormones, infertility, endometriosis, hysterectomy, tubal sterilization, diabetes mellitus, chronic obstructive pulmonary disease or asthma, education, use of statins, low-dose aspirin, and NSAIDs
		Nonuse	1	1945	
		Ever use	0.82 (0.72–0.94)	317	
		Recent use	0.74 (0.63–0.87)	193	
		Former use	0.98 (0.81–1.18)	124	
		Daily dose (per DDDs)	0.66 (0.44–0.98)	NR	
		Duration (per year)	0.98 (0.95–1.01)	NR	
		ICD-O-3: 84413, 84603, 84613, and 90143: OR, Duration of use >10 yrs			
		Nonuse	1	1945	
		Low-intensity use	0.76 (0.38–1.5)	9	
		Medium-intensity use	0.59 (0.29–1.22)	8	
		High-intensity use	0.37 (0.16–0.84)	6	
		Trend-test *p*-value: 0.29			
Trabert et al. (2014) Case-Control USA (n=9), Denmark (n=1), UK (n=1), Australia (n=1) **Enrollment or follow-up:** 1992-2007	**Population:** OCAC; 12 population-based case–control studies Cases: 7733; Controls: 11204 **Exposure assessment method:** records or questionnaire	**OR, Regular use**			Age, race, oral contraceptive use, parity, Menopausal status, BMI, first-degree family history of breast cancer
		No regular use (<1 per wk)	1	3478	
		Use	1.03 (0.91–1.18)	777	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Hannibal et al. (2018) Case-Control Denmark **Enrollment or follow-up:** 1997-2015	**Population:** Cases: 1379; Controls: 20686 **Exposure assessment method:** records	**Serous borderline tumors[a]: OR, Overall use**			Age, educational status, income group, parity, oral contraceptive use, HRT use, infertility, endometriosis, pelvic inflammatory disease, hysterectomy, tubal sterilization, salpingectomy, diabetes mellitus, COPD or asthma, use of statins
		Non-use	1	1198	
		Ever use	1.03 (0.86–1.23)	181	
		Recent use	1.05 (0.85–1.3)	125	
		Former use	0.95 (0.71–1.26)	56	
		DDD/day (per 1 DDD/day increase)	0.86 (0.52–1.42)	NR	
		Duration of use (per 1 year increase)	0.98 (0.94–1.01)	NR	
		Time since last redeemed prescription (per 1 year increase)	1.03 (0.97–1.09)	NR	
Ovary (others)					
Cramer et al. (1998) Case-Control Massachusetts, New Hampshire, US **Enrollment or follow-up:** 1992-1997	**Population:** Cases: 563; Controls: 523 **Exposure assessment method:** interview	**Endometrial/clear-cell: OR, OTC use**			Age, study center, education, religion, parity, use of oral contraceptives, and menstrual, headache, or arthritic pain
		Use	0.82 (0.36–1.82)	130	
		Borderline: OR, OTC use			
		Use	0.56 (0.25–1.25)	152	
		Moderately/well differentiated: OR, OTC use			
		Use	0.76 (0.35–1.62)	151	
		Poorly differentiated: OR, OTC use			
		Use	0.38 (0.17–0.83)	227	
		Ungraded: OR, OTC use			
		Use	0.37 (0.05–2.85)	33	
Rosenberg et al. (2000) Case-Control US **Enrollment or follow-up:** 1976-1998	**Population:** Cases: 780; Controls: 2053, 2570 **Exposure assessment method:** questionnaire	**Epithelial: OR, Acetaminophen use ≥1 d/wk for ≥6 months: noncancer controls**			Age, geography, interview year
		No use	1	750	
		Regular use	1 (0.6–1.5)	28	
		Duration ≥5 yr	1.1 (0.6–1.9)	18	
		First use ≥10 yr ago	0.8 (0.3–1.8)	7	
		Last use <1 yr ago	1 (0.6–1.5)	26	
		Epithelial: OR, Acetaminophen use ≥4 d/wk for ≥6 months: noncancer controls			
		No use	1	765	
		Regular use	0.9 (0.5–1.6)	14	
		Duration ≥5 yr	1.2 (0.5–2.6)	10	
		First use ≥10 yr ago	1.1 (0.4–3.2)	5	
		Last use <1 yr ago	0.9 (0.5–1.7)	13	
		Metastatic epithelial: OR, Acetaminophen use ≥1 d/wk for ≥6 months: noncancer controls			
		No use	1	466	
		Regular use	1 (0.6–1.7)	18	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
		Metastatic epithelial: OR, Acetaminophen use ≥4 d/wk for ≥6 months: noncancer controls			
		No use	1	477	
		Regular use	0.7 (0.3–1.6)	7	
		Nonmetastatic epithelial: OR, Acetaminophen use ≥1 d/wk for ≥6 months: noncancer controls			
		No use	1	284	
		Regular use	1 (0.5–1.9)	10	
		Nonmetastatic epithelial: OR, Acetaminophen use ≥4 d/wk for ≥6 months: noncancer controls			
		No use	1	288	
		Regular use	1.3 (0.6–3)	7	
Schildkraut et al. (2006) Case-Control NC, US **Enrollment or follow-up:** 1999-2003	**Population:** Cases: 586; Controls: 627 **Exposure assessment method:** questionnaire	**Epithelial: OR, Use of acetaminophen**			Age, race, education, oral contraceptive use, total months breastfeeding, months of pregnancy, tubal ligation, family history of first-dgree relative with breast or ovarian cancer, history of endometriosis, pelvic inflammatory disease, hysterectomy, severe menstrual cramping
		Nonuser	1	86	
		User	0.78 (0.56–1.08)	14	
		<8 times/mo	0.97 (0.59–1.61)	6	
		≥8 times/mo for <3 yr	0.62 (0.36–1.08)	4	
		≥8 times/mo for ≥3 yr	0.77 (0.42–1.41)	4	
Hannibal et al. (2008) Case-Control Washington State, US **Enrollment or follow-up:** 2002-2005	**Population:** Cases: 812; Controls: 1313 **Exposure assessment method:** interview	**Epithelial: OR, Acetaminophen use only, duration of use (yrs)**			Age, county of residence, calendar year, number of full-term pregnancies, duration of hormonal contraceptive use
		Never users	1	406	
		Ever users	1.4 (0.9–2)	53	
		0.5-10	1.2 (0.7–1.9)	32	
		>10	1.7 (0.9–3.3)	21	
		Epithelial: OR, Acetaminophen use only, time since first use (yrs)			
		Never users	1	406	
		<5	1.2 (0.6–2.5)	13	
		5-9	1.2 (0.5–2.8)	11	
		10-14	1.1 (0.4–3.2)	6	
		≥15	1.7 (0.9–3)	23	
Baandrup et al. (2014) Case-Control Denmark **Enrollment or follow-up:** 2000-2009	**Population:** Cases: 3471 total epithelial, 577 endometrioid, 221 clear cell; Controls: 50576 **Exposure assessment method:** records	**Epithelial: OR, Overall use**			Age, parity, use of oral contraceptives, use of menopausal hormones, infertility, endometriosis, hysterectomy, tubal sterilization, diabetes mellitus, chronic obstructive pulmonary disease or asthma, education, use of statins, low-dose aspirin, nonaspirin NSAIDs
		Nonuse	1	3012	
		Ever use	0.82 (0.74–0.92)	459	
		Recent use	0.78 (0.69–0.89)	292	
		Former use	0.9 (0.76–1.06)	167	
		Daily dose (per DDDs)	0.7 (0.51–0.96)	NR	
		Duration (per year)	0.98 (0.96–1.01)	NR	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
		Epithelial: OR, Duration of use >10 yrs			
		Nonuse	1	3012	
		Low-intensity use	0.75 (0.42–1.35)	12	
		Medium-intensity use	0.8 (0.47–1.37)	15	
		High-intensity use	0.45 (0.24–0.86)	10	
		Trend-test *p*-value: 0.25			
		Endometrioid, ICD-O-3: 83803, 83813, 85703, 89503, 89513, 89803, and 89333: OR, Regular use			
		Nonuse	1	502	
		Ever use	0.85 (0.65–1.11)	75	
		Recent use	0.79 (0.57–1.1)	47	
		Former use	0.95 (0.63–1.42)	28	
		Clear cell, ICD-O-3: 83103, 83133, and 84903: OR, Regular use			
		Nonuse	1	201	
		Ever use	0.72 (0.44–1.19)	20	
		Recent use	0.96 (0.55–1.66)	16	
		Former use	0.37 (0.13–1.01)	4	
Trabert et al. (2014) Case-Control USA (n=9), Denmark (n=1), UK (n=1), Australia (n=1) **Enrollment or follow-up:** 1992-2007	**Population:** OCAC; 12 population-based case–control studies Cases: 1112 endometrioid, 676 clear cell; Controls: 11541 endometrioid, 12292 clear cell **Exposure assessment method:** records or questionnaire	**Endometrioid: OR, Regular use**			Age, race, oral contraceptive use, parity, Menopausal status, BMI, first-degree family history of breast cancer
		No regular use (<1 per wk)	1	920	
		Use	0.83 (0.66–1.05)	192	
		Clear cell: OR, Regular use			
		No regular use (<1 per wk)	1	510	
		Use	1.22 (0.91–1.64)	166	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Peres et al. (2016) Case-Control US **Enrollment or follow-up:** 2010-2015	**Population:** The African American Cancer Epidemiology Study (AACES). Cases: 541; Controls: 731 **Exposure assessment method:** questionnaire	**Epithelial: OR, Acetaminophen use**			Age, study site, education, income, parity, family history of first-dgree relative with breast or ovarian cancer, tubal ligation, BMI, oral contraceptive use, menopausal status, endometriosis, pelvic inflammatory disease, physical activity, use of other analgesics
		Never users	1	362	
		Ever use	0.89 (0.49–1.62)	54	
		Acetaminophen only	0.98 (0.53–1.81)	23	
		Epithelial: OR, Frequency of use			
		Never users	1	362	
		<30 days/month	0.94 (0.49–1.81)	35	
		Daily	0.79 (0.33–1.92)	19	
		Epithelial: OR, Duration of use			
		Never users	1	362	
		<5 yrs	0.97 (0.42–2.22)	15	
		≥5 yrs	0.87 (0.44–1.74)	39	
		Epithelial: OR, Frequency and duration of use			
		Never users	1	362	
		<30 days/month for <5 yrs	0.94 (0.34–2.63)	8	
		Daily for <5 yrs	1.12 (0.32–3.96)	7	
		<30 days/month for ≥5 yrs	1 (0.45–2.21)	27	
		Daily for ≥5 yrs	0.58 (0.19–1.79)	12	

[a] Hannibal et al. (2018) assessed the association between use of acetaminophen and risk of serous or mucinous borderline tumors, which are belived to be precursor lesions for low-grade serous and mucinous ovarian cancer, respectively.

Table B5. Cohort and case-control studies of cervical cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 171: SIR, Prescribed acetaminophen (1989-1997)**			Age, calendar year
		Prescription	0.5 (0.1–1.1)	4	
Friel et al. (2015) Case-Control NY, US **Enrollment or follow-up:** 1982-1998	**Population:** Cases: 328; Controls: 1312 **Exposure assessment method:** questionnaire	**OR, Acetaminophen use**			Age, year survey conducted, education, age at first pregnancy, cigarette smoking status, Menopausal status, genital tract disease, circulatory system disease, blood and blood-forming organs disease, oral, barrier, and spermicide contraceptive use
		Nonuser	1	227	
		Regular user	1.13 (0.73–1.75)	50	
		1-6 tablets/wk	1.23 (0.77–1.97)	43	
		≥7 tablets/wk	0.76 (0.29–2)	7	
		0.5-10 yrs of use	1.4 (0.85–2.31)	35	
		≥11 yrs of use	0.71 (0.34–1.48)	15	
		<7 tablets/wk and ≥5 yrs of use	1.22 (0.78–1.91)	44	
		≥7 tablets/wk and ≥5 yrs of use	0.42 (0.08–2.12)	2	
		Trend-test *p*-value: 0.58			

Table B6. Cohort and case-control studies of uterine endometrium cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Cohort					
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 172: SIR, Prescribed acetaminophen (1989-1997)**			Age, calendar year
		Prescription	1.2 (0.7–2)	15	
Viswanathan et al. (2008) Cohort US **Enrollment or follow-up:** 1976 to 2004	**Population:** Women in the Nurses' Health Study (NHS) cohort N=82,971 **Exposure assessment method:** questionnaire	**Invasive endometrial cancer: RR, frequency of use (1990-2004)**			BMI, duration of oral contraceptive use, pack-years of smoking, age at menopause, parity, age at menarche, hypertension, diabetes, type of postmenopausal hormone use
		Nonuser	1	370	
		1 d/wk	1.21 (0.92–1.6)	68	
		2-3 d/wk	0.86 (0.6–1.25)	32	
		4-5 d/wk	0.98 (0.59–1.62)	16	
		6-7 d/wk	0.86 (0.57–1.3)	26	
		Trend-test *p*-value: 0.20			
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** VITAL (VITamins And Lifestyle) study N=62,841 **Exposure assessment method:** questionnaire	**HR, 10-yr use prior to baseline in women**			Age, education, race, marital status, height, BMI, physical activity, pack-years of smoking, alcohol intake at 45 yrs, fruit and vegetable intake, red meat intake, multivitamin use, self-rated health, family history of colon, lung, and hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/chronic joint pain, migraine/chronic headaches, use of NSAIDs, family history of breast cancer, mammogram in past 2 yrs, age at menarche, age at menopause, age at first birth, years of estrogen therapy, years of combined hormone therapy, hysterectomy
		No use	1	156	
		Low use (<4 d/wk or <4 yrs)	1.07 (0.71–1.63)	47	
		High use (≥4 d/wk and ≥4 yrs)	0.99 (0.48–2.01)	11	
		Trend-test *p*-value: 0.88			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Setiawan et al. (2012) Cohort Los Angeles County, CA and HI, US **Enrollment or follow-up:** 1993-2008	**Population:** Women in the Multiethnic Cohort (MEC) study N=64,828 **Exposure assessment method:** questionnaire	**RR, Acetaminophen use**			Age, BMI, smoking status, Age at menarche, oral contraceptive use, postmenopausal hormone use, parity, race/ethnicity
		Never use	1	407	
		Ever use	0.96 (0.81–1.13)	213	
		≤1 y	0.88 (0.66–1.19)	50	
		2-5 y	1.24 (0.97–1.58)	79	
		≥6 y	0.8 (0.61–1.06)	59	
		Trend-test *p*-value: 0.55			
Pooled analysis					
Webb et al. (2019) Nested Case-Control Multiple locations: Australia, USA, Sweden **Enrollment or follow-up:**	**Population:** Australian National Endometrial Cancer Study (ANECS); Iowa Women's Health Study (IOWA), Multiethnic Cohort Study(MEC), NIH-AARP Diet and Health Study (NIH); Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO); Black Women's Health Study (BWHS), Swedish Women's Lifestyle and Health Study (SWLHS) Cases: 7,120; Controls: 6,069 **Exposure assessment method:** other (specify in exposure assessment notes)	**OR, Regular acetaminophen use**			Age, parity, BMI, oral contraceptive use, education, smoking
		Overall	0.98 (0.87–1.1)	3849	
		Normal weight	1.1 (0.91–1.33)	211	
		Overweight	0.79 (0.64–0.96)	199	
		Obese	1.04 (0.86–1.24)	469	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Case-Control					
Moysich et al. (2005) Case-Control NY, US **Enrollment or follow-up:** 1982-1998	**Population:** Cases: 427; Controls: 427 **Exposure assessment method:** questionnaire	**OR, Regular use**			Age, education, BMI, parity, Age at menarche, age at menopause and parity
		No regular acetaminophen use	1	361	
		Regular acetaminophen use	0.96 (0.6–1.54)	66	
		OR Frequency of use			
		Used 1-6 times per week	0.92 (0.55–1.56)	53	
		Used >7 times per week	1.12 (0.42–2.98)	13	
		OR Duration of use			
		Used for 1-10 years	1.55 (0.65–2.05)	45	
		Used >7 times per week	0.69 (0.33–1.46)	21	
		OR, Cumulative use defined as combined measure of frequency and duration by computing tablet years (tablets per day X years of use).			
		Moderate use (≤ 10 tablet-yrs)	1.08 (0.65–1.77)	59	
		High use (> 10 tablet-yrs)	0.49 (0.15–1.6)	7	
Bodelon et al. (2009) Case-Control King, Pierce, and Snohomish counties, WA, US **Enrollment or follow-up:** July 1, 2003 to Nov 31, 2005	**Population:** Cases: 410; Controls: 356 **Exposure assessment method:** interview	**OR, Ever users (≥5 d/month for ≥ 6 consecutive months)**			Age, county of residence, calendar year, BMI, hormone therapy use
		Never	1	183	
		Ever users	1.11 (0.7–1.77)	80	
		OR, Duration of use in years			
		Never	1	183	
		0.5-9.9	0.82 (0.46–1.49)	37	
		≥ 10	1.8 (0.91–3.56)	39	
		OR, Age at first use in years			
		Never	1	183	
		< 35	0.86 (0.42–1.77)	25	
		35-44	1.85 (0.72–4.73)	22	
		45-54	1.37 (0.58–3.24)	17	
		≥ 55	0.92 (0.32–2.6)	12	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
		OR, Time since first use in years			
		Never	1	183	
		<5	1.07 (0.4–2.84)	13	
		5-9.9	1.07 (0.36–3.21)	9	
		≥10	1.19 (0.68–2.07)	54	
		OR, Time since last use in years			
		Never	1	183	
		<5	1.02 (0.58–1.78)	45	
		≥5	1.44 (0.69–2.99)	31	
Neill et al. (2013) Case-Control Australia **Enrollment or follow-up:** 2005-2007	**Population:** Cases: 1398; Controls: 740 **Exposure assessment method:** interview	**OR, Acetaminophen use**			Age, age at menarche, parity, HRT use, OC use, BMI, type 2 diabetes, smoking status
		Never	1	154	
		Ever users	1.19 (0.86–1.65)	1199	
		OR, Frequency of use			
		Never	1	154	
		Occasionally	1.4 (0.99–2)	556	
		≤ 1 week	0.99 (0.7–1.41)	438	
		≥ 2 week	1.23 (0.8–1.9)	205	
		Trend-test *p*-value: 0.31			

Table B7. Cohort, nested case-control, and case-control studies of prostate cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Prostate (Overall) – Cohort					
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 177: SIR, Prescribed acetaminophen (1989-1997)**			Age, calendar year
		Prescription	0.8 (0.5–1.3)	16	
Lipworth et al. (2003) Cohort Denmark **Enrollment or follow-up:** 1989-1996	**Population:** Individuals who filled acetaminophen prescriptions identified through the Pharmacoepidemiological Prescription Database N = 17,760 men; 32,130 women **Exposure assessment method:** records	**Mortality: SMR, Number of prescriptions**			Age, calendar year
		1	2.7 (2.2–3.2)	104	
		2-4	4.4 (3.7–5.2)	130	
		5-9	5.3 (4.3–6.5)	89	
		≥10	3.5 (2.7–4.4)	68	
		Mortality: SMR, Prescribed acetaminophen, stratified by latency period			
		Total	3.7 (3.4–4.1)	391	
		<1 yr	7.5 (6.6–8.6)	222	
		1-2 yrs	3 (2.5–3.6)	128	
		3-4 yrs	1.1 (0.7–1.6)	25	
		≥5 yrs	1.6 (0.9–2.6)	16	
Platz et al. (2005) Cohort Baltimore, MD, US **Enrollment or follow-up:** 1980 to 2004	**Population:** Men in the Baltimore Longitudinal Study of Aging (BLSA) N=1,244 **Exposure assessment method:** interview	**RR, Ever use**			Age, calendar year, aspirin and other NSAIDs
		No	1	102	
		Yes	0.89 (0.59–1.34)	39	
		RR, Current use			
		No	1	127	
		Yes	0.69 (0.39–1.2)	14	
		RR, Duration of use			
		Never	1	102	
		<4 yr	0.93 (0.61–1.44)	28	
		≥4 yr	0.88 (0.34–2.33)	11	
Jacobs et al. (2011) Cohort US **Enrollment or follow-up:** 1992-1997	**Population:** Men in the Cancer Prevention Study-II (CPS-II) Nutrition Cohort N=78,485 **Exposure assessment method:** questionnaire	**All: RR, Regular use**			Age, race, education, BMI, diabetes, NSAID use, history of PSA testing
		Never reported use	1	5092	
		Past or < regular use	0.96 (0.92–1.01)	2664	
		Current regular use, <5 years	1 (0.89–1.12)	304	
		Current regular use, ≥5 years	0.62 (0.44–0.87)	32	
		Nonaggressive: RR, Regular use			
		Never reported use	1	2832	
		Past or < regular use	0.95 (0.89–1.01)	1449	
		Current regular use, <5 years	1 (0.85–1.17)	165	
		Current regular use, ≥5 years	0.75 (0.49–1.15)	21	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Veitonmäki et al. (2014) Cohort Finland **Enrollment or follow-up:** 1996-2009	**Population:** Men from the Finnish Prostate Cancer Screening Trial N=78,615 **Exposure assessment method:** records	**Overall risk: HR, Prescription use in screening arm**			Age, use of cholesterol-lowering medication, antihypertensive medication, benign prostatic hyperplasia medication, antidiabetic medication, number of screening rounds attended
		None	1	2068	
		Current	1.51 (1.25–1.81)	801	
		Previous	0.98 (0.82–1.18)	801	
		Localized cancer: HR, Prescription use in screening arm			
		None	1	1967	
		Current	1.42 (1.16–1.73)	748	
		Previous	0.96 (0.79–1.17)	748	
		Metastatic cancer: HR, Prescription use in screening arm			
		None	1	76	
		Current	3.24 (1.71–6.14)	35	
		Previous	0.87 (0.32–2.39)	35	
		High-grade cancer: HR, Prescription use in screening arm			
		None	1	674	
		Current	2 (1.56–2.58)	317	
		Previous	1.13 (0.83–1.54)	317	
		Overall risk: HR, Prescription use in control arm			Age, use of cholesterol-lowering medication, antihypertensive medication, benign prostatic hyperplasia medication, antidiabetic medication
		None	1	2532	
		Current	1.65 (1.44–1.9)	1136	
		Previous	1 (0.86–1.15)	1136	
		Localized cancer: HR, Prescription use in control arm			
		None	1	2249	
		Current	1.52 (1.31–1.78)	1012	
		Previous	1.02 (0.88–1.19)	1012	
		Metastatic cancer: HR, Prescription use in control arm			
		None	1	210	
		Current	1.79 (1.12–2.86)	87	
		Previous	2.85 (1.46–5.55)	87	
		High-grade cancer: HR, Prescription use in control arm			
		None	1	1171	
		Current	1.82 (1.51–2.18)	574	
		Previous	1.24 (1.03–1.5)	574	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Prostate (Overall) – Nested Case-Control					
García Rodriguez and González-Pérez (2004b) Nested Case-Control United Kingdom **Enrollment or follow-up:** 1995 - 2001	**Population:** Data from the General Practice Research Database (GPRD). Cases: 2183; Controls: 10,000 **Exposure assessment method:** records	**OR, Duration of use (years)**			Age, calendar year, prior BPH history, number of visits to general practitioners, referrals, hospitalizations, use of aspirin or other NSAIDs
		No use	1	972	
		Current use	0.95 (0.84–1.07)	721	
		0-1	1.14 (1–1.31)	516	
		1-2	0.67 (0.49–0.92)	58	
		2-4	0.87 (0.66–1.15)	76	
		≥4	0.5 (0.38–0.65)	71	
		Trend-test *p*-value: 0.02			
		OR, Dose among current long-term users (>1 yr)			
		No use	1	972	
		Low-medium (≤2000 mg/d)	0.66 (0.54–0.8)	180	
		High (>2000 mg/d)	0.59 (0.38–0.93)	25	
Murad et al. (2011) Nested Case-Control UK **Enrollment or follow-up:** 2001-2008	**Population:** Data from the Prostate testing for cancer and Treatment (ProtecT)trial Cases: 1016; Controls: 5043 **Exposure assessment method:** questionnaire	**OR, Any acetaminophen use**			Age, recruitment center, any aspirin or other NSAID use
		Nonusers	1	949	
		Any use	1.15 (0.86–1.53)	67	
Prostate (Overall) – Case-Control					
Nelson and Harris (2000) Case-Control Ohio,US **Enrollment or follow-up:** 1992-1995	**Population:** Cases: 417; Controls: 420 **Exposure assessment method:** interview	**OR, Acetaminophen use**			Age, race, other factors
		No NSAID intake	1	308	
		<1 pills per day	0.69 (0.21–2.32)	8	
		≥ 1 pills per day	0.81 (0.25–3.23)	3	
		Trend-test *p*-value: <0.50			
Salinas et al. (2010) Case-Control King County, WA, US **Enrollment or follow-up:** 2002-2005	**Population:** Cases: 1001; Controls: 942 **Exposure assessment method:** interview	**OR, Men - acetaminophen use**			Age, race, prostate cancer screening within 5 years before reference date
		Ever use ≥1 time/wk for ≥3 months	1.03 (0.75–1.41)	998	
		Use > 5 yrs	1.15 (0.71–1.48)	998	
Wright et al. (2016) Case-Control King County, Washington, US **Enrollment or follow-up:** 2002-2005	**Population:** Cases: 346; Controls: 942 **Exposure assessment method:** questionnaire	**OR, Regular use stratified by negative gene fusion status**			Age, race, family history of prostate cancer, prostate cancer screening within 5 years before reference date
		No(reference)	-	163	
		Yes	1.12 (0.58–2.18)	12	
		OR, Regular use stratified by positive gene fusion status			
		No(reference)	-	162	
		Yes	0.93 (0.43–1.98)	9	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Prostate (Aggressive/Advanced) – Cohort					
Jacobs et al. (2011) Cohort US **Enrollment or follow-up:** 1992-1997	**Population:** Men in the Cancer Prevention Study-II (CPS-II) Nutrition Cohort N=78,485 **Exposure assessment method:** questionnaire	**RR, Regular use**			Age, race, education, BMI, diabetes, NSAID use, history of PSA testing
		Never reported use	1	2034	
		Past or < regular use	0.97 (0.9–1.04)	1108	
		Current regular use, <5 years	1.03 (0.86–1.23)	129	
		Current regular use, ≥5 years	0.49 (0.27–0.88)	11	
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** VITAL (VITamins And Lifestyle) study 62,841 **Exposure assessment method:** questionnaire	**HR, 10-yr use prior to baseline in men**			Age, education, race, marital status, height, BMI, physical activity, pack-years of smoking, alcohol intake at 45 yrs, fruit and vegetable intake, red meat intake, multivitamin use, self-rated health, family history of colon, lung, and hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/chronic joint pain, migraine/chronic headaches, use of NSAIDs, family history of prostate cancer, history of PSA testing
		No use	1	649	
		Low use (<4 d/wk or <4 yrs)	0.97 (0.76–1.25)	99	
		High use (≥4 d/wk and ≥4 yrs)	0.74 (0.45–1.21)	20	
		Trend-test *p*-value: 0.32			

Table B8. Cohort and case-control studies of skin cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Non-melanoma skin cancer (overall) – Cohort					
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 191: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year
		Prescription	1.3 (1.1–1.6)	114	
Wysong et al. (2014) Cohort US **Enrollment or follow-up:** 1993-2005	**Population:** Women in the Women's Health Initiative Observational Study (WHI OS) cohort N=54,728 **Exposure assessment method:** interview	**OR, Acetaminophen use**			Age, regional solar radiation, education, BMI, smoking status, vitamin D use, physical activity, history of NMSC, history of melanoma, skin reaction to the sun, current and childhood summer sun exposure, sunscreen use, time since last medical visit, history of cardiovascular disease, arthritis, and migraines, any NSAID use
		No/low use	1	NR	
		Inconsistent	1.07 (0.99–1.14)	NR	
		Regular, <5 yrs	1.08 (0.9–1.29)	NR	
		Regular, ≥5 yrs	1.07 (0.92–1.24)	NR	
		Trend-test *p*-value: 0.26			
Skin (basal cell carcinoma) – Cohort					
Cahoon et al. (2012) Cohort US **Enrollment or follow-up:** 1994-2005	**Population:** Caucasian participants in the United States Radiologic Technologists (USRT) study N=58,213 **Exposure assessment method:** questionnaire	**HR, Any intake**			Age, sex, solar UV exposure quartile
		No	1	779	
		Yes	1.14 (1.04–1.25)	1452	
		HR, Days per month			
		No	1	779	
		0-4	1.18 (1.07–1.3)	1009	
		5-14	1 (0.87–1.16)	281	
		15-21	1.19 (0.93–1.52)	69	
		≥22	1.22 (0.98–1.53)	93	
		Trend-test *p*-value: 0.08			
Jeter et al. (2012) Cohort US **Enrollment or follow-up:** 1980-2008	**Population:** Caucasian women in the Nurses' Health Study, aged 30-55 yr N=92,125 **Exposure assessment method:** questionnaire	**RR, Use**			Age, questionnaire cycle, use of aspirin or other NSAIDs, reaction of skin to sun exposure, ability to tan, number of severe sunburns, number of moles on left arm, family history of melanoma, UV availability at state of residence, menopausal status, use of postmenopausal hormones, height, BMI, physical activity, intake of vitamin C from foods, intake of vitamin D from foods and supplements
		Never	1	3733	
		Past	1.02 (0.96–1.07)	2811	
		Current	1.05 (1–1.1)	3088	
		RR, Tablets/week			
		Never	1	3733	
		1-2	1.07 (0.96–1.19)	469	
		3-5	0.99 (0.88–1.12)	338	
		6-14	1.13 (1.02–1.25)	560	
		≥15	0.89 (0.75–1.04)	164	
		Trend-test *p*-value: 0.40			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
		RR, Days/week			
		Never	1	3733	
		1	1.05 (0.98–1.12)	1080	
		2-3	1.01 (0.93–1.09)	740	
		4-5	1.12 (1.01–1.25)	360	
		6-7	1.07 (0.98–1.17)	675	
		Trend-test *p*-value: 0.60			
		RR, Years of use			
		Never	1	3733	
		<5	1.03 (0.98–1.08)	2906	
		5-9	1.06 (1–1.12)	2198	
		10-14	1 (0.91–1.09)	633	
		≥15	1.1 (0.93–1.3)	162	
		Trend-test *p*-value: 0.35			
Pandeya et al. (2019) Cohort Queensland, Australia **Enrollment or follow-up:** 2010-2014	**Population:** QSkin Sun and Health Study N=34,630 **Exposure assessment method:** questionnaire	**HR, Use among low risk participants (no history of skin cancer excision and ≤ 5 actinic lesions treated)**			Age, sex, private health insurance, education, general health, smoking, other illnesses, cumulative lifetime sun exposure, tanning, skin check
		Never use	1	NR	
		Infrequent use	0.89 (0.68–1.16)	461 (total exposed cases)	
		Frequent use	0.83 (0.59–1.17)	461 (total exposed cases)	
Skin (basal cell carcinoma) – Case-Control					
Torti et al. (2011) Case-Control New Hampshire, US **Enrollment or follow-up:** 1997-2000	**Population:** Cases: 487; Controls: 462 **Exposure assessment method:** interview	**OR, Paracetamol (acetaminophen) use**			Age, gender, number of cigarettes smoked per day, skin type, lifelong number of painful sunburns, lifelong cumulative number of hours of sun exposure
		No use	1	438	
		Use	0.65 (0.42–1.01)	49	
		Yes, current use	0.56 (0.33–0.97)	30	
		Yes, past use	0.82 (0.41–1.63)	19	
		Duration ≤ 7 yrs	0.74 (0.42–1.32)	26	
		Duration ≥ 7 yrs	0.54 (0.29–1.03)	23	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Skin (squamous cell carcinoma) – Cohort					
Jeter et al. (2012) Cohort US **Enrollment or follow-up:** 1980-2008	**Population:** Caucasian women in the Nurses' Health Study, aged 30-55 yr N=92,125 **Exposure assessment method:** questionnaire	**RR, Tablets/week**			Age, questionnaire cycle, use of aspirin or other NSAIDs, reaction of skin to sun exposure, ability to tan, number of severe sunburns, number of moles on left arm, family history of melanoma, UV availability at state of residence, menopausal status, use of postmenopausal hormones, height, BMI, physical activity, intake of vitamin C from foods, intake of vitamin D from foods and supplements
		Never	1	428	
		Past	1.07 (0.92–1.23)	385	
		Current	0.88 (0.75–1.02)	310	
		1-2	0.94 (0.71–1.25)	63	
		3-5	1.11 (0.83–1.49)	58	
		6-14	0.68 (0.5–0.92)	51	
		≥15	0.92 (0.6–1.39)	25	
		Trend-test *p*-value: 0.30			
		RR, Days/week			
		Never	1	428	
		1	1.1 (0.89–1.35)	116	
		2-3	0.87 (0.68–1.11)	83	
		4-5	0.74 (0.52–1.07)	32	
		6-7	0.78 (0.59–1.02)	64	
		Trend-test *p*-value: 0.04			
		RR, Years of use			
		Never	1	428	
		<5	1 (0.86–1.16)	319	
		5-9	0.97 (0.83–1.14)	264	
		10-14	0.95 (0.75–1.2)	95	
		≥15	0.63 (0.38–1.04)	17	
		Trend-test *p*-value: 0.14			
Pandeya et al. (2019) Cohort Queensland, Australia **Enrollment or follow-up:** 2010-2014	**Population:** QSkin Sun and Health Study N=34,630 **Exposure assessment method:** questionnaire	**HR, Use among low risk participants (no history of skin cancer excision and at most five actinic lesions treated)**			Age, sex, private health insurance, education, general health, smoking, other illnesses, cumulative lifetime sun exposure, tanning, skin check
		Never use	1	NR	
		Infrequent use	0.96 (0.58–1.58)	136 (total exposed cases)	
		Frequent use	1.34 (0.75–2.42)	136 (total exposed cases)	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Skin (squamous cell carcinoma) – Case-Control					
Torti et al. (2011) Case-Control New Hampshire, US **Enrollment or follow-up:** 1997-2000	**Population:** Cases: 535; Controls: 462 **Exposure assessment method:** interview	**OR, acetaminophen use**			Age, gender, number of cigarettes smoked per day, skin type, lifelong number of painful sunburns, lifelong cumulative number of hours of sun exposure
		No use	1	490	
		Use	0.62 (0.4–0.97)	45	
		Yes, current use	0.56 (0.33–0.97)	31	
		Yes, past use	0.72 (0.34–1.5)	14	
		Duration ≤ 7 yrs	0.51 (0.27–0.97)	19	
		Duration ≥ 7 yrs	0.68 (0.37–1.23)	25	
Malignant melanoma – Cohort					
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 190: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year
		Prescription	0.6 (0.2–1.3)	7	
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** VITAL (VITamins And Lifestyle) study 62,841 **Exposure assessment method:** questionnaire	**HR, 10-yr use prior to baseline in men and women combined**			Age, education, race, marital status, height, BMI, physical activity, pack-years of smoking, alcohol intake at 45 yrs, fruit and vegetable intake, red meat intake, multivitamin use, self-rated health, family history of colon, lung, and hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/chronic joint pain, migraine/chronic headaches, use of NSAIDs
		No use	1	229	
		Low use (<4 d/wk or <4 yrs)	0.9 (0.6–1.33)	38	
		High use (≥4 d/wk and ≥4 yrs)	0.79 (0.39–1.58)	12	
		Trend-test *p*-value: 0.42			
Jeter et al. (2012) Cohort US **Enrollment or follow-up:** 1980-2008	**Population:** Caucasian women in the Nurses' Health Study, aged 30-55 yr N=92,125 **Exposure assessment method:** questionnaire	**RR, Use**			Age, questionnaire cycle, use of aspirin or other NSAID, reaction of skin to sun exposure, ability to tan, number of severe sunburns, number of moles on left arm, family history of melanoma, UV availability at state of residence, postmenopausal hormone use, menopausal status, height, BMI, physical activity, intake of vitamin C from foods, intake of vitamin D from foods and supplements, prevalent SCC and BCC
		Never	1	176	
		Past	0.97 (0.77–1.23)	142	
		Current	0.9 (0.71–1.13)	133	
		RR, Tablets/week			
		Never	1	176	
		1-2	0.81 (0.51–1.3)	22	
		3-5	0.85 (0.51–1.44)	17	
		≥6	0.79 (0.52–1.2)	31	
		Trend-test *p*-value: 0.90			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
		RR, Days/week			
		Never	1	176	
		1	0.87 (0.62–1.23)	43	
		2-3	0.73 (0.48–1.09)	28	
		4-5	1.31 (0.83–2.06)	22	
		6-7	1.06 (0.72–1.56)	33	
		Trend-test *p*-value: 0.11			
		RR, Years of use			
		Never	1	176	
		<5	0.95 (0.75–1.19)	130	
		5-9	0.96 (0.74–1.24)	109	
		≥10	0.81 (0.55–1.19)	36	
		Trend-test *p*-value: 0.51			
Gamba et al. (2013) Cohort US **Enrollment or follow-up:** 1993-2010	**Population:** Postmenopausal women in the Women's Health Initiative Observational Study (WHI OS), aged 50-79 yr N=59,806 **Exposure assessment method:** questionnaire	**HR, Medication use**			Age, education, BMI, smoking status, vitamin D intake, physical activity, history of nonmelanoma skin cancer, history of melanoma, skin reaction to the sun, regional solar radiation, current and childhood summer sun exposure, sunscreen use, time since last medical visit, duration of NSAID use, medical indication for NSAID use
		Non-users	1	440	
		Users	0.89 (0.68–1.17)	32	
		Trend-test *p*-value: 0.2			

Table B9. Cohort, nested case-control, and case-control studies of colorectal cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Colon – Cohort					
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 153: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year
		Prescription	0.9 (0.7–1.3)	43	
Colon – Case-Control					
Hardell et al. (1996) Case-Control Sweden **Enrollment or follow-up:** 1984-1986	**Population:** Cases: 301; Controls: 621 **Exposure assessment method:** questionnaire	**OR, acetaminophen use**			Sex, age, job-related physical activity
		Overall	2.5 (0.6–8.5)	7	
		Women	2.2 (0.5–9.2)	5	
		Men	-	2	
Meier et al. (2002) Case-Control United Kingdom **Enrollment or follow-up:** 1992-1997	**Population:** Cases: 635; Controls: 2434 **Exposure assessment method:** records	**OR, Acetaminophen use compared to noncancer controls**			Smoking status
		0	1	238	
		1-9	1.3 (1–1.5)	261	
		10-19	1.1 (0.7–1.5)	54	
		20-29	1 (0.6–1.6)	25	
		≥30	1 (0.7–1.4)	57	
Harris et al. (2008) Case-Control USA **Enrollment or follow-up:** 2003-2004	**Population:** Cases: 326; Controls: 652 **Exposure assessment method:** questionnaire	**OR, Acetaminophen use (OTC ≥ once/wk for > 1yr**			Body mass, hypertension, family history of colon cancer among first or second degree relatives, smoking, alcohol intake
		Acetaminophen	0.81 (0.35–1.61)	12	
Rectum – Cohort					
Thun et al. (1993) Cohort US **Enrollment or follow-up:** 1982 to 1988	**Population:** Participants from the Cancer Prevention Study II (CPS II) N=635,031 **Exposure assessment method:** questionnaire	**Rectum cancer mortality: RR (95% CI) Frequency of use**			Age, race, sex, BMI, family history of specific cancer subsite, cigarette smoking, ever pipe or cigar, alcohol, fruit/vegetable/grain intake, fat consumption
		No NSAID use	1	NR	
		≥16 times/month	3.08 (1.11–8.54)	4	
		Trend-test *p*-value: 0.31			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 154: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year
		Prescription	1.2 (0.8–1.7)	27	
Colon & Rectum – Cohort					
García Rodríguez and Huerta-Alvarez (2001) Cohort United Kingdom **Enrollment or follow-up:** 1994-1997	**Population:** General Practice Research Database N=943,903 **Exposure assessment method:** records	**RR, Current and past use**			Age, sex
		Non-use	1	1023	
		Current, <1 yr	1.7 (1.5–2.1)	221	
		Current, ≥1 yr	0.9 (0.7–1.1)	79	
		Past, <1 yr	1 (0.9–1.1)	663	
		Past, ≥1 yr	0.9 (0.5–1.4)	16	
Friis et al. (2009) Cohort Denmark **Enrollment or follow-up:** 1995 to 2006	**Population:** Participants in the Diet, Cancer, and Health Study, aged 50-64 yr N=24,352 men and 26,701 women **Exposure assessment method:** other (specify in exposure assessment notes)	**IRR, Baseline medication use**			Age, sex, BMI, use of hormone replacement therapy, alcohol intake, history of colorectal endoscopy, statin use, use of aspirin or other NSAID
		Non-use (<2 pills/month)	1	439	
		Any use (≥2 pills/month)	0.93 (0.78–1.12)	176	
		2-3 pills/month	1 (0.8–1.25)	103	
		1-6 pills/week	0.86 (0.64–1.14)	54	
		≥1 pill/day	0.82 (0.51–1.33)	19	
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** VITAL (VITamins And Lifestyle) study 62,841 **Exposure assessment method:** questionnaire	**HR, 10-yr use prior to baseline in men and women combined**			Age, education, race, marital status, height, BMI, physical activity, pack-years of smoking, alcohol intake at 45 yrs, fruit and vegetable intake, red meat intake, multivitamin use, self-rated health, family history of colon, lung, and hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/chronic joint pain, migraine/chronic headaches, use of NSAIDs
		No use	1	344	
		Low use (<4 d/wk or <4 yrs)	0.79 (0.56–1.12)	56	
		High use (≥4 d/wk and ≥4 yrs)	0.8 (0.46–1.37)	19	
		Trend-test *p*-value: 0.18			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Colon & Rectum – Nested Case-Control					
Cea Soriano et al. (2016b) Nested Case-Control United Kingdom **Enrollment or follow-up:** 2000-2012	**Population:** Study 1: new-users of low-dose aspirin Cases: 3,033 (Study 1); 3,174 (Study 2); 12,333; Controls: 10,000 (Studies 1 and 2); 20,000 (Study 3) **Exposure assessment method:** records	**RR, Study 1: Use of paracetamol (acetaminophen)**			Age, sex, follow-up time to CRC occurrence, number of PCP visits, smoking, NSAIDs, BMI, use of insulin and oral steroids
		No use	1	2640	
		Any use	1.04 (0.91–1.19)	393	
		RR, Study 2: New users of acetaminophen			
		No use	1	2394	
		New use	1 (0.88–1.14)	780	
		RR, Study 3: Use of acetaminophen			Age, sex, follow-up time to CRC occurrence, number of PCP visits, smoking, NSAIDs, BMI
		No use	1	10886	
		Any use	1 (0.93–1.09)	1447	
Colon & Rectum – Case-Control					
Logan et al. (1993) Case-Control United Kingdom **Enrollment or follow-up:** 1981-1988	**Population:** Cases: 147; Controls: 176 **Exposure assessment method:** questionnaire	**RR, paracetamol (acetaminophen) use**			Age, sex, social class
		Acetaminophen only	1.03 (0.6–1.9)	69	
Muscat et al. (1994) Case-Control USA **Enrollment or follow-up:** 1989-1992	**Population:** Non-users: Cases: 7 males cases, 15 female cases; Controls: 8 males controls, 24 female controls **Exposure assessment method:** questionnaire	**OR, Men- acetaminophen use**			Body mass index, dietary intake of red meat, cheese, fruits, and vegetables, physical activity, cigarette smoking, alcohol consumption, coffee consumption
		Nonuser	1	276	
		Daily use ≥1 yr	1.07 (0.35–3.23)	7	
		OR, Women - acetaminophen use			
		Non users	1	212	
		Daily use ≥ 1 yr	0.59 (0.27–1.25)	17	
Peleg et al. (1996) Case-Control Atlanta, GA **Enrollment or follow-up:** 1987-1992	**Population:** Cases: 113 colorectal adenoma; 93 colorectal adenocarcinoma; Controls: 186 for carcinoma cases; 226 for adenoma cases **Exposure assessment method:** records	**Adenoma: OR, Cumulative acetaminophen dose**			Age, gender
		Nonusers	1	NR	
		<100 g	0.78 (0.38–1.6)	NR	
		100-400 g	1.2 (0.59–2.41)	NR	
		≥400 g	1.08 (0.51–2.28)	NR	
		Adenocarcinoma: OR, Cumulative acetaminophen dose			Age, gender
		Nonusers	1	NR	
		<100 g	1.89 (0.84–4.28)	NR	
		100-500 g	1.97 (0.88–4.43)	NR	
		≥500 g	1.28 (0.53–3.09)	NR	

Table B10. Cohort and case-control studies of brain cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Brain (Overall)					
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 193: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year
		Prescription	0.8 (0.3–1.6)	7	
Brain (Glioma)					
Sivak-Sears et al. (2004) Case-Control US (6 San Francisco Bay Area counties) **Enrollment or follow-up:** 1997-2000	**Population:** Cases: 236; Controls: 401 **Exposure assessment method:** interview	**Glioblastoma multiforme: OR, Acetaminophen and other NSAIDs use**			Age
		< 600 pills	1	161	
		≥ 600 pills	0.92 (0.6–1.5)	31	
		≥ 600 pills for <10 yrs	0.72 (0.3–1.5)	11	
		≥ 600 pills for >10 yrs	1.08 (0.6–2)	20	
Egan et al. (2016) Case-Control US (TN, FL, AL, GA, KY) **Enrollment or follow-up:** 2004-2012	**Population:** Cases: 1123; Controls: 1296 **Exposure assessment method:** interview	**OR, acetaminophen use**			Age, gender, race, education, state of residence
		Acetaminophen	1.2 (0.93–1.54)	182	
Brain (Meningioma)					
Egan et al. (2016) Case-Control US (TN, FL, AL, GA, KY) **Enrollment or follow-up:** 2004-2012	**Population:** Cases: 310; Controls: 1296 **Exposure assessment method:** interview	**OR, acetaminophen use**			Age, gender, race, education, state of residence
		Acetaminophen	1.85 (1.29–2.65)	72	
Brain (Childhood cancer)					
Stålberg et al. (2010) Case-Control Sweden **Enrollment or follow-up:** 1975-1984	**Population:** Cases: 512; Controls: 525 **Exposure assessment method:** interview	**Unadjusted OR, paracetamol (acetaminophen) use**			None
		Acetaminophen	1.7 (0.5–5.1)	8	
		Adjusted OR, acetaminophen use			Maternal age at birth, parity, mothers country of birth, level of hospital
		Acetaminophen	1.7 (0.6–5.4)	8	

Table B11. Cohort, nested case-control, and case-control studies of the respiratory tract cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Lung – Cohort					
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 162: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year
		Prescription	1.6 (1.2–2)	73	
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** VITAL (VITamins And Lifestyle) study 62,841 **Exposure assessment method:** questionnaire	**HR, 10-yr use prior to baseline in men and women combined**			Age, education, race, marital status, height, BMI, physical activity, pack-years of smoking, alcohol intake at 45 yrs, fruit and vegetable intake, red meat intake, multivitamin use, self-rated health, family history of colon, lung, and hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/chronic joint pain, migraine/chronic headaches, use of NSAIDs
		No use	1	458	
		Low use (<4 d/wk or <4 yrs)	1.22 (0.95–1.55)	122	
		High use (≥4 d/wk and ≥4 yrs)	1.06 (0.73–1.54)	42	
		Trend-test *p*-value: 0.32			
Bittoni et al. (2017) Cohort US **Enrollment or follow-up:** 1988-2006	**Population:** Participants of NHANES III, aged ≥40 yr. Subcohort of 5,882 individuals who smoked N=10,735 **Exposure assessment method:** interview	**Fatal lung cancer: HR, Any intake**			Other drugs, years in study, gender, current smoking, comorbid conditions (myocardial infarction, stroke, diabetes, or malignancies other than lung cancer)
		No NSAID use	1	83	
		Any use	0.88 (0.66–1.16)	69	
Lung – Nested Case-Control					
Olsen et al. (2008) Nested Case-Control Denmark **Enrollment or follow-up:** 2002-2005	**Population:** Danish Diet, Cancer and Health prospective cohort study Cases: 573; Controls: 857 **Exposure assessment method:** records	**RR, Any use of acetaminophen ≥ 1 yr before index date**			Age, sex
		Any use	1.34 (1.07–1.68)	NR	
		RR, Any use of acetaminophen ≥ 1 yr before index date			Age, sex, smoking habits, education reported as 'occupational training'
		Any use	1.11	NR	

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Lung – Case-Control					
Harris et al. (2007) Case-Control Ohio,US **Enrollment or follow-up:** 2002-2004	**Population:** Cases: 492; Controls: 984 **Exposure assessment method:** interview	**OR, Acetaminophen use ≥2 times/wk for ≥2 yrs. No use of any NSAID or infrequent use of ≤ 1 pill/wk for <1 yr**			Pack years of cigarette smoking, age, body mass, gender, ethnicity, family history, arthritis, alcohol intake
		None/Infrequent use	1	315	
		Acetaminophen use	1.36 (0.55–3.37)	18	
Van Dyke et al. (2008) Case-Control Michigan, US **Enrollment or follow-up:** 2001-2005	**Population:** Cases: 581; Controls: 541 **Exposure assessment method:** questionnaire	**Non-small cell lung cancer: OR, Never smokers or <100 cigarettes in lifetime**			Age, race, years of educational level, smoking pack-years, BMI, family history of lung cancer, history of chronic obstructive lung disease, history of arthritis, history of cardiovascular disease and BMI x years of education
		Acetaminophen use- never	1	451	
		Acetaminophen use- ever	0.94 (0.65–1.36)	129	
		Non-small cell lung cancer: OR, Duration of use			
		0	1	451	
		1-5	1.03 (0.63–1.7)	74	
		>5	0.94 (0.72–1.22)	53	
		Trend-test *p*-value: 0.63			
Lim et al. (2012) Case-Control Singapore **Enrollment or follow-up:** 2005-2008	**Population:** Cases: 398; Controls: 640 **Exposure assessment method:** interview	**OR, Non-smokers: Regular use of Panadol (acetaminophen) (≥2 times/wk for ≥1 month)**			Age at diagnosis/admission, fruit and vegetable consumption, education, housing type, history of cancer in 1st degree relative
		No	1	243	
		Yes	1.02 (0.47–2.21)	10	
		OR, Smokers: Regular use of acetaminophen (≥2 times/wk for ≥1 month)			Age at diagnosis/admission, fruit and vegetable consumption, years of education, housing type, history of cancer in 1st degree relative, smoking
		No	1	128	
		Yes	2.58 (0.62–10.78)	10	
Erickson et al. (2018) Case-Control Maryland, US **Enrollment or follow-up:** 2005-2015	**Population:** Cases: 1220; Controls: 1634 **Exposure assessment method:** questionnaire	**Non-small Cell Lung Cancer: OR, All users**			Age, gender, smoking, pack-years
		No(reference)	1	1060	
		Yes	1.51 (1.13–2.03)	160	
		Non-small Cell Lung Cancer: OR, All-daily Tylenol (acetaminophen) users			
		Never	1	1060	
		< 1 tablet per day	1.36 (0.79–2.35)	35	
		≥ 1 tablet per day	1.59 (1.13–2.23)	125	
		Trend-test *p*-value: 0.004			
		Non-small Cell Lung Cancer: OR, Duration of acetaminophen use			
		Never	1	1060	
		< 3 years	3.18 (2.06–4.92)	98	
		≥ 3 years	0.72 (0.46–1.15)	48	
		Trend-test *p*-value: 0.25			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Respiratory tract – Cohort					
Lipworth et al. (2003) Cohort Denmark **Enrollment or follow-up:** 1989-1996	**Population:** Individuals who filled acetaminophen prescriptions identified through the Pharmacoepidemiological Prescription Database N = 17,760 men; 32,130 women **Exposure assessment method:** records	**Mortality: SMR, Number of prescriptions**			Age, sex, calendar year
		1	2.9 (2.6–3.3)	307	
		2-4	4.4 (4–4.9)	367	
		5-9	3.6 (3.1–4.1)	172	
		≥10	2.5 (2.1–2.9)	159	
		Mortality: SMR, Prescribed acetaminophen (1989-1996)			
		Total	3.4 (3.1–3.6)	1005	
		Men	3.6 (3.3–3.9)	564	
		Women	3.1 (2.8–3.4)	441	
		Mortality: SMR, Prescribed acetaminophen, stratified by latency period			
		<1 yr	8 (7.3–8.6)	629	
		1-2 yrs	1.9 (1.7–2.2)	233	
		3-4 yrs	1.4 (1.1–1.7)	92	
		≥5 yrs	1.6 (1.2–2.1)	51	

Table B12. Cohort and case-control studies of gastrointestinal cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Buccal cavity and pharynx					
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 140-148: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year
		Prescription	1.3 (0.7–2.3)	12	
Esophagus					
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 150: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year
		Prescription	2.5 (1.2–4.7)	10	
Anderson et al. (2006) Case-Control Northern Ireland **Enrollment or follow-up:** 2002-2004	**Population:** Cases: 224; Controls: 260 population controls **Exposure assessment method:** interview	**Barrett's esophagus: OR acetaminophen use**			Sex, age at interview, job type, education, BMI, smoking, alcohol, location
		Any use	1.09 (0.63–1.91)	NR	
Esophagus (Adenocarcinoma)					
Anderson et al. (2006) Case-Control Northern Ireland **Enrollment or follow-up:** 2002-2004	**Population:** Cases: 227 (131 esophageal, 92 esophagogastric junction, 4 unclassified); Controls: 260 population controls **Exposure assessment method:** interview	**OR, acetaminophen use**			Sex, age at interview, job type, education, BMI, smoking, alcohol, location, gastroesophageal reflux symptoms, upper GI disorders
		Any use	0.82 (0.45–1.52)	NR	
Sadeghi et al. (2008) Case-Control Australia **Enrollment or follow-up:** 2001-2005	**Population:** Cases: 367 esophageal, 426 esophagogastric junction; Controls: 1580 **Exposure assessment method:** questionnaire	**Esophageal adenocarcinoma: OR, acetaminophen in last 5 yrs**			Age, sex, state of residence, household income, cumulative smoking history, mean alcohol consumption, frequency of gastroesophageal reflux symptoms 10 y before diagnosis, BMI
		Never	1	44	
		Occasionally	0.94 (0.61–1.45)	168	
		< Weekly	1.31 (0.81–2.1)	96	
		≥ Weekly	0.85 (0.5–1.44)	59	
		Trend-test *p*-value: 0.76			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
		Esophagogastric junction adenocarcinoma: OR, Acetaminophen in last 5 yrs			
		Never	1	48	
		Occasionally	1.04 (0.71–1.54)	214	
		< Weekly	1 (0.64–1.55)	96	
		≥ Weekly	1.03 (0.64–1.7)	67	
		Trend-test *p*-value: 0.84			
Esophagus (Squamous cell carcinoma)					
Sadeghi et al. (2008) Case-Control Australia **Enrollment or follow-up:** 2001-2005	**Population:** Cases: 420; Controls: 1580 **Exposure assessment method:** questionnaire	**OR, Acetaminophen in last 5 yrs**			Age, sex, state of residence, household income, cumulative smoking history, mean alcohol consumption, BMI, frequency of gastroesophageal reflux symptoms 10 yr before diagnosis
		Never	1	26	
		Occasionally	1.67 (1.01–2.75)	176	
		< Weekly	1.15 (0.65–2.03)	52	
		≥ Weekly	1.26 (0.69–2.29)	50	
		Trend-test *p*-value: 0.10			
Stomach/gastric cancer					
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 151: SIR, Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year
		Prescription	1 (0.6–1.7)	14	
Epplein et al. (2009) Cohort (Hawaii and Los Angeles, CA), US **Enrollment or follow-up:** 1993-2004	**Population:** Multiethnic Cohort study N=169,292 **Exposure assessment method:** questionnaire	**Gastric cardia adenocarcinoma: HR, Duration of use, years**			Age, sex, ethnicity, smoking, BMI, alcohol consumption
		No regular NSAID or acetaminophen use	1	35	
		Any regular use	1.09 (0.67–1.77)	27	
		≤ 1 yr	0.9 (0.37–2.19)	6	
		2-5 yrs	1.1 (0.49–2.46)	7	
		≥ 6 yrs	1.3 (0.62–2.71)	11	
		Trend-test *p*-value: 0.35			
		Distal gastric adenocarcinoma: HR, Duration of use, years			Age, sex, ethnicity, smoking, education, processed meat intake, family history of gastric cancer
		No regular NSAID or acetaminophen use	1	259	
		Any regular use	1.15 (0.94–1.4)	161	
		≤ 1 yr	1.51 (1.11–2.04)	57	
		2-5 yrs	0.95 (0.66–1.36)	39	
		≥ 6 yrs	1 (0.7–1.42)	40	
		Trend-test *p*-value: 0.71			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Other digestive tract					
Lipworth et al. (2003) Cohort Denmark **Enrollment or follow-up:** 1989-1996	**Population:** Individuals who filled acetaminophen prescriptions identified through the Pharmacoepidemiological Prescription Database N = 17,760 men; 32,130 women **Exposure assessment method:** records	**Digestive tract tumor mortality: SMR, Prescribed acetaminophen (1989-1996)**			Age, sex, calendar year
		All	2.3 (2.1–2.4)	864	
		Men	2.7 (2.4–2.9)	400	
		Women	2 (1.8–2.2)	464	
		Digestive tract tumor mortality: SMR, Prescribed acetaminophen , stratified by latency period			
		<1 yr	4.9 (4.4–5.3)	498	
		1-2 yrs	1.5 (1.3–1.7)	225	
		3-4 yrs	1.2 (1–1.4)	100	
		≥5 yrs	1 (0.7–1.4)	41	
		Digestive tract tumor mortality: SMR, Number of prescriptions			
		1	2.4 (2.1–2.7)	304	
		2-4	2.7 (2.4–3.1)	293	
		5-9	2.2 (1.9–2.6)	145	
		≥10	1.5 (1.2–1.7)	122	
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** VITAL (VITamins And Lifestyle) study 62,841 **Exposure assessment method:** questionnaire	**Gastrointestinal: HR 10-yr use prior to baseline in men and women combined**			Age, education, race, marital status, height, BMI, physical activity, pack-years of smoking, alcohol intake at 45 yrs, fruit and vegetable intake, red meat intake, multivitamin use, self-rated health, family history of colon, lung, and hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/chronic joint pain, migraine/chronic headaches, use of NSAIDs
		No use	1	623	
		Low use (<4 d/wk or <4 yrs)	0.9 (0.71–1.14)	120	
		High use (≥4 d/wk and ≥4 yrs)	0.84 (0.57–1.22)	40	
		Trend-test *p*-value: 0.24			

Table B13. Cohort and case-control studies of pancreatic cancer and acetaminophen use

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 157: SIR (95% CI) Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year
		Prescription	0.8 (0.4–1.4)	11	
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** VITAL (VITamins And Lifestyle) study 62,841 **Exposure assessment method:** questionnaire	**HR 10-yr use prior to baseline in men and women combined**			Age, education, race, marital status, height, BMI, physical activity, pack-years of smoking, alcohol intake at 45 yrs, fruit and vegetable intake, red meat intake, multivitamin use, self-rated health, family history of colon, lung, and hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/chronic joint pain, migraine/chronic headaches, use of NSAIDs
		No use	1	105	
		Low use (<4 d/wk or <4 yrs)	0.9 (0.51–1.58)	21	
		High use (≥4 d/wk and ≥4 yrs)	0.4 (0.12–1.31)	6	
		Trend-test *p*-value: 0.16			
Tan et al. (2011) Case-Control MN, US **Enrollment or follow-up:** 2004-2010	**Population:** Cases: 1500; Controls: 2119 **Exposure assessment method:** questionnaire	**OR, d/month**			Age, sex, BMI, smoking status, pack-years of smoking, long-standing diabetes mellitus (>3 yrs of duration)
		Acetaminophen use <1	1	431	
		Acetaminophen use ≥ 1	1.04 (0.82–1.32)	232	
		Trend-test *p*-value: 0.758			
		OR, frequency of use, d/wk			
		Acetaminophen use <1	1	613	
		Acetaminophen use 2-5	0.97 (0.6–1.57)	36	
		Acetaminophen use 6+	0.74 (0.35–1.56)	14	
		Trend-test *p*-value: 0.730			
		OR, typical dosage			
		None	1	249	
		1-2 tablets/d	0.98 (0.75–1.29)	251	
		3+ tablets/d	1.11 (0.76–1.62)	93	
		Trend-test *p*-value: 0.789			

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Kho et al. (2016) Case-Control Australia **Enrollment or follow-up:** 2007-2011	**Population:** Cases: 522; Controls: 653 **Exposure assessment method:** interview	**OR, acetaminophen use over the past 5 years**			Age, sex, pack years of smoking, alcohol use, diabetes, adult BMI
		Never	1	177	
		Occasionally	0.94 (0.7–1.26)	190	
		1/month-3/month	0.68 (0.41–1.15)	29	
		1/week-3/week	0.96 (0.59–1.56)	41	
		4+/week	0.92 (0.62–1.36)	85	

Table B14. Cohort studies of all cancers combined and acetaminophen

Reference, study-design, location, and year	Population description & exposure assessment method	Exposure category or level	Risk estimate (95% CI)	Cases/ deaths	Co-variates controlled
Walter et al. (2011a) Cohort US, 13 counties of the Seattle-Puget Sound area **Enrollment or follow-up:** 2000-2008; mean follow-up of 6.5 ± 1.7 yrs	**Population:** Men and women in the VITamins And Lifestyle (VITAL) study N=62,841 **Exposure assessment method:** questionnaire	**HR, 10-yr use prior to baseline in men and women combined**			Age, education, race, marital status, height, BMI, physical activity, pack-years of smoking, alcohol intake at 45 yrs, fruit and vegetable intake, red meat intake, multivitamin use, self-rated health, family history of colon, lung, and hematologic cancers, sigmoidoscopy in past 10 yrs, diabetes, osteoarthritis/chronic joint pain, migraine/chronic headaches, use of NSAIDs
		No use	1	4467	
		Low use (<4 d/wk or <4 yrs)	1.08 (0.99–1.17)	978	
		High use (≥4 d/wk and ≥4 yrs)	1.02 (0.89–1.17)	305	
		Trend-test *p*-value: 0.25			
		HR, 10-yr use prior to baseline in women			Above factors plus: Family history of breast cancer, mammogram in past 2 yrs, age at menopause, age at menarche, age at first birth, years of estrogen therapy, years of combined hormone therapy, hysterectomy
		No use	1	1741	
		Low use (<4 d/wk or <4 yrs)	1.04 (0.92–1.17)	523	
		High use (≥4 d/wk and ≥4 yrs)	0.97 (0.8–1.17)	177	
		Trend-test *p*-value: 0.97			
		HR, 10-yr use prior to baseline in men			Above factors plus: Family history of prostate cancer, PSA test in past 2 yrs
		No use	1	2726	
		Low use (<4 d/wk or <4 yrs)	1.18 (0.99–1.26)	455	
		High use (≥4 d/wk and ≥4 yrs)	1.08 (0.88–1.31)	128	
		Trend-test *p*-value: 0.11			
Friis et al. (2002) Cohort Denmark **Enrollment or follow-up:** 1989-1997	*See Table B1 for description*	**ICD-7, 140-205: SIR (95% CI) Prescribed acetaminophen (1989-1997)**			Age, sex, calendar year
		Men	1.21 (1.05–1.38)	215	
		Women	1.03 (0.92–1.16)	315	
		ICD-7, 140-205: SIR (95% CI) Number of prescriptions			
		Any prescription	1.1 (1–1.2)	530	
		1	0.9 (0.8–1.1)	155	
		2-4	1.2 (1–1.4)	146	
		5-9	1.5 (1.2–1.8)	120	
		≥10	1 (0.8–1.1)	109	

Appendix B References

Ammundsen HB, Faber MT, Jensen A, Høgdall E, Blaakær J, Høgdall C, et al. 2012. Use of analgesic drugs and risk of ovarian cancer: Results from a Danish case-control study. Acta obstetricia et gynecologica Scandinavica 91:1094-1102.

Anderson LA, Johnston BT, Watson RG, Murphy SJ, Ferguson HR, Comber H, et al. 2006. Nonsteroidal anti-inflammatory drugs and the esophageal inflammation-metaplasia-adenocarcinoma sequence. Cancer research 66:4975-4982.

Ashok V, Dash C, Rohan TE, Sprafka JM, Terry PD. 2011. Selective cyclooxygenase-2 (COX-2) inhibitors and breast cancer risk. Breast (Edinburgh, Scotland) 20:66-70.

Baandrup L, Friis S, Dehlendorff C, Andersen KK, Olsen JH, Kjaer SK. 2014. Prescription use of paracetamol and risk for ovarian cancer in Denmark. Journal of the National Cancer Institute 106:dju111.

Bittoni MA, Carbone DP, Harris RE. 2017. Ibuprofen and fatal lung cancer: A brief report of the prospective results from the Third National Health and Nutrition Examination Survey (NHANES III). Molecular and clinical oncology 6:917-920.

Bodelon C, Doherty JA, Chen C, Rossing MA, Weiss NS. 2009. Use of nonsteroidal antiinflammatory drugs and risk of endometrial cancer. American journal of epidemiology 170:1512-1517.

Bosco JL, Palmer JR, Boggs DA, Hatch EE, Rosenberg L. 2011. Regular aspirin use and breast cancer risk in US Black women. Cancer causes & control : CCC 22:1553-1561.

Brasky TM, Bonner MR, Moysich KB, Ambrosone CB, Nie J, Tao MH, et al. 2010. Non-steroidal anti-inflammatory drug (NSAID) use and breast cancer risk in the Western New York Exposures and Breast Cancer (WEB) Study. Cancer causes & control : CCC 21:1503-1512.

Cahoon EK, Rajaraman P, Alexander BH, Doody MM, Linet MS, Freedman DM. 2012. Use of nonsteroidal anti-inflammatory drugs and risk of basal cell carcinoma in the United States radiologic technologists study. International journal of cancer 130:2939-2948.

Cea Soriano L, Soriano-Gabarro M, Garcia Rodriguez LA. 2016. The Protective Effect of Low-Dose Aspirin against Colorectal Cancer Is Unlikely Explained by Selection Bias: Results from Three Different Study Designs in Clinical Practice. PloS one 11:e0159179.

Clarke CA, Canchola AJ, Moy LM, Neuhausen SL, Chung NT, Lacey JV, Jr., et al. 2017. Regular and low-dose aspirin, other non-steroidal anti-inflammatory medications and prospective risk of HER2-defined breast cancer: the California Teachers Study. Breast cancer research : BCR 19:52.

Cramer DW, Harlow BL, Titus-Ernstoff L, Bohlke K, Welch WR, Greenberg ER. 1998. Over-the-counter analgesics and risk of ovarian cancer. Lancet (London, England) 351:104-107.

Egan KM, Nabors LB, Thompson ZJ, Rozmeski CM, Anic GA, Olson JJ, et al. 2016. Analgesic use and the risk of primary adult brain tumor. European journal of epidemiology 31:917-925.

Eliassen AH, Chen WY, Spiegelman D, Willett WC, Hunter DJ, Hankinson SE. 2009. Use of aspirin, other nonsteroidal anti-inflammatory drugs, and acetaminophen and risk of breast cancer among premenopausal women in the Nurses' Health Study II. Archives of internal medicine 169:115-121; discussion 121.

Epplein M, Nomura AMY, Wilkens LR, Henderson BE, Kolonel LN. 2009. Nonsteroidal antiinflammatory drugs and risk of gastric adenocarcinoma. American journal of epidemiology 170:507-514.

Erickson P, Gardner LD, Loffredo CA, St. George DM, Bowman ED, Deepak J, et al. 2018. Racial and ethnic differences in the relationship between aspirin use and non–small cell lung cancer risk and survival. Cancer Epidemiology Biomarkers and Prevention 27:1518-1526.

Fairfield KM, Hunter DJ, Fuchs CS, Colditz GA, Hankinson SE. 2002. Aspirin, other NSAIDs, and ovarian cancer risk (United States). Cancer causes & control : CCC 13:535-542.

Friel G, Liu CS, Kolomeyevskaya NV, Hampras SS, Kruszka B, Schmitt K, et al. 2015. Aspirin and Acetaminophen Use and the Risk of Cervical Cancer. Journal of lower genital tract disease 19:189-193.

Friis S, Nielsen GL, Mellemkjaer L, McLaughlin JK, Thulstrup AM, Blot WJ, et al. 2002. Cancer risk in persons receiving prescriptions for paracetamol: a Danish cohort study. International journal of cancer 97:96-101.

Friis S, Thomassen L, Sorensen HT, Tjonneland A, Overvad K, Cronin-Fenton DP, et al. 2008. Nonsteroidal anti-inflammatory drug use and breast cancer risk: a Danish cohort study. European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP) 17:88-96.

Friis S, Poulsen AH, Sørensen HT, Tjønneland A, Overvad K, Vogel U, et al. 2009. Aspirin and other non-steroidal anti-inflammatory drugs and risk of colorectal cancer: A Danish cohort study. Cancer Causes and Control 20:731-740.

Gallicchio L, McSorley MA, Newschaffer CJ, Thuita LW, Huang H-Y, Hoffman SC, et al. 2006. Nonsteroidal antiinflammatory drugs, cyclooxygenase polymorphisms, and the risk of developing breast carcinoma among women with benign breast disease. Cancer (Hoboken, NJ, U S) 106:1443-1452.

Gallicchio L, Visvanathan K, Burke A, Hoffman SC, Helzlsouer KJ. 2007. Nonsteroidal anti-inflammatory drugs and the risk of developing breast cancer in a population-based prospective cohort study in Washington County, MD. International journal of cancer 121:211-215.

Gamba CA, Swetter SM, Stefanick ML, Kubo J, Desai M, Spaunhurst KM, et al. 2013. Aspirin is associated with lower melanoma risk among postmenopausal Caucasian women: the Women's Health Initiative. Cancer 119:1562-1569.

Garcia Rodriguez LA, Huerta-Alvarez C. 2001. Reduced risk of colorectal cancer among long-term users of aspirin and nonaspirin nonsteroidal antiinflammatory drugs. Epidemiology (Cambridge, Mass) 12:88-93.

Garcia Rodriguez LA, Gonzalez-Perez A. 2004a. Risk of breast cancer among users of aspirin and other anti-inflammatory drugs. British journal of cancer 91:525-529.

Garcia Rodriguez LA, Gonzalez-Perez A. 2004b. Inverse association between nonsteroidal anti-inflammatory drugs and prostate cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 13:649-653.

Gill JK, Maskarinec G, Wilkens LR, Pike MC, Henderson BE, Kolonel LN. 2007. Nonsteroidal antiinflammatory drugs and breast cancer risk: the multiethnic cohort. American journal of epidemiology 166:1150-1158.

Hannibal CG, Rossing MA, Wicklund KG, Cushing-Haugen KL. 2008. Analgesic drug use and risk of epithelial ovarian cancer. American journal of epidemiology 167:1430-1437.

Hannibal CG, Dehlendorff C, Kjaer SK. 2018. Use of paracetamol, low-dose aspirin, or non-aspirin non-steroidal anti-inflammatory drugs and risk of ovarian borderline tumors in Denmark. Gynecologic oncology 151:513-518.

Hardell L, Fredrikson M, Axelson O. 1996. Case-control study on colon cancer regarding previous diseases and drug intake. International journal of oncology 8:439-444.

Harris RE, Kasbari S, Farrar WB. 1999. Prospective study of nonsteroidal anti-inflammatory drugs and breast cancer. Oncology reports 6:71-73.

Harris RE, Chlebowski RT, Jackson RD, Frid DJ, Ascenseo JL, Anderson G, et al. 2003. Breast cancer and nonsteroidal anti-inflammatory drugs: prospective results from the Women's Health Initiative. Cancer research 63:6096-6101.

Harris RE, Beebe-Donk J, Alshafie GA. 2006. Reduction in the risk of human breast cancer by selective cyclooxygenase-2 (COX-2) inhibitors. BMC cancer 6:27.

Harris RE, Beebe-Donk J, Alshafie GA. 2007. Reduced risk of human lung cancer by selective cyclooxygenase 2 (COX-2) blockade: results of a case control study. International journal of biological sciences 3:328-334.

Harris RE, Beebe-Donk J, Alshafie GA. 2008. Similar reductions in the risk of human colon cancer by selective and nonselective cyclooxygenase-2 (COX-2) inhibitors. BMC cancer 8:237.

Jacobs EJ, Newton CC, Stevens VL, Gapstur SM. 2011. A large cohort study of long-term acetaminophen use and prostate cancer incidence. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 20:1322-1328.

Jeter JM, Han J, Martinez ME, Alberts DS, Qureshi AA, Feskanich D. 2012. Non-steroidal anti-inflammatory drugs, acetaminophen, and risk of skin cancer in the Nurses' Health Study. Cancer causes & control : CCC 23:1451-1461.

Kehm RD, Hopper JL, John EM, Phillips KA, MacInnis RJ, Dite GS, et al. 2019. Regular use of aspirin and other non-steroidal anti-inflammatory drugs and breast cancer risk for women at familial or genetic risk: a cohort study. Breast cancer research : BCR 21:52.

Kho PF, Fawcett J, Fritschi L, Risch H, Webb PM, Whiteman DC, et al. 2016. Nonsteroidal anti-inflammatory drugs, statins, and pancreatic cancer risk: a population-based case-control study. Cancer causes & control : CCC 27:1457-1464.

Kwan ML, Habel LA, Slattery ML, Caan B. 2007. NSAIDs and breast cancer recurrence in a prospective cohort study. Cancer causes & control : CCC 18:613-620.

Lacey Jr JV, Sherman ME, Hartge P, Schatzkin A, Schairer C. 2004. Medication use and risk of ovarian carcinoma: A prospective study. International journal of cancer 108:281-286.

Lim WY, Chuah KL, Eng P, Leong SS, Lim E, Lim TK, et al. 2012. Aspirin and non-aspirin non-steroidal anti-inflammatory drug use and risk of lung cancer. Lung cancer (Amsterdam, Netherlands) 77:246-251.

Lipworth L, Friis S, Mellemkjaer L, Signorello LB, Johnsen SP, Nielsen GL, et al. 2003. A population-based cohort study of mortality among adults prescribed paracetamol in Denmark. Journal of clinical epidemiology 56:796-801.

Lo-Ciganic WH, Zgibor JC, Bunker CH, Moysich KB, Edwards RP, Ness RB. 2012. Aspirin, nonaspirin nonsteroidal anti-inflammatory drugs, or acetaminophen and risk of ovarian cancer. Epidemiology (Cambridge, Mass) 23:311-319.

Logan RF, Little J, Hawtin PG, Hardcastle JD. 1993. Effect of aspirin and non-steroidal anti-inflammatory drugs on colorectal adenomas: case-control study of subjects participating in the Nottingham faecal occult blood screening programme. BMJ (Clinical research ed) 307:285-289.

Marshall SF, Bernstein L, Anton-Culver H, Deapen D, Horn-Ross PL, Mohrenweiser H, et al. 2005. Nonsteroidal anti-inflammatory drug use and breast cancer risk by stage and hormone receptor status. Journal of the National Cancer Institute 97:805-812.

Meier CR, Schmitz S, Jick H. 2002. Association between acetaminophen or nonsteroidal antiinflammatory drugs and risk of developing ovarian, breast, or colon cancer. Pharmacotherapy 22:303-309.

Moysich KB, Mettlin C, Piver MS, Natarajan N, Menezes RJ, Swede H. 2001. Regular use of analgesic drugs and ovarian cancer risk. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 10:903-906.

Moysich KB, Baker JA, Rodabaugh KJ, Villella JA. 2005. Regular analgesic use and risk of endometrial cancer. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 14:2923-2928.

Murad AS, Down L, Davey Smith G, Donovan JL, Athene Lane J, Hamdy FC, et al. 2011. Associations of aspirin, nonsteroidal anti-inflammatory drug and paracetamol use with PSA-detected prostate cancer: findings from a large, population-based, case-control study (the ProtecT study). International journal of cancer 128:1442-1448.

Muscat JE, Stellman SD, Wynder EL. 1994. Nonsteroidal antiinflammatory drugs and colorectal cancer. Cancer 74:1847-1854.

Neill AS, Nagle CM, Protani MM, Obermair A, Spurdle AB, Webb PM. 2013. Aspirin, nonsteroidal anti-inflammatory drugs, paracetamol and risk of endometrial cancer: a case-control study, systematic review and meta-analysis. International journal of cancer 132:1146-1155.

Nelson JE, Harris RE. 2000. Inverse association of prostate cancer and non-steroidal anti-inflammatory drugs (NSAIDs): results of a case-control study. Oncology reports 7:169-170.

Olsen JH, Friis S, Poulsen AH, Fryzek J, Harving H, Tjonneland A, et al. 2008. Use of NSAIDs, smoking and lung cancer risk. British journal of cancer 98:232-237.

Pandeya N, Olsen CM, Thompson BS, Dusingize JC, Neale RE, Green AC, et al. 2019. Aspirin and nonsteroidal anti-inflammatory drug use and keratinocyte cancers: a large population-based cohort study of skin cancer in Australia. The British journal of dermatology.

Peleg II, Lubin MF, Cotsonis GA, Clark WS, Wilcox CM. 1996. Long-term use of nonsteroidal antiinflammatory drugs and other chemopreventors and risk of subsequent colorectal neoplasia. Digestive diseases and sciences 41:1319-1326.

Peres LC, Camacho F, Abbott SE, Alberg AJ, Bandera EV, Barnholtz-Sloan J, et al. 2016. Analgesic medication use and risk of epithelial ovarian cancer in African American women. British journal of cancer 114:819-825.

Pinheiro SP, Tworoger SS, Cramer DW, Rosner BA, Hankinson SE. 2009. Use of nonsteroidal antiinflammatory agents and incidence of ovarian cancer in 2 large prospective cohorts. American journal of epidemiology 169:1378-1387.

Pinheiro SP, Gates MA, Devivo I, Rosner BA, Tworoger SS, Titus-Ernstoff L, et al. 2010. Interaction between use of non-steroidal anti-inflammatory drugs and selected genetic polymorphisms in ovarian cancer risk. International Journal of Molecular Epidemiology and Genetics 1:320-331.

Platz EA, Rohrmann S, Pearson JD, Corrada MM, Watson DJ, De Marzo AM, et al. 2005. Nonsteroidal anti-inflammatory drugs and risk of prostate cancer in the Baltimore Longitudinal Study of Aging. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 14:390-396.

Rahme E, Ghosn J, Dasgupta K, Rajan R, Hudson M. 2005. Association between frequent use of nonsteroidal anti-inflammatory drugs and breast cancer. BMC cancer 5.

Rosenberg L, Palmer JR, Rao RS, Coogan PF, Strom BL, Zauber AG, et al. 2000. A case-control study of analgesic use and ovarian cancer. Cancer Epidemiology, Biomarkers & Prevention 9:933-937.

Sadeghi S, Bain CJ, Pandeya N, Webb PM, Green AC, Whiteman DC. 2008. Aspirin, nonsteroidal anti-inflammatory drugs, and the risks of cancers of the esophagus. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 17:1169-1178.

Salinas CA, Kwon EM, FitzGerald LM, Feng Z, Nelson PS, Ostrander EA, et al. 2010. Use of aspirin and other nonsteroidal antiinflammatory medications in relation to prostate cancer risk. American journal of epidemiology 172:578-590.

Schildkraut JM, Moorman PG, Halabi S, Calingaert B, Marks JR, Berchuck A. 2006. Analgesic drug use and risk of ovarian cancer. Epidemiology (Cambridge, Mass) 17:104-107.

Setiawan VW, Matsuno RK, Lurie G, Wilkens LR, Carney ME, Henderson BE, et al. 2012. Use of nonsteroidal anti-inflammatory drugs and risk of ovarian and endometrial cancer: the Multiethnic Cohort. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 21:1441-1449.

Sivak-Sears NR, Schwartzbaum JA, Miike R, Moghadassi M, Wrensch M. 2004. Case-control study of use of nonsteroidal antiinflammatory drugs and glioblastoma multiforme. American journal of epidemiology 159:1131-1139.

Stålberg K, Haglund B, Strömberg B, Kieler H. 2010. Prenatal exposure to medicines and the risk of childhood brain tumor. Cancer epidemiology 34:400-404.

Tan XL, Reid Lombardo KM, Bamlet WR, Oberg AL, Robinson DP, Anderson KE, et al. 2011. Aspirin, nonsteroidal anti-inflammatory drugs, acetaminophen, and pancreatic cancer risk: a clinic-based case-control study. Cancer prevention research (Philadelphia, Pa) 4:1835-1841.

Terry MB, Gammon MD, Zhang FF, Tawfik H, Teitelbaum SL, Britton JA, et al. 2004. Association of frequency and duration of aspirin use and hormone receptor status with breast cancer risk. Jama 291:2433-2440.

Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath CW, Jr. 1993. Aspirin use and risk of fatal cancer. Cancer research 53:1322-1327.

Torti DC, Christensen BC, Storm CA, Fortuny J, Perry AE, Zens MS, et al. 2011. Analgesic and nonsteroidal anti-inflammatory use in relation to nonmelanoma skin cancer: a population-based case-control study. Journal of the American Academy of Dermatology 65:304-312.

Trabert B, Ness RB, Lo-Ciganic WH, Murphy MA, Goode EL, Poole EM, et al. 2014. Aspirin, nonaspirin nonsteroidal anti-inflammatory drug, and acetaminophen use and risk of invasive epithelial ovarian cancer: a pooled analysis in the Ovarian Cancer Association Consortium. Journal of the National Cancer Institute 106:djt431.

Trabert B, Poole EM, White E, Visvanathan K, Adami HO, Anderson GL, et al. 2019. Analgesic Use and Ovarian Cancer Risk: An Analysis in the Ovarian Cancer Cohort Consortium. Journal of the National Cancer Institute.

Van Dyke AL, Cote ML, Prysak G, Claeys GB, Wenzlaff AS, Schwartz AG. 2008. Regular adult aspirin use decreases the risk of non-small cell lung cancer among women. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 17:148-157.

Veitonmaki T, Murtola TJ, Maattanen L, Taari K, Stenman UH, Tammela TL, et al. 2014. Prostate cancer risk and nonsteroidal antiinflammatory drug use in the Finnish prostate cancer screening trial. British journal of cancer 111:1421-1431.

Viswanathan AN, Feskanich D, Schernhammer ES, Hankinson SE. 2008. Aspirin, NSAID, and acetaminophen use and the risk of endometrial cancer. Cancer research 68:2507-2513.

Walter RB, Brasky TM, White E. 2011. Cancer risk associated with long-term use of acetaminophen in the prospective VITamins and lifestyle (VITAL) study. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 20:2637-2641.

Wright JL, Chery L, Holt S, Lin DW, Luedeke M, Rinckleb AE, et al. 2016. Aspirin and NSAID use in association with molecular subtypes of prostate cancer defined by TMPRSS2:ERG fusion status. Prostate cancer and prostatic diseases 19:53-56.

Wu AH, Pearce CL, Tseng CC, Templeman C, Pike MC. 2009. Markers of inflammation and risk of ovarian cancer in Los Angeles county. International journal of cancer 124:1409-1415.

Wysong A, Ally MS, Gamba CS, Desai M, Swetter SM, Seiffert-Sinha K, et al. 2014. Non-melanoma skin cancer and NSAID use in women with a history of skin cancer in the Women's Health Initiative. Preventive medicine 69:8-12.

Zhang X, Smith-Warner SA, Collins LC, Rosner B, Willett WC, Hankinson SE. 2012. Use of aspirin, other nonsteroidal anti-inflammatory drugs, and acetaminophen and postmenopausal breast cancer incidence. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 30:3468-3477.

Appendix C Other study designs in animals: Administration of acetaminophen with carcinogens and other modifying factors

Mice

Studies of tumor occurrence: Effects of acetaminophen with carcinogens and other modifying factors

11-month studies in NIH general purpose mice (Weisburger et al. 1973)

Groups of 20-40 male and female six-week-old NIH general purpose Swiss mice were given either control diet or diets containing either 1.1% acetaminophen, 0.05% N-2-fluorenylacetamide (FAA or 2-AAF), 0.0535% N-hydroxy-2-fluorenylacetamide (N-OHAAF, the hydroxylated metabolite of 2-AAF), acetaminophen plus 2-AAF, or acetaminophen plus N-OHAAF for 11 months (Weisburger et al. 1973).

Male mice exposed to acetaminophen alone developed liver and urinary bladder tumors; no tumors were observed in female mice fed acetaminophen alone (see discussion of these studies in Section 3.2.1). 2-AAF alone and N-OHAAF alone induced liver tumors in male and female mice and bladder tumors in male mice. Animals fed diets containing both acetaminophen and 2-AAF (or N-OHAAF) developed fewer tumors than animals exposed to 2-AAF (or N-OHAAF) alone. Sulfonation of 2-AAF (and N-OHAAF) generates N-sulphoxy-AAF, a highly reactive genotoxic carcinogen (Luch 2005). It is possible that co-administration of acetaminophen with 2-AAF (or N-OHAAF) reduced tumor incidences as a result of either competition between acetaminophen and 2-AAF (or N-OHAAF) for phase two sulfotransferase enzymes, or inhibition of these enzymes, resulting in reduced formation of N-sulphoxy-AAF.

30- and 52-week studies in female Swiss mice (Cohen and Bryan 1978)

The effects of acetaminophen, sodium sulfate, and L-methionine on lymphocytic leukemia, urinary bladder carcinomas, and forestomach papillomas induced by *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide (NFTA) were studied in female Swiss mice (Cohen and Bryan 1978). Groups of 30 five-week-old mice were fed control diets or diets containing combinations of acetaminophen, NFTA, sodium sulfate, L-methionine, formic acid, and/or *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) at various doses. Animals received experimental diets for 14 or 33 weeks followed by control diets for 16 or 19 weeks, respectively, and were then sacrificed. No treatment-related increases in tumors were observed in animals receiving acetaminophen alone. Exposure to acetaminophen plus the carcinogen NFTA resulted in a decreased incidence of leukemia compared to animals fed NFTA alone. However, when either sodium sulfate or L-methionine were included in the acetaminophen plus NFTA diet, the tumor

incidences were similar to those in animals fed NFTA alone. Similar trends were observed with forestomach papillomas, and with the carcinogen FANFT and urinary bladder carcinomas. These results suggest that sulfonation plays an important role in the carcinogenicity of NFTA and FANFT, and that acetaminophen interferes with that process by depleting sulfate sources.

25-week study in female A/J mice (Rioux and Castoguay 1998)

The effect of acetaminophen on 4-(methylnitrosamino)-1-(3-pryidyl)-1-butanone (NNK)-induced lung tumorigenesis was assessed in female A/J mice (Rioux and Castoguay 1998). Acetaminophen (99% pure) was fed in the diet to seven-week-old mice for two weeks at a dose of 1520 mg/kg-day. After two weeks, NNK (2.2 mmol/kg-bw) was added to the drinking water. After seven weeks, NNK was removed from the drinking water, and the mice were exposed to only acetaminophen in the diet for an additional 16 weeks, at which time they were sacrificed. Mice given basal diet and tap water served as negative controls, and mice given basal diet and NNK in drinking water for seven weeks served as positive controls. All mice that received NNK alone or NNK with acetaminophen developed lung tumors (29/29; 25/25, respectively).

Studies of pre-neoplastic lesions: Promotion studies

24-week promotion study in male B6C3F1 mice (Hagiwara and Ward 1986)

A single intraperitoneal (i.p.) injection of a highly genotoxic carcinogen, N-nitrosodiethylamine (DEN, 40 mg/kg), was given to three groups of male $B6C3F_1$ mice at four weeks of age (Hagiwara and Ward 1986). Two weeks later, acetaminophen was administered in feed at 5,000 or 10,000 ppm to 30 and 60 mice, respectively, for 22 weeks to investigate the effects of acetaminophen on focal hepatocellular proliferative lesions induced by DEN. Mice given DEN-only served as controls (n=30). Significant increases in the number of eosinophilic and basophilic focal hepatocellular proliferative lesions were observed in mice given DEN plus the higher dose of acetaminophen, compared to mice given only DEN. No liver tumors were observed in any treatment group.

Rats

Studies of tumor occurrence: Promotion studies

32-week study in male F344 rats (Tsuda et al. 1984a)

The effects of acetaminophen on the promotion of liver or kidney tumors initiated by N-ethyl-N-hydroxyethylnitrosamine (EHEN) were examined in a two-stage (initiation and promotion) carcinogenesis model in male F344 rats. Thirty animals (age not specified)

were given 0.1% EHEN as the initiator in drinking water for two weeks. One week after the end of EHEN treatment, animals were fed diets containing 1.3% (13,000 ppm) acetaminophen until study termination. The study also included EHEN-only and acetaminophen-only groups. All animals were sacrificed at the end of week 32. Body weights were significantly decreased in the EHEN plus acetaminophen group and the acetaminophen-only group, compared to animals in the EHEN-only group.

EHEN alone, but not acetaminophen alone, induced hepatocellular carcinomas and preneoplastic liver lesions (gamma-glutamyl transferase [GGT] positive foci) (Table C1). Animals treated with EHEN plus acetaminophen had significantly decreased incidences of hepatocellular carcinomas and GGT positive foci per cm^2 compared to EHEN alone. EHEN alone, but not acetaminophen alone, also induced kidney adenomas (Table C1). Animals treated with EHEN plus acetaminophen had a significantly increased incidence of kidney adenomas or adenocarcinomas combined, compared to EHEN alone. Thus acetaminophen inhibited the progression of pre-neoplastic and neoplastic lesions in the liver, but enhanced development of neoplastic lesions in the kidney. It is possible that acetaminophen induced hepatotoxicity, which affected the development of pre-neoplastic liver lesions, and induced nephrotoxicity which caused tubular injury in the kidneys that affected renal tubular cell proliferation.

Table C1. Liver and kidney tumor incidence[a] in EHEN-treated male F344 rats administered acetaminophen (Tsuda et al. 1984a)

Tumor site and type		EHEN	Acetaminophen	EHEN + Acetaminophen[b]
Liver	**Hepatocellular adenoma**	22/23	0/25	20/27*
	Hepatocellular carcinoma	11/23	0/25	3/27*
Kidney	**Renal cell adenoma**	5/23	0/25	15/27**
	Renal cell adenocarcinoma	0/23	0/25	1/27
	Nephroblastoma	1/23	0/25	0/27

[a] Number of animals with lesion per number of animals with organ examined microscopically, as reported by Tsuda et al. (1984a). The authors did not report the use of an untreated control group.

[b] Tumor incidences with asterisks indicate statistically significant differences compared to the EHEN only group by Student's t-test [conducted by Tsuda et al. (1984a)]. * $p < 0.05$; ** $p < 0.01$

52-week study in male F344 rats (Kurata et al. 1987)

The effects of acetaminophen and its metabolite *para*-aminophenol (PAP) on the promotion of liver or kidney tumors initiated by EHEN were examined in a two-stage carcinogenesis model in male F344 rats. Groups of 25 six-week-old animals were given 0.1% EHEN in drinking water for two weeks. One week after the end of EHEN treatment, rats were fed diets containing 0.8% (8,000 ppm) acetaminophen or 0.8% (8,000 ppm) PAP for 49 weeks. The study also included groups treated with EHEN only, acetaminophen only, or PAP only. All animals were sacrificed at the end of 52 weeks.

As shown in Table C2, treatment with either acetaminophen or PAP alone did not induce liver or kidney tumors. Acetaminophen, but not PAP, significantly decreased the incidence of hepatocellular carcinomas and pre-neoplastic liver lesions (glutathione S-transferase placental form [GST-P] positive foci) induced by EHEN. Acetaminophen and PAP significantly increased the incidence of pre-neoplastic microadenomas and adenomas of the kidney induced by EHEN. One adenocarcinoma was also observed in a rat that received EHEN and PAP.

Table C2. Incidence[a] of pre-neoplastic and neoplastic liver and kidney lesions in EHEN-treated male F344 rats administered acetaminophen or PAP (Kurata et al. 1987)

Tumor site and type		EHEN	Acetaminophen	PAP	EHEN + Acetaminophen	EHEN + PAP[b]
Liver	**GST-P positive foci**	21/21	0/25	0/25	17/23*	21/24
	Hepatocellular carcinoma	15/21	0/25	0/25	8/23*	15/24
Kidney	**Renal cell microadenoma[c]**	5/21	0/25	0/25	15/23**	12/24
	Renal cell adenoma	0/21	0/25	0/25	7/23**	5/24*
	Renal cell adenocarcinoma	0/21	0/25	0/25	0/23	1/24

[a] Number of animals with lesion per number of animals with organ examined microscopically, as reported by Kurata et al. (1987). The authors did not report the use of an untreated control group.
[b] Treatment group tumor incidences with asterisks indicate statistically significant differences compared to the EHEN-only group by Chi-square test [conducted by Kurata et al. (1987)]. * $p < 0.05$; ** $p < 0.01$
[c] Lesions of <0.5 mm in diameter (approximately three times the diameter of a glomerulus) were classified as microadenomas

36-week study in male F344 rats (Kurata et al. 1986)

The potential for acetaminophen to promote urinary bladder tumors initiated by N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) was examined in male F344 rats. In the initiator plus promoter treatment, 25 six-week-old animals were administered 0.05% BBN in drinking water for four weeks then fed a diet containing 1.3% (13,000 ppm) acetaminophen for 32 weeks. The study also included an initiator-only and a promoter-only group. The use of an untreated control group was not reported. All rats were sacrificed at the end of week 36. Body weights in animals administered acetaminophen were significantly decreased compared to the BBN only group at the end of the study.

Acetaminophen alone did not induce urinary bladder tumors, and did not promote or otherwise effect the incidence of BBN-induced urinary bladder tumors.

40-week study in male F344 rats (Shibata et al. 1995)

The promoting effects of acetaminophen on tumors initiated by dihydroxy-di-N-propylnitrosamine (DHPN) and uracil were investigated in male F344 rats. Twenty six-week-old rats were treated with 0.1% DHPN in drinking water and 3.0% uracil in the diet for four weeks. One week later, rats were fed a diet containing 0.8% (8,000 ppm) acetaminophen from week six until the end of the study. The study also include initiator-only (n = 20) and acetaminophen-only (n = 10) groups. All animals were sacrificed at week 40 for histopathological examination.

Acetaminophen alone did not induce tumors (0/10) in any sites reported. DHPN/uracil treatment induced tumors of the kidney, esophagus, lung and thyroid, but not tumors of the liver, urinary bladder or ureter. Acetaminophen increased the incidences of esophageal papilloma (DHPN/uracil, 5/19; DHPN/uracil + acetaminophen, 8/20) and kidney nephroblastoma (DHPN/uracil, 2/19; DHPN/uracil + acetaminophen, 7/20), but the increases were not statistically significant. The authors concluded that acetaminophen did not have tumor promoting activity in DHPN/uracil-initiated carcinogenesis.

Studies of tumor occurrence: Effects of acetaminophen with carcinogens and other modifying factors

44-week study in male F344 rats (Williams and Iatropoulos 1997)

The effect of acetaminophen on 3,2′-dimethyl-4-aminobiphenyl (DMAB)-induced intestinal tumors was studied. Six-week-old male F344 rats (48 animals per group) were fed 0, 250 (low-dose) or 5,000 ppm (high-dose) acetaminophen in the diet for 44 weeks. Two weeks after starting acetaminophen administration, animals were given 50 mg/kg DMAB by subcutaneous (*s.c.*) injection once a week for 20 weeks. The study

also included room control, vehicle control, DMAB-only, and high-dose acetaminophen-only groups. Animals were sacrificed at the end of 44 weeks.

No intestinal tumors were observed in the room control (0/27), vehicle control (0/27) or acetaminophen-only (0/48) groups. DMAB induced tumors of the small and large intestine, including adenomas, small *in situ* carcinomas and large invasive adenocarcinomas. Acetaminophen administration reduced the incidence of DMAB-induced tumors of the small and large intestine (combined) (DMAB alone, 35/48; DMAB plus low-dose ACETAMINOPHEN, 24/48; DMAB plus high-dose ACETAMINOPHEN, 24/48). The authors concluded that acetaminophen inhibited intestinal carcinogenesis induced by DMAB.

40-week study in male F344 rats with partial hepatectomy (Masui et al. 1986)

The effect of acetaminophen on liver carcinogenesis after treatment with diethylnitrosamine (DEN) and 2-AAF, followed by partial hepatectomy, was investigated in male F344 rats. Twenty six-week-old rats were administered 200 mg/kg DEN in 0.9% NaCl solution by a single *i.p* injection. From week two to eight, animals were fed 0.02% 2-AAF in the diet. Animals were subjected to partial hepatectomy three weeks after the DEN injection. From week 12, animals were given diets containing 1% (10,000 ppm) acetaminophen until week 36. The study also included DEN/2-AAF, with partial hepatectomy-only and acetaminophen-only (without partial hepatectomy) groups. All animals were sacrificed at week 40.

No liver neoplastic lesions were reported in the acetaminophen-only group. In the DEN/2-AAF, with partial hepatectomy-only group, 88% (15/17) of the animals developed hepatocellular carcinomas (HCC). Acetaminophen administration after DEN/2-AAF (with partial hepatectomy) treatment reduced hepatocellular carcinomas to 65% (11/17), but the decrease was not statistically significant.

26- and 32-week studies in male F344 rats (Yamamoto et al. 1973)

The effects of acetaminophen on liver carcinogenesis were studied by co-administration with the carcinogen N-hydroxy-2-fluorenylacetamide (N-OHAAF) in male F344 rats in 26- and 32-week studies. Both studies also investigated the potential for the sulfate ester to restore N-OHAAF carcinogenicity by supplementing with sodium sulfate in the diet. In the 26-week study, six-week-old rats were fed diets containing 0.89% (8,900 ppm) acetaminophen and 0.0213% (low-dose) N-OHAAF for 16 weeks, followed by control diets until study termination. An additional group received a diet containing 2.52% (178 mmoles/kg) sodium sulfate along with acetaminophen and N-OHAAF. The study also included untreated control, acetaminophen only, and N-OHAAF-only groups. In the 32-week study, six-week-old rats were fed diets containing 0.89% (8,900 ppm) acetaminophen and 0.032% (high-dose) N-OHAAF for 16 weeks. Animals were then on

the control diet for 16 weeks until study termination. Additional groups received 0.84% (59 mmoles/kg, low-dose) or 2.52% (178 mmoles/kg, high-dose) sodium sulfate along with acetaminophen and N-OHAAF. The study also included acetaminophen-only and N-OHAAF-only groups.

In the 26-week study, no liver tumors were observed in the untreated controls or acetaminophen-alone groups. Low-dose N-OHAAF induced liver hepatomas (assumed to be hepatocellular adenomas) in all animals examined (10/10). Co-administration of N-OHAAF and acetaminophen reduced the incidence of liver hepatomas to 4/20. No tumors were observed in the group that additionally received sodium sulfate (0/20).

In the 32-week study, acetaminophen alone did not induce liver hepatomas (0/6). High-dose N-OHAAF induced liver hepatomas in all animals examined (5/5). Co-administration with acetaminophen reduced the incidence of liver tumors induced by N-OHAAF (6/12) compared to the N-OHAAF-only group. Supplementation with sodium sulfate did not have any additional effect on liver neoplasms (5/6, 4/12 in the low- and high-dose sodium sulfate groups, respectively). Both studies showed that acetaminophen inhibited N-OHAAF-induced liver tumors. The addition of sulfate did not "restore" the carcinogenicity of N-OHAAF, as hypothesized by the authors.

30-week studies in female Charles River Sprague-Dawley-derived (S-D) rats (Weisburger et al. 1973)

The effects of acetaminophen on mammary carcinogenesis induced by 2-AAF or its hydroxylated metabolite N-OHAAF were investigated in female S-D rats in a co-administration study. Six-week-old female rats (20 animals per group) were fed diets containing 1.1% (11,000 ppm) acetaminophen and either 0.025% 2-AAF or 0.027% N-OHAAF for 20 weeks, then switched to control diet for 10 weeks until study termination at 30 weeks. The study also included control, acetaminophen-only, 2-AAF only, and N-OHAAF-only groups.

Animals in the control (0/20) or acetaminophen-only (0/20) groups did not develop mammary tumors. Co-administration of acetaminophen with 2-AAF significantly reduced the incidence of mammary tumors compared to the 2-AAF-only group (2-AAF, 14/20; ACETAMINOPHEN + 2-AAF, 7/20). Co-administration of acetaminophen with N-OHAAF did not affect mammary tumors compared to the N-OHAAF-only group (N-OHAAF, 15/20; ACETAMINOPHEN + N-OHAAF, 13/20).

52-week study in a rat fatty liver and cirrhosis model (Mayuyama et al. 1990)

The effects of acetaminophen on pre-neoplastic lesions and liver tumors were investigated in male F344 rats with fatty liver and cirrhosis in a 52-week study. A choline-deficient (CD) diet was used to induce cirrhosis via fatty liver changes. A choline-supplemented (CS) diet was also administered to some animals in this study. In

the “control” groups, six-week-old rats were fed the CS diet for 27 weeks, then basal diet (BD) containing 0, 0.45% (4,500 ppm), or 0.9% (9,000 ppm) acetaminophen for 25 weeks. In the cirrhotic groups, six-week-old rats were fed the CD diet for 27 weeks, then the BD containing 0, 0.45% or 0.9% acetaminophen for 25 weeks. The study also included CS-only and CD-only groups in which animals were fed the CS or CD diets for 52 weeks.

Rats fed acetaminophen following the CS diet did not develop cirrhosis, pre-neoplastic nodules or hepatocellular carcinomas (0.45% acetaminophen, 0/13; 0.9% acetaminophen, 0/12). All rats on the CD diet developed cirrhosis with pre-neoplastic liver nodules (BD, 12/12; 0.45% acetaminophen, 13/13; 0.9% acetaminophen, 13/13). One hepatocellular carcinoma was observed in the CD-only group (1/7). No hepatocellular carcinomas were associated with acetaminophen treatment with either the CS or CD diets. In cirrhotic livers (CD animals), the high dose of acetaminophen increased hepatic pre-neoplastic lesions (measured as GGT-positive foci), while the low dose of acetaminophen had no affect on the incidence of GGT-positive foci. The authors concluded that acetaminophen did not demonstrate any carcinogenic potential in animals with cirrhosis.

Studies of pre-neoplastic lesions: Effects of acetaminophen with carcinogens and other modifying factors

13- and 18-week studies in male F344 rats with and without partial hepatectomy (Hasegawa et al. 1988)

The effect of acetaminophen on pre-neoplastic liver lesions was investigated in a two-stage model in male F344 rats. In the 13-week study, six-week-old animals were administered a single dose of 500 mg/kg acetaminophen via gavage 24 hours after partial hepatectomy (H) (initiation stage), and one week later, administered 0.1% phenobarbital (PB) in drinking water for 12 weeks (promotion stage). The study also included an initiator-only (acetaminophen + H), promoter-only (PB + H), and no partial hepatectomy (with acetaminophen or PB) groups. Animals were euthanized at 13 weeks. In the 18-week study, six-week-old rats were given 1 g/kg bw acetaminophen by gavage twice a week for five weeks. The promotion stage started from week seven by administration of 0.1% PB in the drinking water for 12 weeks. All animals were euthanized at the end of 18 weeks. The study also included control (vehicle), initiator-only (acetaminophen), and promoter-only (PB) groups.

PB alone, but not acetaminophen alone, induced liver GST-P positive foci in the 13- and 18-week studies. Among rats with partial hepatectomy, a single dose of acetaminophen administration followed by the PB treatment increased GST-P positive foci by number of foci per unit liver area compared to the PB only group, but the difference was not statistically significant. Compared to the PB only group, treatment of rats with repeated

doses of acetaminophen followed by PB significantly increased total number of foci when single cells, mini-foci, and solitary positive hepatocytes were included, but it is not clear if these are indeed preneoplastic lesions. The authors concluded that acetaminophen does not have tumor-initiating activity in rat liver in the 13- or 18-week studies.

Ten-week study in male Crj:CD (SD) rats with partial liver hepatectomy (Omura et al. 2014)

The effect of acetaminophen on pre-neoplastic liver lesions was investigated in a two-stage model in male *Crj:CD (SD)* rats. Six-week-old male rats were treated with 1,000 mg/kg-d acetaminophen in 0.5% methylcellulose suspension for two weeks by daily oral administration, followed by a two-week recovery period. Animals were then exposed to 500 ppm PB in drinking water from week four to ten. One week after PB treatment, all animals were subjected to partial hepatectomy. The study also included control (vehicle), promoter-only (PB), and positive control (DEN + PB) groups. No information on an acetaminophen-alone treatment group was reported. The study was terminated at the end of week ten.

No hepatic GST-P positive foci were observed in the PB-only group. The positive control group showed 100% induction (DEN + PB, 12/12) of GST-P positive foci. One animal in the acetaminophen plus PB group developed GST-P positive foci. The authors concluded that acetaminophen has no tumor initiating activity in the liver of Crj:CD (SD) rats.

Eight-week study in male F344 rats with partial liver hepatectomy (Uehara et al. 1999)

The effect of acetaminophen on promoting pre-neoplastic lesions was investigated using a two-stage model in an eight-week study in male F344 rats. Six-week-old rats were administered a single *i.p.* injection of 200 mg/kg of the tumor initiator DEN. From two to eight weeks, animals were fed diets containing 0.3% acetaminophen (3,000 ppm) as the promotion regimen. All animals were subjected to partial hepatectomy (H) three weeks after the DEN injection. The study also included an initiator only (DEN + H) group. The use of an untreated control or acetaminophen alone group was not reported. Animals were sacrificed at the end of week eight.

The DEN + H treatment induced the formation of GST-P positive foci, measured as number of foci per unit liver area. Acetaminophen treatment after DEN initiation significantly reduced the number of GST-P positive foci in rat liver. The authors concluded that acetaminophen treatment following tumor induction inhibited liver carcinogenesis.

Eight-week studies in different ages of male F344 rats with partial liver hepatectomy (Hasegawa et al. 1991)

The age-related effects of acetaminophen on promoting pre-neoplastic lesions were investigated in male F344 rats using a two-stage model in eight-week studies in male F344 rats. Groups of 6-, 26-, and 46-week-old rats (groups 1, 2, and 3, respectively) were treated with a single *i.p.* injection of 200 mg/kg DEN as the initiator. After two weeks on basal diet, animals were administered 1.3% acetaminophen (13,000 ppm) in feed for six weeks. All animals were subjected to partial hepatectomy (H) three weeks after the DEN injection. The study also included initiator-only (DEN + H) groups for the corresponding ages (groups 4, 5, and 6). The study did not report that there were any acetaminophen-alone groups. Animals were sacrificed at the end of eight weeks. For the DEN plus acetaminophen treatment groups, 22, 12 and 19 animals were reported in groups 1, 2 and 3, respectively. For the initiator only groups, 17, 9 and 18 animals were reported in groups 4, 5 and 6, respectively.

DEN alone induced pre-neoplastic lesions in all three age groups, measured as number and area of GST-P positive foci per unit liver area. The addition of acetaminophen decreased the DEN-induced GST-P foci in rats of all age groups by number and area of GST-P positive foci per unit tissue area. Acetaminophen treatment showed the greatest reduction of DEN-induced GST-P positive foci in the six-week-old rats compared to the 26- and 46-week-old rats.

Eight-week study in male F344 rats with partial liver hepatectomy (Tsuda et al. 1984b)

The potential effect of acetaminophen on promoting pre-neoplastic lesions was investigated using a two-stage model in an eight-week study. Male F344 rats (age not specified) were treated with a single *i.p.* injection of 200 mg/kg DEN then fed basal diets for two weeks. All animals were subjected to partial hepatectomy (H) three weeks after DEN injection. For the promoting stage, animals were fed diets containing 1.3% (13,000 ppm) acetaminophen from week three to eight. The study also included initiator-only (DEN + H) and promoter-only (acetaminophen + H) groups. Animals were sacrificed at the end of week eight.

The initiator-only (DEN + H) treatment induced liver GGT positive foci as measured by number and area per unit tissue area. Acetaminophen + H alone did not induce GGT positive foci. DEN + H with acetaminophen significantly the reduced number and area of GGT positive foci per unit area compared to DEN +H alone. The authors concluded that acetaminophen did not promote but inhibited DEN-induced pre-neoplastic lesion development.

Nine-week study in male F344 rats (Williams et al. 2007)

The effects of acetaminophen on the progression of 2-AAF-induced pre-neoplastic lesions was investigated in male F344 rats. Eight-week-old rats (four animals per group) were fed 2,400 (144 mg/kg-day) or 4,800 ppm (288 mg/kg-day) acetaminophen in the diet for nine weeks. One week after starting acetaminophen treatment, animals were treated with 2.6 mg/kg 2-AAF by gavage three times per week for eight weeks. The experiment was terminated 24 hours after the final 2-AAF dosing, i.e., at the end of nine weeks. The study also included control (vehicle), low-dose acetaminophen-only, high-dose acetaminophen-only and 2-AAF-only groups.

Acetaminophen-only treatments showed similar number of hepatic GST-P positive foci per unit liver area to that of the controls (control, 0.14 /cm^2, low-dose acetaminophen, 0.20 /cm^2; high-dose acetaminophen, 0.22 /cm^2). 2-AAF-only treatment significantly increased GST-P positive foci compared to the control. Treatment with acetaminophen and 2-AAF reduced the number of GST-P positive foci compared to the 2-AAF-only group. The authors concluded that acetaminophen displayed an inhibitory effect on 2-AAF-induced liver pre-neoplastic lesions.

23-day study in male Fischer rats (Hiruma et al. 2001)

The effect of acetaminophen on the progression of aflatoxin B_1 (AFB_1)-induced pre-neoplastic lesions was studied in male F344 rats. Rats were administered 300 or 600 mg/kg acetaminophen in DMSO via *i.p.* injection. Forty-eight hours later, animals were administered 0.5 or 2.5 mg/kg AFB_1 via *i.p.* injection. Four days after AFB_1 administration, all the animals received 0.1% PB in drinking water. The study also included control (vehicle), acetaminophen-only (300 or 600 mg/kg), and AFB_1-only (0.5 or 2.5 mg/kg) groups. Animals were euthanized three weeks after DMSO or AFB_1 administration. The total study duration was 23 days. Each group consisted of four to eight animals.

Treatment with acetaminophen alone did not increase hepatic GST-P positive foci. Treatment with either dose of AFB_1 alone induced GST-P positive foci. Acetaminophen administration with AFB_1 did not affect the induction of GST-P positive foci compared to AFB_1 alone.

Nine-week study in male F344 rats with partial hepatectomy in a fatty liver and cirrhosis model (Maruyama et al. 1990)

The effects of acetaminophen on pre-neoplastic lesions were studied in F344 rats with partial hepatectomy in a nine-week study. Similar to the 52-week study by Maruyama et al. (1990) referenced above, groups of six-week-old F344 rats were fed CS or CD diets for four weeks. Animals from each dietary group were then administered a single gavage dose of 0 (vehicle control), 0.5, 1.0, or 1.5 g/kg acetaminophen or a single *i.p.*

dose of 20 mg/kg DEN. Five rats fed the CD diet died after acetaminophen treatment due to hepatic failure. Four hours later, surviving rats were subjected to partial hepatectomy. After a two-week recovery period, enzyme-altered foci were induced by a diet containing 20 mg/kg 2-AAF for two weeks and a single gavage dose of 1 ml/kg bw carbon tetrachloride (CCl_4). Surviving animals were sacrificed at the end of week nine. In the CS groups, 14, 22, 16 and 14 animals were reported in 0, 0.5, 1.0 and 1.5 g/kg acetaminophen groups, respectively. In the CD groups, 7, 11, 12 and 8 animals were reported in 0, 0.5, 1.0 and 1.5 g/kg acetaminophen group, respectively.

In rats fed the CS diet, acetaminophen did not alter liver GST-P positive foci (except for an increase in the mid-dose group). In rats fed the CD diet, increases in GST-P positive lesions were observed in all groups when compared to the respective CS-treated group. Acetaminophen treatment following the CD diet did not affect the numbers or sizes of GST-P positive foci compared to the CD control group. The authors concluded that acetaminophen did not initiate hepatic pre-neoplastic lesions in rats with fatty liver and cirrhosis.

Hamsters

Studies of tumor occurrence: Effects of acetaminophen with administration of carcinogens

47-week studies of acetaminophen on neoplastic lesions (Weisburger et al. 1973)

The effects of acetaminophen on liver and gastric carcinogenesis induced by 2-AAF or N-OHAAF were investigated in male and female Syrian golden hamsters. Groups of 30 six-week-old animals were fed diets containing 0.04% 2-AAF or 0.043% N-OHAAF and 1.1% (11,000 ppm) acetaminophen for 47 weeks. The study also included control, acetaminophen-only, 2-AAF-only, and N-OHAAF-only groups for males and females. Some hamsters receiving 2-AAF or N-OHAAF only were necropsied after 39 weeks. Remaining hamsters were sacrificed at week 47.

Acetaminophen alone did not induce liver tumors in male or female hamsters. Co-administration of acetaminophen with either carcinogen (2-AAF or N-OHAAF) significantly reduced the incidence of liver tumors (described as “bile duct proliferation; cholangiomas”) compared to corresponding carcinogen-alone treatment in both males and females. Acetaminophen alone, 2-AAF alone or 2-AAF plus acetaminophen did not induce gastric papillomas or gastric “squamous cell carcinomas; adenocarcinomas” in males or females. Acetaminophen increased the incidence of gastric papilloma and decreased the incidences of “squamous cell carcinoma; adenocarcinoma” induced by N-OHAAF. This was significant only in males for papillomas.

Studies of pre-neoplastic lesions: Effects of acetaminophen with administration of carcinogens

23- or 24-day study of acetaminophen on pre-neoplastic lesions in male hamsters (Hiruma et al. 2001)

The effects of acetaminophen pre-treatment on liver pre-neoplastic lesions induced by AFB_1 were investigated in male Syrian golden hamsters. Groups of hamsters were given acetaminophen (0, 300, 600 mg/kg) in DMSO via *i.p.* injection. Animals were then administered the carcinogen AFB_1 (0, 1, or 2 mg/kg) via *i.p.* injection 48 or 72 hours after either DMSO or the acetaminophen pre-treatment. Four days after AFB_1 administration, all animals received 0.1% PB in the drinking water. Animals were sacrificed three weeks after DMSO or AFB_1 administration. The total study duration was 23 or 24 days.

As shown in Table C3, the low-dose acetaminophen-alone group did not differ significantly from the control group in the number of single cell GST-P positive foci. Increases in GST-P positive single hepatocytes were observed in the other treated groups compared to controls, and increases in mini-foci were seen in the high-dose acetaminophen groups with AFB_1 treatment, compared to AFB_1 alone. The authors attributed the hepatic necrosis-mediated compensatory cell proliferation to be responsible for the increase in GST-P positive hepatic foci in hamsters.

Table C3. Acetaminophen pre-treatment on AFB_1-induced liver GST-P positive foci in Syrian golden hamsters[a] (Hiruma et al. 2001)

Treatment	Number of foci/cm^2 (mean ± SD)	
	Single cells	Mini-foci (2-9 cells)
Control (DMSO)	0.5 ± 0.4	0
300 mg/kg APAP 48h	0.7 ± 0.3	0
600 mg/kg APAP 48h	1.4 ± 0.7**[b]	0
600 mg/kg APAP 72h	2.8 ± 2.6***[b]	0
1 mg/kg AFB_1	5.9 ± 2.2***[b]	0
300 mg/kg APAP 48h + 1 mg/kg AFB_1	5.7 ± 2.3***[b]	0
600 mg/kg APAP 72h + 1 mg/kg AFB_1	18.3 ± 12.5***[b]	0.2 ± 0.2***[c]
2 mg/kg AFB_1	26.1 ± 9.7**[d]	0
600 mg/kg APAP 48h + 2 mg/kg AFB_1	121 ± 40***[e]	4.5 ± 1.7***[f]

[a] Each group consisted of six to eight animals. APAP, acetaminophen.

[b] Significantly different compared with control group, as reported by Hiruma et al. (2001).

[c] Significantly different compared with respective data of animals without APAP pretreatment, as reported by Hiruma et al. (2001).

[d] Significantly different compared with control group and respective data of animals with 1 mg/kg AFB_1 dosing, as reported by Hiruma et al. (2001).

[e] Significantly different compared with control group and with respective data of animals with 2.0 mg/kg AFB_1 dosing and without APAP pretreatment, as reported by Hiruma et al. (2001).

[f] Significantly different compared with control group and with respective data of animals treated with 1.0 mg/kg AFB_1 and 600 mg/kg APAP dosing at 72 h, as reported by Hiruma et al. (2001).

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, by multiple pairwise comparisons with the Dunn-Bonferroni adjustment (as reported by Hiruma et al. 2001).

Appendix C References

Cohen SM, Bryan GT. 1978. Effect of p-hydroxyacetanilide, sodium sulfate, and L-methionine on the leukemogenicity of N-[4-(5-nitro-2-furyl)-2-thiazolyl]acetamide. Cancer Research 38:1398-405.

Hagiwara A, Ward JM. 1986. The chronic hepatotoxic, tumor-promoting, and carcinogenic effects of acetaminophen in male B6C3F1 mice. Fundamental and Applied Toxicology 7:376-86.

Hasegawa R, Furukawa F, Toyoda K, Jang JJ, Yamashita K, Sato S, Takahashi M, Hayashi Y. 1988. Study for tumor-initiating effect of acetaminophen in two-stage liver carcinogenesis of male F344 rats. Carcinogenesis. 9:755-9.

Hasegawa R, Takahashi S, Imaida K, Yamaguchi S, Shirai T, Ito N. 1991. Age-dependent induction of preneoplastic liver cell foci by 2-acetylammo-fluorene, phenobarbital and acetaminophen in f344 rats initially treated with diethylnitrosamine. Japanese Journal of Cancer Research 82:293-7.

Hiruma S, Gopalan-Kriczky P, Qin G, Gaughan JP, Lotlikar PD. 2001. Differential effects of acetaminophen pretreatment on hepatic aflatoxin B1-DNA binding, cellular proliferation, and aflatoxin B1-induced hepatic foci in rats and hamsters. Cancer Letters 170:117-24.

Kurata Y, Asamoto M, Hagiwara A, Masui T, Fukushima S. 1986. Promoting effects of various agents in rat urinary bladder carcinogenesis initiated by N-butyl-N-(4-hydroxybutyl)nitrosamine. Cancer Letters 32:125-35.

Kurata Y, Tsuda H, Sakata T, Yamashita T, Ito N. 1987. Reciprocal modifying effects of isomeric forms of aminophenol on induction of neoplastic lesions in rat liver and kidney initiated by N-ethyl-N-hydroxyethylnitrosamine. Carcinogenesis. 8:1281-5.

Luch A. 2005. Nature and nurture - lessons from chemical carcinogenesis. Nat Rev Cancer 5:113-125.

Maruyama H, Takashima Y, Murata Y, Nakae D, Eimoto H, Tsutsumi M, Denda A, Konishi Y. 1990. Lack of hepatocarcinogenic potential of acetaminophen in rats with liver damage associated with a choline-devoid diet. Carcinogenesis. 11:895-901.

Masui T, Tsuda H, Inoue K, Ogiso T, Ito N. 1986. Inhibitory effects of ethoxyquin, 4,4'-diaminodiphenylmethane and acetaminophen on rat hepatocarcinogenesis. Japanese Journal of Cancer Research 77:231-7

Omura K, Uehara T, Morikawa Y, Hayashi H, Mitsumori K, Minami K, Kanki M, Yamada H, Ono A, Urushidani T. 2014. Detection of initiating potential of non-genotoxic carcinogens in a two-stage hepatocarcinogenesis study in rats. Journal of Toxicological Sciences 39:785-94.

Rioux N, Castonguay A. 1998. Prevention of NNK-induced lung tumorigenesis in A/J mice by acetylsalicylic acid and NS-398. Cancer Research 58:5354-60.

Shibata MA, Sano M, Hagiwara A, Hasegawa R, Shirai T. 1995. Modification by analgesics of lesion development in the urinary tract and various other organs of rats pretreated with dihydroxy-di-N-propylnitrosamine and uracil. Japanese Journal of Cancer Research 86: 160–167

Tsuda H, Sakata T, Masui T, Imaida K, Ito N. 1984a. Modifying effects of butylated hydroxyanisole, ethoxyquin and acetaminophen on induction of neoplastic lesions in rat liver and kidney initiated by N-ethyl-N-hydroxyethylnitrosamine. Carcinogenesis. 5:525-31.

Tsuda H, Hasegawa R, Imaida K, Masui T, Moore MA, Ito N. 1984b. Modifying potential of thirty-one chemicals on the short-term development of gamma-glutamyl transpeptidase-positive foci in diethylnitrosamine-initiated rat liver. Gann 75:876-83.

Uehara H, Otsuka H, Izumi K. 1999. Modifying effects of a mixture of acetaminophen, aspirin, dipyrone and ethenzamide on a multiorgan initiation model and its carcinogenicity in male F344 rats. Cancer Letters 135:83-90.

Weisburger JH, Weisburger EK, Madison RM, Wenk ML, Klein DS. 1973. Effect of acetanilide and p-hydroxyacetanilide on the carcinogenicity of N-2-fluorenylacetamide and N-hydroxy-N-2-fluorenylacetamide in mice, hamsters, and female rats. Journal of the National Cancer Institute 51:235-240.

Williams GM, Iatropoulos MJ.1997. Inhibition by acetaminophen of intestinal cancer in rats induced by an aromatic amine similar to food mutagens. European Journal of Cancer Prevention 6:357-62.

Williams GM, Iatropoulos MJ, Jeffrey AM, Duan JD, Perrone CE. 2007. Inhibition by acetaminophen of neoplastic initiation elicited in rat liver by the DNA-reactive hepatocarcinogen N-acetyl-2-aminofluorene. European Journal of Cancer Prevention 16:528-34.

Yamamoto RS, Williams GM, Richardson HL, Weisburger EK, Weisburger JH.1973. Effect of p-hydroxyacetanilide on liver cancer induction by N hydroxy-N-2-fluorenylacetamide. Cancer Research 33:454-7.