OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Proposition 65

Prioritization: Chemicals Identified for Consultation with the Carcinogen Identification Committee

September 2020

California Environmental Protection Agency
Office of Environmental Health Hazard Assessment
Reproductive and Cancer Hazard Assessment Branch

Page Intentionally Left Blank

Table of Contents

Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is proposing seven chemicals or chemical groups for prioritization review by the Carcinogen Identification Committee (CIC), using the prioritization process endorsed by the CIC and adopted by OEHHA in 2004. These chemicals are bisphenol A, chlorpyrifos, coal dust, decabromodiphenyl ether (decaBDE), methyl bromide, perfluorooctane sulfonate (PFOS) and its salts and transformation and degradation precursors, and trifluralin. These chemicals are not proposed for listing at this time. OEHHA is seeking public comment and the CIC's consultation regarding which, if any, of these chemicals should proceed to the next stage of the listing process. The public comment period will end on October 19, 2020.

After receiving advice on priority from the CIC, OEHHA will choose chemical(s) for consideration for potential listing by the CIC at a future meeting.

Part I. 2020 Application of the Prioritization Process to Identify Chemicals for Consultation with the Carcinogen Identification Committee

Introduction

OEHHA's 2004 "Process for Prioritizing Chemicals for Consideration under Proposition 65 by the "State's Qualified Experts" (available at http://oehha.ca.gov/media/downloads/proposition-65/document/finalpriordoc.pdf), describes the process OEHHA follows to identify chemicals for CIC consultation. This process can be briefly summarized as follows:

- OEHHA maintains a tracking database of chemicals that have come to OEHHA's attention through a variety of avenues (e.g., literature searches, suggestions from the CIC, other state programs, the scientific community, or the public) for carcinogenicity evaluation.
- OEHHA identifies chemicals with some evidence of cancer hazard *and* the potential for human exposure in California as "candidate chemicals".
- Hazard data screens are applied to the results of focused literature searches conducted on candidate chemicals.

- Chemicals that pass at least one of the applied data screens are then subjected to a preliminary toxicological evaluation. The preliminary toxicological evaluation entails consideration of the available overall evidence of carcinogenicity (e.g., epidemiology, animal bioassay, other relevant information), but it is of necessity an initial, abbreviated appraisal of the information identified through screening-level literature searches.
- Based on this preliminary toxicological evaluation procedure, OEHHA identifies chemicals or chemical groups for consultation with the CIC.

In this most recent application of the prioritization process, OEHHA applied both a human and an animal data screen to candidate chemicals in its tracking database. OEHHA identified seven chemicals or chemical groups (see Table 1 below) for committee consultation, discussion, and advice.

This document presents information on the seven chemicals or chemical groups. For each of the seven, an initial, abbreviated appraisal of the information identified through the screening-level literature search and the preliminary toxicological evaluation is presented.

At its next meeting, the CIC will provide advice and consultation regarding possible development of hazard identification materials for these chemicals, as described in "Next Steps" below. The following is a description of the process OEHHA conducted that led to the identification of the seven chemicals that will be presented to the CIC.

Chemicals Screened

Under this process, only candidate chemicals (or chemical groups) are screened. These are chemicals in the tracking database with data suggesting that they cause cancer and have exposure potential in California. The evaluation of exposure potential is qualitative, based primarily on production, use or monitoring data.

OEHHA applied both a human and an animal data screen to candidate chemicals in the tracking database as of June 2020. Chemicals meeting either the human epidemiology or animal data screen are subjected to preliminary toxicological evaluation.

Chemicals that are candidates for listing via an administrative mechanism were not screened.

Applying the Epidemiology Data Screen

The epidemiology data screen was applied to candidate chemicals. The screen entails the identification of chemicals with epidemiological studies suggesting evidence of

carcinogenicity. The screen involves finding relevant epidemiology studies through a literature search and evaluating them to identify studies reporting an association between exposure to the chemical and increased cancer risk. More weight was given to analytical studies, and less weight to descriptive studies and case reports. Single case reports were not sufficient to satisfy the screen. For those chemicals with studies available, the studies were reviewed to determine whether there was a positive report of cancer associated with exposure to the chemical. The studies were further reviewed to determine whether the cancer effect might be attributed to the chemical with some confidence.

For each chemical, the steps used in applying the epidemiology data screen were as follows:

1. The chemical's Chemical Abstracts Service (CAS) Registry Number and synonyms were identified using the US EPA Chemical Dashboard (https://comptox.epa.gov/dashboard).
2. The chemical identifiers were used in a search of the literature, using PubMed (https://pubmed.ncbi.nlm.nih.gov). The search included cancer-related search terms entered into PubMed. Further refinement of the search was performed if necessary (e.g., enormous volume of articles returned).
3. Epidemiological studies were identified from the titles retrieved in the online search.
4. Abstracts of the identified articles were reviewed for relevance to the possible finding of cancer in humans exposed to the chemical. The full article was retrieved if the study appeared relevant upon review of the abstract. For articles lacking abstracts, copies of those with titles suggesting possible relevance were requested for review.
5. All articles identified as potentially relevant were considered in assessing whether evidence existed of human cancer related to exposure to the chemical.

Applying the Animal Data Screen

After the epidemiology data screen, OEHHA applied the animal data screen to candidate chemicals. The animal data screen is based on "positive" bioassays and involved finding relevant animal cancer bioassays through a literature search and evaluating them with regard to the screening criteria. A positive animal cancer bioassay is a study in which a treatment-related increase in the incidence of malignant or combined malignant and benign tumors was reported in a given tissue or organ, or for a given type of tumor (e.g., hemangiosarcoma). An increased incidence is either statistically significant ($p < 0.05$) by pairwise comparison with controls or by trend test, or biologically significant (e.g., an increased incidence of a rare tumor type).

The animal screen identified chemicals with:

- Two or more positive animal cancer bioassays;
- One positive animal cancer bioassay with findings of tumors at multiple sites or with malignant (or combined malignant and benign) tumors occurring to an unusual degree with regard to incidence, site, type of tumor or age at onset;
- One positive animal cancer bioassay and evidence from a second animal cancer bioassay of benign tumors of a type known to progress to malignancy.

For each chemical, the steps used in applying the animal data screen were as follows:

1. The chemical identifiers were used in a search of the literature, using PubMed (https://pubmed.ncbi.nlm.nih.gov). The search included cancer-related search terms entered into PubMed. Further refinement of the search was performed if necessary (e.g., enormous volume of articles returned). Searches of PubChem and other databases were also conducted, as appropriate.
2. Animal cancer bioassays were identified from the titles retrieved in the online search.
3. Abstracts of the identified articles were reviewed. The full article was retrieved if the abstract indicated that animal cancer bioassay findings were presented or discussed in the article. For articles lacking abstracts, copies of those with titles suggesting possible relevance were requested for review.
4. All articles identified as potentially relevant were considered in assessing whether the animal data screen employed in this round of prioritization had been met for the chemical in question.

Preliminary Toxicological Evaluation

OEHHA conducted a preliminary toxicological evaluation of chemicals identified through application of the human and animal data screens. For these chemicals, OEHHA reviewed results of a further search of the literature designed to identify additional information relevant to carcinogenicity, such as studies on key characteristics of carcinogens (IARC 2019; Smith et al. 2016), metabolism, and pharmacokinetics. This additional information was used to conduct a preliminary evaluation of the overall evidence of carcinogenicity for each of the chemicals identified by the data screens. Chemicals for which a preliminary evaluation of the overall evidence indicated that carcinogenicity may be a concern have been proposed here for CIC consideration. Chemicals previously brought for consultation are not brought back to the CIC unless additional human or animal data indicative of a carcinogenicity concern are identified.

Chemicals Proposed for CIC Consideration

OEHHA identified the seven chemicals or chemical groups listed in Table 1 below for possible preparation of hazard identification materials. At its next meeting, the CIC will provide OEHHA with advice on the prioritization of these chemicals for possible preparation of hazard identification materials.

Table 1. Chemicals Identified through Prioritization and Proposed for Consideration by the CIC

Chemical	CAS Registry Number
Bisphenol A	80-05-7
Chlorpyrifos	2921-88-2
Coal dust	Not applicable
Decabromodiphenyl ether (DecaBDE)	1163-19-5
Methyl bromide	74-83-9
Perfluorooctane sulfonate (PFOS) and its salts and transformation and degradation precursors	1763-23-1 for PFOS[1]
Trifluralin	1582-09-8

[1] There are a number of PFOS salts and precursors. Their CAS Registry Numbers are not provided here.

For each of the chemicals, OEHHA has compiled a separate summary of the relevant studies that were identified during the preliminary toxicological evaluation; these summaries are presented later in this document. An overview of the exposure characteristics and types of studies providing evidence relevant to carcinogenicity for each of these chemicals is presented in Table 2, below.

Table 2. Chemicals for CIC Consultation: Exposure Characteristics and Types of Studies Providing Evidence Relevant to Carcinogenicity

Chemical	Exposure				Human Data				Animal Data					Other Relevant Data		
	Widespread	High in frequent consumers	Limited / occupational	High in infrequent consumers	Analytical	Descriptive	Analytical: mixed / poorly defined exposure	Case series/ reports	Two or more studies	One study w/ unusual incidence, site/type, age at onset	One study and second study with benign tumors only	One study	Tumor initiation / promotion or co-carcinogenicity studies	key characteristics of carcinogens (KCs)[1]	Carcinogenic metabolites	Structural similarity with P65 carcinogens
Bisphenol A	✓	✓			✓	✓			✓				✓	1-6, 8-10	✓	✓
Chlorpyrifos	✓				✓							✓		2, 4, 5, 6, 8, 10		
Coal dust			✓		✓				✓					2, 5, 6, 9		
DecaBDE	✓				✓				✓					2, 5, 8, 10		✓
Methyl bromide			✓		✓				✓[2]					1-5, 7		✓
PFOS and its salts and transformation and degradation precursors	✓				✓				✓				✓	2, 4-10		✓
Trifluralin			✓		✓				✓					2, 3, 5, 8		✓

[1] KCs, key characteristics of carcinogens (Smith et al. 2016; IARC 2019). KC1: Is electrophilic or metabolically activated; KC2: Is genotoxic; KC3: Alters DNA repair or causes genomic instability; KC4: Induces epigenetic alterations; KC5: Induces oxidative stress; KC6: Induces chronic inflammation; KC7: Is immunosuppressive; KC8: Modulates receptor-mediated effects; KC9: Causes immortalization; KC10: Alters cell proliferation, cell death or nutrient supply.

[2] Danse et al. (1984) reported that methyl bromide increased forestomach tumors in two studies in male and female rats, but these tumor findings were later questioned by others. US EPA (1989) reported, "A panel of NTP scientists reevaluated the histological slides and concluded that the lesions were hyperplasia and inflammation rather than neoplasia."

Next Steps

The CIC will consider the chemicals in Table 1 at its next meeting, providing advice and consultation regarding possible development of hazard identification materials by OEHHA. Written public comments received by OEHHA will be provided to the CIC for consideration. The public is also given the opportunity at the CIC meeting to comment on the chemicals being proposed for hazard identification materials preparation.

The CIC may also suggest chemicals other than these seven for which hazard identification materials should be prepared. The CIC can provide informal advice to OEHHA concerning which chemicals should be brought back for their consideration for listing.

OEHHA will then choose which chemical(s) to prepare hazard identification materials summarizing the available scientific evidence on the chemicals' carcinogenic potential following a comprehensive search and evaluation of the scientific literature. These materials will be provided to the CIC, and released for public comment, prior to the public meeting at which the CIC deliberates on a listing decision.

Further details on prioritization, the development of hazard identification materials and CIC consideration of the listing of chemicals under Proposition 65 are given in OEHHA (2004).

References cited in Part I

International Agency for Research on Cancer (IARC). 2019. Preamble. IARC Monographs on the Identification of Carcinogenic Hazards to Humans. Available at: https://monographs.iarc.fr/wp-content/uploads/2019/07/Preamble-2019.pdf

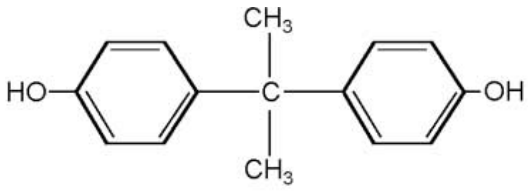
Office of Environmental Health Hazard Assessment (OEHHA). 2004. Process for Prioritizing Chemicals for Consideration under Proposition 65 by the "State's Qualified Experts". California Environmental Protection Agency, OEHHA, Sacramento, CA, December 2004. Available online at: http://oehha.ca.gov/media/downloads/proposition-65/document/finalpriordoc.pdf

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. Environ Health Perspect. 124(6):713-721. doi:10.1289/ehp.1509912

Part II. Chemicals Identified for Consultation with the Carcinogen Identification Committee

Bisphenol A (BPA)

(4,4'-isopropylidenediphenol, BPA; CAS No. 80-05-7)



Bisphenol A (BPA) has numerous uses, including in the manufacture of polycarbonate plastics, and some polyvinyl chloride (PVC) plastics, epoxy resins, thermal papers, dental sealants, and adhesives. Exposures to BPA can result from the use of polycarbonate plastic tableware, cookware, water bottles, water cooler bottles, and food storage containers, from the use of some PVC plastic wrap and gloves, from the use of some epoxy resins to line metal jar lids and bottle caps, from some cash register, gas pump, and automated teller machine receipts, and from some dental sealants and adhesives. In 2018, US production of BPA was approximately 2.3 billion pounds[1] (Cornwall 2020). Recent Biomonitoring California studies indicate that human exposure to BPA is widespread[2].

BPA passed the animal data screen in 2011 and was brought to the Carcinogen Identification Committee (CIC) for consultation. At that time, the CIC recommended that BPA be placed in the 'medium' priority group for development of hazard identification materials. Since 2011, additional epidemiology data, animal carcinogenicity data, and mechanistic data have become available. In 2020, BPA passed both the human and the animal data screens, underwent a preliminary toxicological evaluation, and is being brought again to the CIC for consultation. This is a summary of the relevant studies identified during the preliminary toxicological evaluation. Studies identified since consultation with the CIC in 2011 are marked with an asterisk (*).

[1] From www.statista.com, Results for Bisphenol A (BPA) (accessed June 24, 2020)
[2] From Biomonitoring California, Results for Bisphenol A (BPA) (accessed June 24, 2020)

Epidemiological data

- Breast cancer
    - *Population-based case-control study of the association of BPA with breast cancer incidence and mortality in the Long Island Breast Cancer Study Project: Parada et al. (2019)
        - Exposure assessed through spot urine samples collected within three months of first diagnosis of breast cancer.
        - No significant difference in levels between women with and without breast cancer (p = 0.13).
        - No increased risk of breast cancer in highest quintile (3.63-388 micrograms per gram (µg/g) creatinine) compared to lowest quintile (<0.95) (odds ratio (OR), 0.91; 95% confidence interval (CI), 0.80–1.02; 128 cases).
        - After median follow-up of 17.6 years, no increased risk of breast cancer-specific mortality (OR, 0.93; 95% CI, 0.50–1.73; 18 deaths).
    - *Population-based case-control study of the association of urinary BPA with breast cancer and blood methylation profiles in women in Taiwan: Yang et al. (2018)
        - Exposure assessed through spot urine samples.
        - Significantly higher concentration of BPA in breast cancer cases compared to controls (p = 0.033).
        - BPA levels not associated with altered methylation status or ADAM33 expression (a gene for a transmembrane glycoprotein that mediates changes in cell adhesion and plays a role in cancer progression).
    - *Population-based case-control study of urinary BPA-glucuronide (BPA-G) and postmenopausal breast cancer in Poland: Trabert et al. (2014)
        - Exposure assessed through one-time collection of overnight 12-hour (h) urine samples.
        - Increased risk of breast cancer in 2nd quartile (2.06–4.16 nanogram (ng) BPA-G/mg creatinine) compared to lowest quartile (<2.06 ng/mg) (OR, 1.70; 95% CI, 1.15–2.52; 176 cases).
        - Risk of breast cancer not increased for highest quartile (>7.80 ng/mg) compared to lowest quartile (<2.06 ng/mg) (OR, 1.09; 95% CI, 0.73–1.63; 128 cases).
        - Higher risks observed with estrogen receptor (ER)-negative breast cancer than with ER-positive cases.

- *Population-based case-control study of occupational exposure to estrogenic chemicals and risk of breast cancer in Cape Cod: Aschengrau et al. (1998)
    - Assessed exposure through data from job history (National Institute for Occupational Safety and Health (NIOSH) survey, chemical production and usage information, and expert judgment of industrial hygienist).
    - No association of exposure to any BPA with risk of breast cancer (OR, 0.8; 95% CI, 0.5–1.4).
- *Cross-sectional study of BPA in breast adipose tissue of breast cancer cases and controls: Reeves et al. (2018)
    - Measured BPA in breast adipose tissue from women undergoing mastectomy for breast cancer treatment (“cases”) or elective reduction mammoplasty (“controls”).
    - No significant difference in BPA levels between cases and controls.
- *Cross-sectional study of BPA and breast cancer with National Health and Nutrition Examination Survey (NHANES) data: Morgan et al. (2017)
    - Exposure assessed through urinary sample.
    - Risk of breast cancer not significantly associated with higher BPA levels (0.42-1.23 ng/mg creatinine adjusted) compared to lower levels (<0.42 ng/mg) (OR, 0.76; 95% CI, 0.45–1.30) in model adjusted for age and race/ethnicity.
- *Cross-sectional study of BPA levels and breast cancer in South Korea: Yang et al. (2009)
    - Exposure assessed through single blood sample.
    - No significant difference in blood BPA levels between cases and controls (p = 0.42)
    - Authors note that there was a negative association of BPA levels with ‘age at first birth’, which is a risk factor for breast cancer in this study; this interaction may attenuate the effects of BPA on breast cancer.

- Lung cancer

    - *Hospital-based case-control study of BPA and interaction with rs2046210 (a breast cancer susceptible locus upstream of ESR1 and involved in estrogen signaling) polymorphism on risk of non-small cell lung cancer (NSCLC) in China: Li et al. (2020)
        - Exposure assessed through single urinary BPA measurement within 24 hours after subjects admitted to hospital.

        - Increased risk of NSCLC in highest exposed quartile (Q4) (>1.32 µg/g creatinine) compared to lowest quartile (Q1) (≤0.39 µg/g) (OR, 1.91; 95% CI, 1.39–2.62, 257 cases; P_{trend} <0.001), adjusted for age, sex, smoking, drinking, Body Mass Index (BMI).
        - Significantly increased risk in people with the rs2046210 variant A allele (genotype GA or AA) for Q4 vs Q1 (OR, 3.02; 95% CI, 1.89–4.83, 132 cases) but not GG wild-type (OR, 1.68; 95% CI, 0.94–3.01, 69 cases) ($P_{interaction}$ <0.05).
    - *Cross-sectional study of lung cancer and BPA levels in the Korean Cancer Prevention Study II: Pamungkas et al. (2016)
        - BPA measured in serum samples from 70 healthy people and 35 lung cancer patients.
        - BPA was statistically significantly elevated in lung cancer subjects compared to controls (p < 0.05).

- Prostate cancer

    - *Hospital-based case-control study of environmental BPA and prostate cancer in Hong Kong: Tse et al. (2018); Tse et al. (2017)
        - Exposure assessed through a "cumulative BPA exposure index" (CBPAI) based on amount of use of food or beverage containers from a questionnaire. CBPAI = Σ(BPA intensity^2 × frequency of use × net years of using specific container).
        - Increased risk of prostate cancer in middle tertile compared to lowest tertile (OR, 1.54; 95% CI, 1.05–2.26; 232 cases) and highest tertile compared to lowest tertile (OR, 1.57; 95% CI, 1.01–2.44; 124 cases; p_{trend} = 0.057) in the fully adjusted model.
    - *Hospital-based cross-sectional study of BPA levels and prostate cancer in men in Ohio: Tarapore et al. (2014)
        - Exposure assessed through single urine sample collected at biopsy
        - Levels of BPA significantly higher in prostate cancer patients than non-prostate cancer patients (p = 0.012). The difference was more significant in patients < 65 years old (p = 0.006).

- Brain cancer

    - *Hospital-based case-control study of the association of BPA exposure with meningioma in China: Duan et al. (2013)
        - Exposure assessed through single urinary BPA measurement

        - Controls were healthy men and women participating in routine physical exams (referred to by the authors as “health examinations”) at the same hospital
        - Increased risk of meningioma in highest exposed quartile (>1.69 ng/ml) compared to lowest quartile (<0.53 ng/ml) (OR, 1.57; 95% CI, 1.12–2.09; 18 cases; $P_{trend} = 0.003$).
        - Association was independent of BMI and independent of hormone replacement therapy (HRT).

- Bone cancer

    - *Hospital-based case-control study of the interactive effect of BPA exposure with a polymorphism (-22G>C in LOX [lysyl oxidase] gene) on the risk of osteosarcoma in China: Jia et al. (2013)
        - Exposure assessed through a single measurement of urinary BPA.
        - Overall osteosarcoma
            - Increased risk overall (OR, 1.41; 95% CI, 1.01–1.72, 63 cases) in those exposed to ≥7.01 micromole per mole (µmol/mol) creatinine compared to <7.01 µmol/mol creatinine (median value).
            - Increased risk with wild-type GG genotype (OR, 1.37; 95% CI, 1.00–7.15, 42 cases) in those exposed to ≥7.01 µmol/mol creatinine.
            - Increased risk with GC or CC polymorphism (OR, 1.48; 95% CI, 1.06–7.37, 21 cases) in those exposed to ≥7.01 µmol/mol creatinine.
            - Significant interaction with BPA level and -22G>C polymorphism ($P_{interaction} = 0.036$).
        - Osteosarcoma of the knee
            - Overall (OR, 1.66; 95% CI, 1.14–2.49, 36 cases)
            - GG (OR, 1.38; 95% CI, 1.01–7.21, 20 cases)
            - GC or CC (OR, 1.72; 95% CI, 1.23–2.24, 16 cases)
            - Significant interaction ($P_{interaction} = 0.024$)
        - Osteosarcoma of the hip
            - Overall (OR, 2.00; 95% CI, 1.30–3.17, 22 cases)
            - GG (OR, 1.67; 95% CI, 1.13–2.12, 12 cases)
            - GC or CC (OR, 2.40; 95% CI, 1.45–3.36, 10 cases)
            - Significant interaction ($P_{interaction} = 0.017$)

- Thyroid cancer
    - *Hospital-based cross-sectional study of BPA levels in patients with thyroid cancer or benign thyroid nodules in Italy: Marotta et al. (2019)
        - Exposure assessed through single blood serum measurement.
        - Levels of BPA were not significantly higher in patients with differentiated thyroid cancer compared to patients with benign thyroid nodules (unadjusted OR, 3.71; 95% CI, 0.67–20.34; p = 0.142). Multivariate adjusted analysis not reported for BPA.
        - Authors conclude that BPA exposure was not associated with progression of thyroid nodules to thyroid cancer.
- Endometrial cancer
    - *Cross-sectional study of BPA levels and endometrial hyperplasia and cancer in Japan: Hiroi et al. (2004)
        - Exposure assessed through single serum BPA measurement.
        - 11 healthy women, 10 with simple endometrial hyperplasia, 9 with complex endometrial hyperplasia, 7 with endometrial carcinoma.
        - Significantly lower BPA levels in women with endometrial cancer or complex endometrial hyperplasia, compared to control women (p < 0.05) or women with simple endometrial hyperplasia (p < 0.01).

Animal carcinogenicity data

- Long-term feeding studies in rats
    - 103-week studies in male and female Fisher 344 rats: NTP (1982)
        - Increase in leukemia (by pairwise comparison and trend), testicular interstitial cell tumors (by pairwise comparison and trend), and mammary gland fibroadenomas (by trend) in males (Table 3).
        - No treatment-related tumor findings in females.

Table 3. Tumor incidence in male Fisher 344 rats exposed to BPA in feed for 103 weeks (NTP 1982)

Organ	Tumor type	Concentration in feed (ppm)			Exact trend test *p*-value
		0	1000	2000	
Hematopoietic system	Leukemia	13/50	12/50	23/50*	$p = 0.021$
Mammary gland	Fibroadenoma	0/50	0/50	4/50	$p = 0.0114$
Testis	Interstitial-cell tumor	35/49	48/50***	46/49**	$p < 0.001$

Tumor incidence is expressed as the number of rats with the specified neoplastic lesion over the number of rats examined at the site. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Exact trend test conducted by OEHHA.

- Long-term feeding studies in mice
    - 103-week studies in male and female B6C3F$_1$ mice: NTP (1982)
        - Increase in pituitary chromophobe carcinoma (by trend), lymphoma (by pairwise comparison) in males (Table 4).
        - No treatment-related tumor findings in females.

Table 4. Tumor incidence in male B6C3F$_1$ mice exposed to BPA in feed for 103 weeks (NTP 1982)

Organ	Tumor type	Concentration in feed (ppm)			Exact trend test *p*-value
		0	5000	10,000	
Hematopoietic system	Lymphoma	2/49	8/50*	3/50	NS
Pituitary	Chromophobe carcinoma	0/37	0/36	3/42	$p < 0.05$

Tumor incidence is expressed as the number of rats with the specified neoplastic lesion over the number of rats examined at the site. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

- Long-term perinatal and chronic gavage studies in rats
    - *One-year interim continuous-dose studies (exposure *in utero* and via gavage from postnatal day [PND] 1 till one year of age) in F1 male and female Sprague-Dawley (S-D) rats: NTP (2018)
        - BPA administered by daily oral gavage to F0 dams from gestational day (GD) 6 through parturition and then by daily oral gavage to pups (F1) from PND 1 until termination at one year.
        - No treatment-related tumor findings in males.
        - Increase in uterine stromal polyps in females (by trend) (Table 5).

Table 5. Tumor incidence in female F1 S-D rats exposed to BPA during gestation and via gavage from PND 1 until one year of age (NTP 2018)

Organ	Tumor type	Dose (gavage) (µg/kg bw/day)						Exact trend test *p*-value
		0	2.5	25	250	2500	25000	
Uterine	Stromal polyps	1/23	0/22	1/21	0/24	3/20	3/24	$p < 0.05$

Tumor incidence is expressed as the number of rats with the specified neoplastic lesion over the number of rats examined at the site. Fisher pairwise comparison and exact trend test conducted by OEHHA.

- *One-year interim stop-dose studies (exposure *in utero* and via gavage from PND 1 through PND 21) in F1 male and female S-D rats: NTP (2018)
    - BPA administered by daily oral gavage to F0 dams from GD 6 through parturition and then by daily oral gavage to pups (F1) from PND1 to PND21. The F1 rats were then held without further treatment until termination at one year of age.
    - No treatment-related tumor findings in males.
    - No treatment-related tumor findings in females.

- *Two-year continuous-dose studies (exposure *in utero* and via gavage from PND 1 till two years of age) in F1 male and female S-D rats: NTP (2018)
    - BPA administered by daily oral gavage to F0 dams from GD 6 through parturition and then by daily oral gavage to pups (F1) from PND1 until termination at two years of age.
    - No treatment-related tumor findings in males.
    - No treatment-related tumor findings in females.

- *Two-year stop-dose studies (exposure *in utero* and via gavage from PND 1 through PND 21) in F1 male and female S-D rats: NTP (2018)
    - BPA administered by daily oral gavage to F0 dams from GD 6 through parturition and then by daily oral gavage to pups (F1) from PND1 to PND21. F1 rats were then held without further treatment until termination at two years of age.
    - Increases in lymphoma in each of these sites: liver, bone marrow, spleen, kidney, dorsal/lateral prostate, and in systemic lymphoma in males (by trend) and in dorsal/lateral prostate lymphoma at the highest dose (by pairwise comparison) in males (Table 6).
    - Increases in mammary gland adenocarcinoma and the combination of adenoma and adenocarcinoma at the lowest dose tested (2.5 µg/kg bw/day) (by pairwise comparison) in females (Table 7).
        - No treatment-related increase in lymphoma in females.

Table 6. Tumor incidence in male F1 S-D rats exposed to BPA during gestation and via gavage from PND1 to PND21 and then held without further treatment until termination at two years of age (NTP 2018)

Organ	Tumor type	Dose (gavage) (µg/kg bw/day)						Poly-3 trend test *p*-value
		0	2.5	25	250	2500	25000	
Liver	Lymphoma	1/37.5	0/33.4	1/35.6	3/32.9	2/37.4	5/30.0	$p < 0.01$
Prostate	Lymphoma	0/35.1	0/33.4	0/34.8	3/32.9	2/37.4	4/29.1*	$p = 0.002$
Bone marrow	Lymphoma	1/36.1	0/32.6	0/34.3	3/32.9	2/37.4	5/29.7	$p < 0.01$
Spleen	Lymphoma	1/36.1	0/32.6	1/35.4	3/32.5	2/37.4	5/29.7	$p < 0.01$
Kidney	Lymphoma	1/37.5	0/33.4	1/35.6	3/32.9	2/37.4	5/29.7	$p < 0.01$
Systemic	Lymphoma	1/37.5	0/33.4	1/35.6	3/32.9	2/37.4	5/30.0	$p < 0.01$

The survival of the treated animals was more than 15% lower than the control animals by week 71; therefore, the tumor incidences presented in the table are survival-adjusted poly-3 incidences reported by NTP. Pairwise comparisons and overall trends were assessed by NTP using a variance- and continuity-corrected poly-3 trend test. Treatment group tumor incidences with asterisks indicate significant results from pairwise comparison with controls: * $p < 0.05$.

Table 7. Tumor incidence in female F1 S-D rats exposed to BPA during gestation and via gavage from PND1 to PND21 and then held without further treatment until termination at two years of age (NTP 2018)

Organ	Tumor type	Dose (gavage) (µg/kg bw/day)						Exact trend test *p*-value
		0	2.5	25	250	2500	25000	
Mammary gland	Adenocarcinoma	3/50	11/50*	5/48	7/49	9/50	5/46	NS
	Adenoma or adenocarcinoma	4/50	12/50*	5/48	9/49	9/50	6/46	NS

Tumor incidence is expressed as the number of rats with the specified neoplastic lesion over the number of rats examined at the site. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

- Perinatal exposure studies
    - *Liver tumor studies in F1 male and female isogenic Agouti $^{+/-}$ C57BL/6J: C3H/HeJ mice exposed to BPA in diet during gestation, via lactation and in feed until 10 months old; evaluation at age 10 months: Weinhouse et al. (2014)
        - No treatment-related tumor findings in males.
        - Increase in liver adenoma and carcinoma (combined) (by pairwise comparison and trend) in female offspring (Table 8).

Table 8. Tumor incidence in female F1 isogenic Agouti $^{+/-}$ C57BL/6J: C3H/HeJ mice exposed to BPA during gestation, via lactation, and in feed until 10 months; tumors were evaluated at age 10 months (Weinhouse et al. 2014)

Organ	Tumor type	Concentration in feed (mg/kg)				Exact trend test *p*-value
		0	50 x 10^{-6}	50 x 10^{-3}	50	
Liver	Adenoma	0/9	0/10	0/10	1/9	NS
	Carcinoma	0/9	2/10	1/10	3/9	NS
	Adenoma or carcinoma	0/9	2/10	1/10	4/9*	$p = 0.018$

Tumor incidence is expressed as the number of mice with the specified neoplastic lesion over the number of mice examined at the site. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

- Prenatal exposure studies
    - Mammary tumor study in female Wistar-Furth rats; evaluation at age 110 days: Murray et al. (2007)
        - Increase (non-significant) in mammary gland carcinoma in situ (BPA vs control; 2/6 vs 0/6) in female offspring.
    - Reproductive system tumor study in female CD-1 mice; evaluation at age 18 months: Newbold et al. (2009)
        - Increase (non-significant) in reproductive system tumors.
- Postnatal exposure studies
    - Prostate tumor study - subcutaneous (*s.c.*) injection of newborn male S-D rats, evaluation at age 28 weeks: Ho et al. (2006)
        - Increase (non-significant) in prostatic intraepithelial neoplasia (PIN) (10 µg/kg BPA vs control; 2/6 vs 1/9).

- Co-carcinogenicity studies

  Mammary tumor studies

  - Prenatal exposure of female Wistar rats plus *N*-nitroso-*N*-methylurea (NMU) exposure at age 50 days; evaluation at age 180 days: Durando et al. (2007)
    - Increase in mammary ductal hyperplasia and carcinoma (by pairwise comparison).
  - Prenatal exposure of female FVB/N mice, plus two single gavage of dimethylbenzanthracene (DMBA) at age 5 and 6 weeks; evaluation at age 110 weeks: Weber Lozada and Keri (2011)
    - Increase in squamous cell carcinoma of mammary gland (by pairwise comparison).
  - Prenatal exposure of female Charles River SD rats, plus single gavage of DMBA on day 50 or 100; evaluation at age 12 months: Betancourt et al. (2010)
    - Increase in mammary carcinomas (by pairwise comparison).
    - Modulation of biomarkers potentially related to mammary carcinogenesis (SRCs, PR-A, Bcl-2, estrogen receptor-α, epidermal growth factor receptor, phospho-insulin-like growth factor 1 receptor, and phospho-Raf).
  - Neonatal and prepubertal exposure of female Charles River SD rats plus DMBA exposure at age 50 days; evaluation at age 12 months: Jenkins et al. (2009)
    - Increase in mammary carcinoma (by pairwise comparison and trend).
    - Modulation of biomarkers potentially related to mammary carcinogenesis (steroid receptor coactivators (SRCs), Akt, phosphorylated Akt, progesterone receptor A and erbB3 proteins) (by pairwise comparison).

  Uterine tumor studies

  - Prenatal and neonatal exposure of female Crj:Donryu rats, plus single intra-uterine administration of N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) at age week 11; evaluation at age 15 months: Yoshida et al. (2004)
    - No treatment-related uterine cancer findings.

Prostate tumor studies

- *Prenatal and neonatal exposure of male S-D rats with 2.5 µg BPA by daily oral gavage from GD 6 through parturition (F0) and then by daily oral gavage to F1 male S-D rats from PND1 to PND21, plus estradiol and testosterone implanted on PND90; evaluation at one year of age: Heindel et al. (2020)
    - Increased carcinoma multiplicity (4-fold) in dorsolateral prostate ducts (by pairwise comparison).
- *s.c.* injection of newborn male S-D rats, plus estradiol and testosterone at age 90 days; evaluation at age 28 weeks: Ho et al. (2006)
    - Increase in PIN (by pairwise comparison) and prostate adenomas.
- Prenatal and neonatal exposure of Fisher 344 rats, plus *s.c.* injections of 3,2'-dimethyl-4-aminobiphenyl (DMAB) on week 5; evaluation at age 60 weeks: Ichihara et al. (2003)
    - No treatment-related prostate cancer findings.

- Xenograft studies

  Mammary tumors

  - Ovariectomized NCR/*nu*/*nu* (athymic) female mice with human breast MCF-7 cancer cell xenografts with BPA exposure by *s.c.* implant for 9 weeks: Weber Lozada and Keri (2011)
      - BPA accelerated human estrogen-dependent breast cancer growth rate (by pairwise comparison).

  Prostate tumors

  - NCR/*nu*/*nu* (athymic) male mice with human prostate LNCaP tumor cell xenografts with BPA exposure by *s.c.* implant, for 21 days: Wetherill et al. (2006)
      - BPA accelerated human prostate tumor growth rate (by pairwise comparison).
  - *NCR/*nu*/*nu* (athymic) male mice with human prostate stem-progenitor cell xenografts with oral BPA exposure for 2 weeks: Prins et al. (2014)
      - Increase in high-grade PIN and adenocarcinoma (by pairwise comparison).

Other relevant data

Key characteristics of carcinogens

There are numerous mechanistic studies examining the cancer-related effects of BPA. In this document, findings related to the key characteristics of carcinogens from a selection of studies are summarized.

- Is electrophilic or can be metabolically activated

  In vivo

  - DNA adducts in CD1 male rat liver (positive): Atkinson and Roy (1995b)

  In vitro

  - *Formation of DNA adducts (i.e., 3-hydroxy-bisphenol A-N7-guanine) by the BPA metabolite bisphenol A 3,4-quinone with 2′-deoxyguanosine (dG), calf thymus DNA, and DNA from MCF-7 cells (positive): Zhao et al. (2018)
  - DNA adducts in cultured Syrian hamster embryo (SHE) cells (positive): Atkinson and Roy (1995a), Tsutsui et al. (1998)

- Is genotoxic

  In vivo

  - *DNA double strand breaks (γH2AX assay) in microglia and astrocytosis in rat offspring at PND17 from pregnant females who received BPA soon after mating and during lactation and weaning (positive): Di Pietro et al. (2020)
  - Meiotic aneuploidy in female C57BL mice
    - Oral gavage expoure (positive): Hunt et al. (2003)
    - *In utero* exposure (positive)*:* Susiarjo et al. (2007)
  - Chromosome abnormalities in *Caenorhabditis elegans* (positive): Allard and Colaiácovo (2010)

  In vitro

  - K-ras mutations (positive): Takahashi et al. (2001)
  - Mammalian gene (HPRT) mutation assay in V79 cells (negative)*:* Keri et al. (2007, pp. 245)
  - Micronucleus formation

        - Human breast cancer MCF-7 cells (positive): Kabil et al. (2008)
        - Human lymphoblastoid MCL-5 cell line (positive): Parry et al. (2002)
        - Chinese hamster V79 cells (positive): Pfeiffer et al. (1997)
    - Aneuploidy in SHE cells (positive): Tsutsui et al. (1998)
    - Chromosomal abnormalities
        - *Chromosome aberrations (CAs) in human TCD4+ and TCD8+ subsets of T lymphocytes (positive): Di Pietro et al. (2020)
        - *CAs in human amniocytes and MCF-7 breast cancer cells (positive): Aghajanpour-Mir et al. (2016)
        - CAs in Chinese Hamster ovary (CHO) cells (positive): Hilliard et al. (1998) and Galloway et al. (1998); CAs in CHO cells (negative): Keri et al. (2007, pp. 245)
        - Aberrations of mitotic cell division in Chinese hamster cell line V79 line (positive)*:* Parry et al. (2002)
        - Chromosome non-disjunction in human MCL-5 cell line (positive)*:* Parry et al. (2002)
        - Sister chromatid exchange in CHO cells (negative)*:* Keri et al. (2007, pp. 245)
    - DNA damage
        - DNA double strand breaks in human breast cancer MCF-7 cells (positive): Iso et al. (2006)
        - *DNA strand breaks (comet assay) in rat insulinoma INS-1 cells (positive): Xin et al. (2014)
        - *DNA strand breaks (comet assay) in immortalized human prostate epithelial (RWPE-1) cells (positive): Kose et al. (2020)
        - *DNA double strand breaks (γH2AX assay) in human hepatocellular carcinoma (HEPG2) cells (positive): Hercog et al. (2019) and Quesnot et al. (2016)
        - Unscheduled DNA synthesis in transformed human embryo fibroblast cells (positive): Takahashi et al. (2001)
        - *BPA caused oxidative damage to DNA bases (both purines and pyrimidines) in human peripheral blood mononuclear cells (positive): Mokra et al. (2018)

In bacteria

- *Salmonella* reverse mutation assay
    - by nitrosylated BPA (positive): Schrader et al. (2002)
    - by BPA (negative): Keri et al. (2007, pp. 245)

- Alters DNA repair or causes genomic instability

  In vitro

  - *Down-regulation of the gene expression of DNA repair proteins (OGG1, Ape-1, and MyH) involved in the base excision repair pathway, as well as p53 protein levels in human prostate epithelial (RWPE-1) cells (positive): Kose et al. (2020)
  - Disruption of double-strand DNA repair in *Caenorhabditis elegans* (positive): Allard and Colaiacovo (2010)

- Induces epigenetic alterations

  In vivo

  - Alteration in DNA methylation patterns of cell signaling genes in male S-D rat prostate (neonatal low dose exposure): Ho et al. (2006) and Prins et al. (2008)
  - DNA hypomethylation and change in phenotype (coat color) (*in utero* exposure) in agouti mice: Dolinoy et al. (2007)

  In vitro

  - *Altered DNA methylation at several genomic clusters of CpG sites and at single CpG sites associated with cancer-related pathways in breast cell lines: Awada et al. (2019)
  - *Decreased global DNA methylation in mouse neuroblastoma cells (N2A): Bastos Sales et al. (2013)
  - Altered DNA methylation and gene expression in normal human breast epithelial cells (low-dose BPA exposure): Weng et al. (2010)
  - Increase of DNA methylation human breast MCF-7 tumor cells: Weng et al. (2010)

- Induces oxidative stress

  In vivo

  - *BPA significantly increased level of 15F2t-isoprostane, a urinary biomarker of oxidative stress, in 7-19 years old healthy students with BPA levels above 6 ng/mg creatinine in Chivasso, Italy (Bono et al. 2019).

In vitro

- *BPA significantly decreased reduced glutathione and glutathione peroxidase, and increased lipid peroxidation in human breast cancer MCF-7 cells (Güzel et al. 2020).
- *BPA significantly increased glutathione levels and glutathione reductase activities, and decreased superoxide dismutase and glutathione peroxidase activities and decreased total antioxidant capacity in human prostate epithelial (RWPE-1) cells (Kose et al. 2020).
- *BPA up-regulated an oxidative stress responsive gene, glutamate-cysteine ligase catalytic (GCLC), in human hepatocellular carcinoma (HepG2) cells (Hercog et al. 2019).
- *BPA caused a significant increase in intracellular reactive oxygen species (ROS) and a significant reduction in the level of reduced glutathione (GSH) in rat insulinoma (INS-1) cells (Xin et al. 2014).

- Induces chronic inflammation

  - *BPA induced six inflammation-related markers (WBC, CRP, IL-10, ALT, AST, and γ-GTP levels) in elderly subjects in Korea (Song et al. 2017).
  - *Perinatal BPA exposure induced chronic colonic and liver inflammation in rabbit offspring (Reddivari et al. 2017).

- Modulates receptor-mediated effects

  In vivo

  - Alters serum levels of progesterone and estradiol; alters their co-regulators SRC-3, SMRT (co-repressor) and estrogen receptor alpha (ERα) in Wistar rats (Durando et al. 2011).
  - *Increases expression of ERα in the mammary gland of male CD-1 mice (F1) (with perinatal exposure) (Vandenberg et al. 2013).
  - Neuroendocrine (hypothalamic-pituitary-gonadal axis) effects following neonatal exposure (*s.c.* injection in SD rats): Fernandez et al. (2010)
    - Decrease in gonadotropin-releasing hormone (GnRH) inter-pulse intervals in adult females.
    - Increase in testosterone and estradiol, but decrease in progesterone in adult females.
    - Increases incidence of polycystic ovarian syndrome (PCOS); humans and animals with PCOS are at increased risk of developing endometrial cancer.

In vitro

- Neoplastic transformation of human breast epithelial MCF-10F cells by estrogen receptor dependent pathway: Fernandez and Russo (2010)
- Promoted human testicular seminoma germ cell proliferation by G-protein coupled estrogen receptor dependent pathways (low dose exposure): Bouskine et al. (2009)
- Meta-analysis review on estrogenicity: Positive in most in vitro estrogenicity assays, including recombinant yeast screen, MCF-7 human breast cell proliferation and luciferase assays: Montano et al. (2010)

- Causes immortalization
    - Cell transformation in SHE cells (positive): Tsutsui et al. (1998)

- Alters cell proliferation, cell death or nutrient supply
    - *Mammary gland cell proliferation, decreased apoptosis and increased number of branching points and ductal area in male CD-1 mice (F1) (with perinatal exposure) (positive)*:* Vandenberg et al. (2013)
    - *Mammary gland cell proliferation and decreased apoptosis in female rats (F1) (with neonatal exposure) (positive)*:* Lamartiniere et al. (2011)
    - *Increased mammary gland terminal end-bud numbers in female offspring rhesus monkeys (with *in utero* exposure) (positive): Tharp et al. (2012)
    - *Mammary hyperplastic ducts in female Wistar rats (F1) (with *in utero* exposure) (positive): Durando et al. (2011)
    - *Increased progesterone-induced cell proliferation in the mammary gland in adult C57/Bl6 female mice (F1) (with perinatal exposure) (positive): Ayyanan et al. (2011)
    - Mammary tubular epithelial ductal cells in female S-D rats (F1) (with *in utero* exposure) (positive): Betancourt et al. (2010)
    - Increased the proliferative activity in developing dorsolateral
    - prostate ducts of gestation day 19 male CD-1 mice (F1) (with in utero exposure) (positive): Timms et al. (2005)
    - Effects on germ line cells
        - *In vivo*, BPA *in utero* exposure disrupts early oogenesis in the female C57BL mouse (positive): Susiarjo et al. (2007)
        - *In vitro*, human testicular seminoma germ cell proliferation (positive): Bouskine et al. (2009)

Reviews

- Keri et al. (2007)

References cited in "BPA"

Aghajanpour-Mir SM, Zabihi E, Akhavan-Niaki H, Keyhani E, Bagherizadeh I, Biglari S, et al. 2016. The genotoxic and cytotoxic effects of bisphenol-a (BPA) in MCF-7 cell line and amniocytes. International journal of molecular and cellular medicine 5:19-29.

Allard P, Colaiacovo MP. 2010. Bisphenol a impairs the double-strand break repair machinery in the germline and causes chromosome abnormalities. Proceedings of the National Academy of Sciences of the United States of America 107:20405-20410.

Aschengrau A, Coogan PF, Quinn M, Cashins LJ. 1998. Occupational exposure to estrogenic chemicals and the occurrence of breast cancer: An exploratory analysis. Am J Ind Med 34:6-14.

Atkinson A, Roy D. 1995a. In vitro conversion of environmental estrogenic chemical bisphenol a to DNA binding metabolite(s). Biochemical and biophysical research communications 210:424-433.

Atkinson A, Roy D. 1995b. In vivo DNA adduct formation by bisphenol a. Environmental and molecular mutagenesis 26:60-66.

Awada Z, Nasr R, Akika R, Cahais V, Cuenin C, Zhivagui M, et al. 2019. DNA methylome-wide alterations associated with estrogen receptor-dependent effects of bisphenols in breast cancer. Clinical epigenetics 11:138.

Ayyanan A, Laribi O, Schuepbach-Mallepell S, Schrick C, Gutierrez M, Tanos T, et al. 2011. Perinatal exposure to bisphenol a increases adult mammary gland progesterone response and cell number. Molecular endocrinology (Baltimore, MD) 25:1915-1923.

Bastos Sales L, Kamstra JH, Cenijn PH, van Rijt LS, Hamers T, Legler J. 2013. Effects of endocrine disrupting chemicals on in vitro global DNA methylation and adipocyte differentiation. Toxicology in vitro : an international journal published in association with BIBRA 27:1634-1643.

Betancourt AM, Eltoum IA, Desmond RA, Russo J, Lamartiniere CA. 2010. In utero exposure to bisphenol a shifts the window of susceptibility for mammary carcinogenesis in the rat. Environmental health perspectives 118:1614-1619.

Bono R, Bellisario V, Tassinari R, Squillacioti G, Manetta T, Bugiani M, et al. 2019. Bisphenol a, tobacco smoke, and age as predictors of oxidative stress in children and adolescents. International journal of environmental research and public health 16.

Bouskine A, Nebout M, Brücker-Davis F, Benahmed M, Fenichel P. 2009. Low doses of bisphenol a promote human seminoma cell proliferation by activating PKa and PKg via a membrane g-protein-coupled estrogen receptor. Environmental health perspectives 117:1053-1058.

Cornwall W. 2020. To replace controversial plastic additive BPA, a chemical company teams up with unlikely allies. Science news.

Di Pietro P, D'Auria R, Viggiano A, Ciaglia E, Meccariello R, Russo RD, et al. 2020. Bisphenol a induces DNA damage in cells exerting immune surveillance functions at peripheral and central level. Chemosphere 254:126819.

Dolinoy DC, Huang D, Jirtle RL. 2007. Maternal nutrient supplementation counteracts bisphenol a-induced DNA hypomethylation in early development. Proceedings of the National Academy of Sciences of the United States of America 104:13056-13061.

Duan B, Hu X, Zhao H, Qin J, Luo J. 2013. The relationship between urinary bisphenol a levels and meningioma in chinese adults. Int J Clin Oncol 18:492-497.

Durando M, Kass L, Piva J, Sonnenschein C, Soto AM, Luque EH, et al. 2007. Prenatal bisphenol a exposure induces preneoplastic lesions in the mammary gland in wistar rats. Environmental health perspectives 115:80-86.

Durando M, Kass L, Perdomo V, Bosquiazzo VL, Luque EH, Munoz-de-Toro M. 2011. Prenatal exposure to bisphenol a promotes angiogenesis and alters steroid-mediated responses in the mammary glands of cycling rats. The Journal of steroid biochemistry and molecular biology 127:35-43.

Fernandez M, Bourguignon N, Lux-Lantos V, Libertun C. 2010. Neonatal exposure to bisphenol a and reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats. Environmental health perspectives 118:1217-1222.

Fernandez SV, Russo J. 2010. Estrogen and xenoestrogens in breast cancer. Toxicologic pathology 38:110-122.

Galloway SM, Miller JE, Armstrong MJ, Bean CL, Skopek TR, Nichols WW. 1998. DNA synthesis inhibition as an indirect mechanism of chromosome aberrations: Comparison of DNA-reactive and non-DNA-reactive clastogens. Mutation research 400:169-186.

Güzel KGU, Nazıroğlu M, Ceyhan D. 2020. Bisphenol a-induced cell proliferation and mitochondrial oxidative stress are diminished via modulation of Trpv1 channel in estrogen positive breast cancer cell by selenium treatment. Biological trace element research.

Heindel JJ, Belcher S, Flaws JA, Prins GS, Ho SM, Mao J, et al. 2020. Data integration, analysis, and interpretation of eight academic clarity-BPA studies. Reproductive toxicology (Elmsford, NY).

Hercog K, Maisanaba S, Filipic M, Sollner-Dolenc M, Kac L, Zegura B. 2019. Genotoxic activity of bisphenol a and its analogues bisphenol s, bisphenol f and bisphenol af and their mixtures in human hepatocellular carcinoma (HepG2) cells. The Science of the total environment 687:267-276.

Hilliard CA, Armstrong MJ, Bradt CI, Hill RB, Greenwood SK, Galloway SM. 1998. Chromosome aberrations in vitro related to cytotoxicity of nonmutagenic chemicals and metabolic poisons. Environmental and molecular mutagenesis 31:316-326.

Hiroi H, Tsutsumi O, Takeuchi T, Momoeda M, Ikezuki Y, Okamura A, et al. 2004. Differences in serum bisphenol a concentrations in premenopausal normal women and women with endometrial hyperplasia. Endocr J 51:595-600.

Ho SM, Tang WY, Belmonte de Frausto J, Prins GS. 2006. Developmental exposure to estradiol and bisphenol a increases susceptibility to prostate carcinogenesis and epigenetically regulates phosphodiesterase type 4 variant 4. Cancer Res 66:5624-5632.

Hunt PA, Koehler KE, Susiarjo M, Hodges CA, Ilagan A, Voigt RC, et al. 2003. Bisphenol a exposure causes meiotic aneuploidy in the female mouse. Curr Biol 13:546-553.

Ichihara T, Yoshino H, Imai N, Tsutsumi T, Kawabe M, Tamano S, et al. 2003. Lack of carcinogenic risk in the prostate with transplacental and lactational exposure to bisphenol a in rats. The Journal of toxicological sciences 28:165-171.

Iso T, Watanabe T, Iwamoto T, Shimamoto A, Furuichi Y. 2006. DNA damage caused by bisphenol a and estradiol through estrogenic activity. Biological & pharmaceutical bulletin 29:206-210.

Jenkins S, Raghuraman N, Eltoum I, Carpenter M, Russo J, Lamartiniere CA. 2009. Oral exposure to bisphenol a increases dimethylbenzanthracene-induced mammary cancer in rats. Environmental health perspectives 117:910-915.

Jia J, Tian Q, Liu Y, Shao ZW, Yang SH. 2013. Interactive effect of bisphenol a (BPA) exposure with -22G/C polymorphism in lox gene on the risk of osteosarcoma. Asian Pac J Cancer Prev 14:3805-3808.

Kabil A, Silva E, Kortenkamp A. 2008. Estrogens and genomic instability in human breast cancer cells--involvement of src/raf/erk signaling in micronucleus formation by estrogenic chemicals. Carcinogenesis 29:1862-1868.

Keri RA, Ho SM, Hunt PA, Knudsen KE, Soto AM, Prins GS. 2007. An evaluation of evidence for the carcinogenic activity of bisphenol a. Reproductive Toxicology 24:240-252.

Kose O, Rachidi W, Beal D, Erkekoglu P, Fayyad-Kazan H, Kocer Gumusel B. 2020. The effects of different bisphenol derivatives on oxidative stress, DNA damage and DNA repair in rwpe-1 cells: A comparative study. Journal of applied toxicology : JAT 40:643-654.

Lamartiniere CA, Jenkins S, Betancourt AM, Wang J, Russo J. 2011. Exposure to the endocrine disruptor bisphenol a alters susceptibility for mammary cancer. Hormone molecular biology and clinical investigation 5:45-52.

Li J, Ji Z, Luo X, Li Y, Yuan P, Long J, et al. 2020. Urinary bisphenol a and its interaction with esr1 genetic polymorphism associated with non-small cell lung cancer: Findings from a case-control study in chinese population. Chemosphere 254:126835.

Marotta V, Russo G, Gambardella C, Grasso M, La Sala D, Chiofalo MG, et al. 2019. Human exposure to bisphenol af and diethylhexylphthalate increases susceptibility to develop differentiated thyroid cancer in patients with thyroid nodules. Chemosphere 218:885-894.

Mokra K, Wozniak K, Bukowska B, Sicinska P, Michalowicz J. 2018. Low-concentration exposure to BPA, BPF and BPAF induces oxidative DNA bases lesions in human peripheral blood mononuclear cells. Chemosphere 201:119-126.

Montano M, Bakker EJ, Murk AJ. 2010. Meta-analysis of supramaximal effects in in vitro estrogenicity assays. Toxicological sciences : an official journal of the Society of Toxicology 115:462-474.

Morgan M, Deoraj A, Felty Q, Roy D. 2017. Environmental estrogen-like endocrine disrupting chemicals and breast cancer. Molecular and cellular endocrinology 457:89-102.

Murray TJ, Maffini MV, Ucci AA, Sonnenschein C, Soto AM. 2007. Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol a exposure. Reproductive toxicology (Elmsford, NY) 23:383-390.

Newbold RR, Jefferson WN, Padilla-Banks E. 2009. Prenatal exposure to bisphenol a at environmentally relevant doses adversely affects the murine female reproductive tract later in life. Environmental health perspectives 117:879-885.

NTP. 1982. Carcinogenesis bioassay of bisphenol a (CAS No. 80-05-7) in F344 rats and B6C3F1 mice (feed study). Natl Toxicol Program Tech Rep Ser 215:1-116.

NTP. 2018. The clarity-BPA core study: A perinatal and chronic extended-dose-range study of bisphenol a in rats. In: Ntp research report on the clarity-BPA core study: A perinatal and chronic extended-dose-range study of bisphenol a in rats. Research Triangle Park (NC):National Toxicology Program.

Pamungkas AD, Park C, Lee S, Jee SH, Park YH. 2016. High resolution metabolomics to discriminate compounds in serum of male lung cancer patients in south korea. Respiratory research 17:100.

Parada H, Jr., Cleveland RJ, North KE, Stevens J, Teitelbaum SL, Neugut AI, et al. 2019. Genetic polymorphisms of diabetes-related genes, their interaction with diabetes status, and breast cancer incidence and mortality: The long island breast cancer study project. Mol Carcinog 58:436-446.

Parry EM, Parry JM, Corso C, Doherty A, Haddad F, Hermine TF, et al. 2002. Detection and characterization of mechanisms of action of aneugenic chemicals. Mutagenesis 17:509-521.

Pfeiffer E, Rosenberg B, Deuschel S, Metzler M. 1997. Interference with microtubules and induction of micronuclei in vitro by various bisphenols. Mutation research 390:21-31.

Prins GS, Tang WY, Belmonte J, Ho SM. 2008. Perinatal exposure to oestradiol and bisphenol a alters the prostate epigenome and increases susceptibility to carcinogenesis. Basic & clinical pharmacology & toxicology 102:134-138.

Prins GS, Hu WY, Shi GB, Hu DP, Majumdar S, Li G, et al. 2014. Bisphenol a promotes human prostate stem-progenitor cell self-renewal and increases in vivo carcinogenesis in human prostate epithelium. Endocrinology 155:805-817.

Quesnot N, Rondel K, Audebert M, Martinais S, Glaise D, Morel F, et al. 2016. Evaluation of genotoxicity using automated detection of gammaH2AX in metabolically competent heparg cells. Mutagenesis 31:43-50.

Reddivari L, Veeramachaneni DNR, Walters WA, Lozupone C, Palmer J, Hewage MKK, et al. 2017. Perinatal bisphenol a exposure induces chronic inflammation in rabbit offspring via modulation of gut bacteria and their metabolites. mSystems 2.

Reeves KW, Schneider S, Xue J, Kannan K, Mason H, Johnson M, et al. 2018. Bisphenol-a in breast adipose tissue of breast cancer cases and controls. Environmental research 167:735-738.

Schrader TJ, Langlois I, Soper K, Cherry W. 2002. Mutagenicity of bisphenol a (4,4'-isopropylidenediphenol) in vitro: Effects of nitrosylation. Teratog Carcinog Mutagen 22:425-441.

Song H, Park J, Bui PTC, Choi K, Gye MC, Hong YC, et al. 2017. Bisphenol a induces cox-2 through the mitogen-activated protein kinase pathway and is associated with levels of inflammation-related markers in elderly populations. Environmental research 158:490-498.

Susiarjo M, Hassold TJ, Freeman E, Hunt PA. 2007. Bisphenol a exposure in utero disrupts early oogenesis in the mouse. PLoS Genet 3:e5.

Takahashi S, Chi XJ, Yamaguchi Y, Suzuki H, Sugaya S, Kita K, et al. 2001. Mutagenicity of bisphenol a and its suppression by interferon-alpha in human RSA cells. Mutation research 490:199-207.

Tarapore P, Ying J, Ouyang B, Burke B, Bracken B, Ho SM. 2014. Exposure to bisphenol a correlates with early-onset prostate cancer and promotes centrosome amplification and anchorage-independent growth in vitro. PloS one 9:e90332.

Tharp AP, Maffini MV, Hunt PA, VandeVoort CA, Sonnenschein C, Soto AM. 2012. Bisphenol a alters the development of the rhesus monkey mammary gland. Proceedings of the National Academy of Sciences of the United States of America 109:8190-8195.

Timms BG, Howdeshell KL, Barton L, Bradley S, Richter CA, vom Saal FS. 2005. Estrogenic chemicals in plastic and oral contraceptives disrupt development of the fetal mouse prostate and urethra. Proceedings of the National Academy of Sciences of the United States of America 102:7014-7019.

Trabert B, Falk RT, Figueroa JD, Graubard BI, Garcia-Closas M, Lissowska J, et al. 2014. Urinary bisphenol a-glucuronide and postmenopausal breast cancer in poland. Cancer causes & control : CCC 25:1587-1593.

Tse LA, Lee PMY, Ho WM, Lam AT, Lee MK, Ng SSM, et al. 2017. Bisphenol a and other environmental risk factors for prostate cancer in Hong Kong. Environment international 107:1-7.

Tse LA, Ho WM, Wang F, He YH, Ng CF. 2018. Environmental risk factors of prostate cancer: A case-control study. Hong Kong medical journal = Xianggang yi xue za zhi 24 Suppl 4:30-33.

Tsutsui T, Tamura Y, Yagi E, Hasegawa K, Takahashi M, Maizumi N, et al. 1998. Bisphenol-a induces cellular transformation, aneuploidy and DNA adduct formation in cultured syrian hamster embryo cells. Int J Cancer 75:290-294.

Vandenberg LN, Schaeberle CM, Rubin BS, Sonnenschein C, Soto AM. 2013. The male mammary gland: A target for the xenoestrogen bisphenol a. Reproductive toxicology (Elmsford, NY) 37C:15-23.

Weber Lozada K, Keri RA. 2011. Bisphenol a increases mammary cancer risk in two distinct mouse models of breast cancer. Biology of reproduction 85:490-497.

Weinhouse C, Anderson OS, Bergin IL, Vandenbergh DJ, Gyekis JP, Dingman MA, et al. 2014. Dose-dependent incidence of hepatic tumors in adult mice following perinatal exposure to bisphenol a. Environmental health perspectives 122:485-491.

Weng YI, Hsu PY, Liyanarachchi S, Liu J, Deatherage DE, Huang YW, et al. 2010. Epigenetic influences of low-dose bisphenol a in primary human breast epithelial cells. Toxicology and applied pharmacology 248:111-121.

Wetherill YB, Hess-Wilson JK, Comstock CE, Shah SA, Buncher CR, Sallans L, et al. 2006. Bisphenol a facilitates bypass of androgen ablation therapy in prostate cancer. Molecular cancer therapeutics 5:3181-3190.

Xin F, Jiang L, Liu X, Geng C, Wang W, Zhong L, et al. 2014. Bisphenol a induces oxidative stress-associated DNA damage in INS-1 cells. Mutation research Genetic toxicology and environmental mutagenesis 769:29-33.

Yang M, Ryu JH, Jeon R, Kang D, Yoo KY. 2009. Effects of bisphenol a on breast cancer and its risk factors. Archives of toxicology 83:281-285.

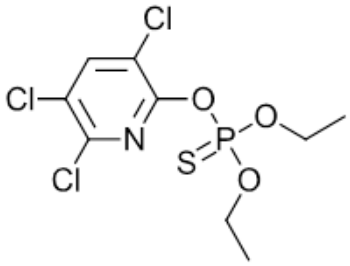
Yang PJ, Hou MF, Tsai EM, Liang SS, Chiu CC, Ou-Yang F, et al. 2018. Breast cancer is associated with methylation and expression of the a disintegrin and metalloproteinase domain 33 (ADAM33) gene affected by endocrinedisrupting chemicals. Oncology reports 40:2766-2777.

Yoshida M, Shimomoto T, Katashima S, Watanabe G, Taya K, Maekawa A. 2004. Maternal exposure to low doses of bisphenol a has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats. The Journal of reproduction and development 50:349-360.

Zhao H, Wei J, Xiang L, Cai Z. 2018. Mass spectrometry investigation of DNA adduct formation from bisphenol a quinone metabolite and MCF-7 cell DNA. Talanta 182:583-589.

Chlorpyrifos

(CAS No. 2921-88-2)



Chlorpyrifos is an organophosphate insecticide, acaricide and miticide that has been used to control a variety of foliage and soil-borne pests, with numerous tolerances for food/feed commodities, and various meats and meat products.

The California Department of Pesticide Regulation announced on October 9, 2019 that virtually all agricultural use of the pesticide chlorpyrifos in California will end by December 31, 2020[3] based on serious health effects, including impaired neurological development in children and other sensitive populations. Chlorpyrifos in granular form will still be allowed for use in California; in the past, the granular form constituted 1% of total use of chlorpyrifos[4]. Past uses on food crops included citrus fruits and vegetables such as broccoli, corn, and root vegetables. The half-life of chlorpyrifos in soil ranges from 2 weeks to over 1 year, depending on the soil type, climate and other conditions[5]. Exposures to the general public after December 31, 2020 in California are expected to occur primarily as a result of consumption of residues present on food grown or raised outside the state.

Chlorpyrifos passed the human data screen, underwent a preliminary toxicological evaluation, and is being brought to the Carcinogen Identification Committee for consultation. This is a summary of the relevant studies identified during the preliminary toxicological evaluation.

---

[3] California Department of Pesticide Regulation (2019). Agreement Reached to End Sale of Chlorpyrifos in California by February 2020. https://www.cdpr.ca.gov/docs/pressrls/2019/100919.htm (published on 10/9/2019; accessed on 7/28/2020)

[4] *Ibid*.

[5] Cornell Cooperative Extension, Pesticide Management Education Program. Pesticide Information Profile. Chlorpyrifos. http://pmep.cce.cornell.edu/profiles/extoxnet/carbaryl-dicrotophos/chlorpyrifos-ext.html (published in 9/1993; accessed on 8/6/2020)

Epidemiological data

- Breast cancer
    - Prospective cohort study (Agricultural Health Study, AHS)
        - Cohort of more than 80,000 people: farmers and pesticide applicators (n>54,000) in Iowa and North Carolina and their spouses (n>30,000)
        - Enrolment period 1993 to 1997 (Alavanja et al. 1996)
        - Followed up until 2015 in Iowa and 2014 in North Carolina
        - Incident cancers identified through linkage to state cancer registries
        - Exposure data collected prospectively through self-administered questionnaire at enrollment, additional take-home questionnaires, and follow-up telephone interview. At enrollment, participants reported ever/never use of 50 pesticides; further details gathered for 22 of the pesticides (i.e., years and days per year each pesticide was applied, use of personal protective equipment, pesticide application method).
        - Engel et al. (2005)
            - Analyzed breast cancer incidence in women who were directly or indirectly exposed to pesticides through use of pesticides themselves or by their husbands.
            - "Female licensed pesticide applicators were not included in the analyses reported here, because of their relatively small numbers (n = 1,347; 15 cases) and differences in the nature and extent of their pesticide use in comparison with women who were not themselves licensed but may have applied pesticides through their husband's license."
            - 309 cases, 4.8 years average follow-up
            - Both direct and indirect chlorpyrifos use were associated with not statistically significant elevated relative risk of breast cancer
                - Women who used chlorpyrifos among all women included in the analysis: Relative risk (RR), 1.4; 95% confidence interval (CI), 0.9–2.4
                - Husband's use of chlorpyrifos among women who never used pesticides: RR, 1.3; 95% CI, 0.9–1.8
            - Stratified by menopausal status, increased relative risks observed only for

- Pre-menopausal, women who used chlorpyrifos among all women included in the analysis: RR, 2.2; 95% CI, 1.0–4.9
- Post-menopausal, husband's use of chlorpyrifos among women who never used pesticides: RR, 1.6; 95% CI, 1.1–2.4

- Lerro et al. (2015)
  - 1059 cases, median follow-up 15.3 years
  - Estrogen receptor and progesterone receptor negative (ER-PR-) tumors: RR, 2.26; 95% CI, 1.07–4.75
  - No significant difference when analyses were stratified by menopausal status.
- Engel et al. (2017)
  - 1081 cases, 14.7 years average follow-up
  - Ever use of chlorpyrifos: Hazard ratio (HR),1.4; 95% CI, 1.0–2.0
  - Direct use by women: HR,1.6; 95% CI, 0.9–2.9
  - No significant trend with quartiles of husband's chlorpyrifos use (*p*-value for trend = 0.72).

- Population-based case-control study from California's Central Valley: Tayour et al. (2019)
  - 155 cases of postmenopausal breast cancer (2007-2008), 150 controls (2001-2011)
  - Exposure assessed through telephone interview, mailed questionnaire, and geographic information system (GIS)-based method:
  - Workplace and residential exposure to chlorpyrifos: Odds ratio (OR), 3.22; 95% CI, 1.38–7.53. Risk estimates were similar when analyses were restricted to residence only and workplace only.

- Non-Hodgkin's Lymphoma (NHL)
  - Pooled analysis of a consortium of 3 cohort studies (AGRICOH): Leon et al. (2019)
    - More than 300,000 farmers or agricultural workers from France, Norway and the USA, accruing more than 3.5 million person-years under risk
      - Includes the AHS
    - Periodic data linkage to cancer incidence registries
    - Exposure to pesticides and/or crop cultivation estimated through questionnaire or census data

        - Chlorpyrifos use and NHL: HR, 0.99; 95% CI, 0.86–1.15
        - No association with NHL subtypes (Chronic lymphocytic leukemia/small lymphocytic lymphoma, Diffuse large B-cell lymphoma, Follicular lymphoma, or Multiple myeloma/plasma-cell leukemia)
    - Prospective cohort of 54,383 pesticide applicators in the AHS study: Lee et al. (2004)
        - Reported on multiple cancer sites
        - Chlorpyrifos use and NHL: RR, 1.03; 95% CI, 0.62–1.70; 37 exposed cases
        - No exposure-response relationship
    - Pooled analysis of 3 population-based case–control studies of NHL in Kansas, Nebraska, Iowa: Waddell et al. (2001)
        - 748 cases, 2236 controls
        - Chlorpyrifos use: OR, 3.2; 95% CI, 1.1–9.2
- Lung cancer
    - Two reports available from the AHS, both adjusted for smoking
    - Alavanja et al. (2004)
        - Highest group of chlorpyrifos use vs. nonexposed reported: OR, 1.8; 95% CI, 1.0–3.2
        - Significant exposure-response associations (*p*-value for trend < 0.05).
    - Lee et al. (2004) [see above for details]
        - Ever chlorpyrifos use: RR, 1.36; 95% CI, 0.96–1.93
        - Significant exposure-response trends and increased risk in the highest categories of intensity-weighted chlorpyrifos exposure-days (RR, 1.80; 95% CI, 1.00–3.23; *p*-value for trend = 0.036) and lifetime chlorpyrifos exposure days (RR, 2.18; 95% CI, 1.31–3.64; *p*-value for trend = 0.002)
            - This pattern persisted in stratified analyses by state of residence, smoking status and histologic subtype
            - *p*-values for trend were 0.019 for North Carolina, 0.001 for current smokers, and 0.022 for adenocarcinoma.

- Brain cancer
    - Case-control study of glioma from eastern Nebraska: Lee et al. (2005)
        - OR, 22.6; 95% CI, 2.7–191
        - 10 exposed cases, 9 were proxy responses
    - Prospective cohort within the AHS: Lee et al. (2004) [see above for details]

        - RR, 1.77; 95% CI, 0.70–4.50; 15 exposed cases

- Kidney cancer
    - Two reports available from the AHS
    - Lee et al. (2004) [see above for details]
        - RR, 1.08; 95% CI, 0.56–2.06; 20 exposed cases
        - No significant trend by categories of lifetime chlorpyrifos exposure days (p = 0.665) or intensity-weighted chlorpyrifos exposure-days (p = 0.904)
    - Andreotti et al. (2020)
        - 308 renal cell carcinoma cases, 19.5 years average follow-up
        - Exposures were presented as unlagged and lagged analyses, which discounted exposure during the years most proximal to cancer diagnosis or other censoring event
        - Highest quartile of chlorpyrifos use lagged 20 years: RR, 1.68; 95% CI, 1.05–2.70
        - Significant exposure-response trend (p-value = 0.01)

- Pancreatic cancer
    - Two reports available from the AHS
    - Prospective cohort analysis: Lee et al. (2004) [see above for details]
        - RR, 0.36; 95% CI, 0.13–0.97; 10 exposed cases
    - Nested case control analysis: Andreotti et al. (2009)
        - 93 incident pancreatic cancer cases, 82,503 cancer-free controls
        - OR, 0.6; 95% CI, 0.4–1.1
        - No exposure response association by intensity-weighted lifetime days (p-value for trend = 0.09)

- Prostate cancer
    - Two reports available from the AHS
    - Alavanja et al. (2003)
        - Ever vs never use: OR, 0.90; 95% CI, 0.74–1.09; 174 exposed cases
        - No significant trend with cumulative exposure score categories of chlorpyrifos use (p-value = 0.23)
        - No association with chlorpyrifos use regardless of whether a family history of prostate cancer was absent (OR, 0.82; 95% CI, 0.66–1.02) or present (OR, 1.29; 95% CI, 0.84–1.98) but there was a statistical interaction between chlorpyrifos use and family history of prostate cancer (OR, 1.65; 95% CI, 1.02–2.66).
    - Lee et al. (2004)

        - RR, 0.91; 95% CI, 0.76–1.09; 297 exposed cases

- Rectal cancer
    - Two reports available from the AHS
    - Lee et al. (2004) [see above for details]
        - Chlorpyrifos use: RR, 1.33 (95% CI, 0.75–2.36; 35 exposed cases)
        - Significantly increased risk in highest categories of use with some evidence of an exposure-response trend
            - Lifetime chlorpyrifos exposure days: RR, 3.25; 95% CI, 1.60–6.62; *p*-value for trend = 0.035
            - Intensity-weighted chlorpyrifos exposure days: RR, 3.16; 95% CI, 1.42–7.03; *p*-value for trend = 0.057
    - Lee et al. (2007)
        - Chlorpyrifos use: RR, 1.4; 95% CI, 0.9–2.2; 42 exposed cases
        - Increased risk in the highest exposure category of lifetime exposure days to chlorpyrifos: RR, 2.7; 95% CI, 1.2–6.4
        - Significant exposure-response trend (*p*-value = 0.008)

- Other cancers
    - Prospective cohort analyses from the AHS reported no significant associations between ever chlorpyrifos use and a number of other cancer sites: bladder, buccal cavity, pharynx, esophagus, stomach, colon, melanoma, hematopoietic cancers (multiple myeloma, leukemia) (Lee et al. 2004) and uterus (Lerro et al. 2015) [see above for details]

Animal carcinogenicity data

- 104-week feeding studies in male and female CDF Fischer rats (Yano et al. 2000)
    - 60 rats/sex/dose; 0; 0.05; 1.0; 10 milligram per kilogram bodyweight per day (mg per kg bw/d); 10 randomly selected rats/sex/dose necropsied at 12 months. No statistically significant difference in mortality rates, BW or feed intake compared to controls.
    - No increase in treatment-related tumor incidence reported in either sex

- 104-week feeding studies in male and female Sherman rats by the Dow Chemical Company, as reviewed by US EPA (1984)
    - Sherman rats of unspecified source; 25 animals/sex/dose. Doses: 0.01, 0.03, 0.1, 1.0, 3.0 mg/kg/d. A supplementary group of 57/sex/dose was maintained for interim pathologic examinations and cholinesterase determinations.

- No increase in treatment-related tumor incidence reported in either sex.

- 104-week feeding studies in male and female Fischer rats by the Dow Chemical Company, as reviewed by US EPA (1989)
  - 50 rats/sex/dose; doses: 0, 0.05, 0.1, 1.0, 10 mg/kg bw/d. 10 additional rats/sex/group were randomly allocated for the 12-month sacrifice.
  - No treatment-related differences in mortality in males or females; at the highest dose, bw gain was significantly lower in treated males and females compared to controls, but the difference was no more than 9%
  - No increase in treatment-related tumor incidence reported in either sex

- 105-week feeding studies in male and female CD-1 mice by the Dow Chemical Company, as reviewed by US EPA (1980)
  - 56 mice/sex/dose; dose: 0, 0.5, 5.0, 15 ppm
    - Male mice
      - Treated male mice had non-significantly greater survival than controls; survival to 105 weeks in high-dose group was 46%; controls 39%.
      - Tumor findings:
        - Lung: Significant increase in lung adenomas in mid-dose group (no tumor incidences reported; no dose-related trend).
        - US EPA memo indicates that highest dose may not have reached the maximum tolerated dose.
    - Female mice
      - No statistically significant difference in survival compared to controls.
      - No increase in treatment-related tumor incidence reported

- 78-week feeding studies in male and female CD-1 mice, as reviewed by US EPA (2000) (submitter unknown)
  - Doses in males: 0, 0.89, 8.84, 45.2 mg/kg bw/d
  - Doses in females: 0, 0.938, 9.79, 48.1 mg/kg bw/d
  - Decreased bw gain and food consumption in males; decreased water consumption in females; increased incidence of gross clinical findings (ocular opacity and hair loss) in both sexes (US EPA 2000)
  - No increase in treatment-related tumor incidence reported in either sex

Other relevant data

Key Characteristics of carcinogens

- Is genotoxic
    - Mutagenicity
        - Positive in bacterial assays where greater cytotoxicity in repair-deficient vs. repair-proficient strains of *B. subtilis* and *E. coli* is indicative of mutagenicity (Simmon et al. 1977)
        - Negative in: Ames test *S. typhimurium* TA 100; TA 1535; TA 1537; TA 1538; *S. typhimurium* (His + reversion); *E. coli WP2* (Tyr + reversion) (Simmon et al. 1977)
    - Chromosomal effects
        - Chromosome loss and missegregation in human peripheral blood lymphocytes *in vitro* (Mužinić et al. 2019)
        - Increase in micronuclei (MN) *in vivo* in rat bone marrow cells (Ezzi et al. 2016) and in male and female Wistar rat blood lymphocytes (Sandhu et al. 2013)
    - Increases in aneuploidy in human peripheral blood lymphocytes *in vitro* (Sultana Shaik et al. 2016)
    - Increases in DNA damage *in vivo* in rats and mice (Comet assay) (Ezzi et al. 2016; Mehta et al. 2008; Rahman et al. 2002; Sandhu et al. 2013)
    - Positive in the dominant lethal test in *Culex quinquefasciatus* (Bhinder and Chaudhry 2013)
    - Negative for unscheduled DNA synthesis (UDS) (Gollapudi et al. 1995, as reviewed by Rahman et al. 2002)
    - Negative in *S. cerevisiae* mitotic recombination test (Simmon et al. 1977)

- Induces epigenetic alterations
    - Female SD rats were treated orally with chlorpyrifos (dissolved in castor oil) daily for 150 days (Ventura et al. 2019)
        - Chlorpyrifos did not change the CpG methylation status in CDKN1B or BRCA1 promoters in mammary tissues.
        - Chlorpyrifos did not change the global CpG methylation status, as measured in long interspersed nucleotide elements 1 (LINE-1).
        - 0.01 mg/kg/day chlorpyrifos-treated animals presented significantly increased expression of HDAC1 ($p < 0.001$). HDAC1 is responsible for histone deacetylation, an important mechanism of epigenetic regulation.

- Induces oxidative stress
    - Chlorpyrifos induced an increase in reactive oxygen species (ROS) levels in estrogen-dependent MCF-7 (58%) and estrogen-independent MDA-MB-231 (108%) breast cancer cells (Ventura et al. 2012).
    - Significant increases in H_2O_2 production and superoxide anion generation ($O_2^{\bullet -}$) in rat lymphocytes (Ojha and Srivastava 2014)
    - In the immortalized skin keratinocyte cell line HaCaT chlorpyrifos induced transcriptional activity at the Romo-1 promoter (measured via luciferase assay), an inducer of endogenous ROS. Chlorpyrifos also increased the number of cells positive for the general oxidative stress indicator H_2DCFDA. [H2DCFDA is known for detecting general ROS, including hydroxyl radical, hydrogen peroxide, peroxynitrate, and peroxyl radical.] (Jang et al. 2015)

- Induces chronic inflammation
    - Chlorpyrifos induced the NLRP3 inflammasome in HaCaT cells (immortalized human keratinocyte cell line) (Jang et al. 2015)
        - "NLRP3 is an intracellular sensor that detects a broad range of microbial motifs, endogenous danger signals and environmental irritants, resulting in the formation and activation of the NLRP3 inflammasome. Assembly of the NLRP3 inflammasome leads to the release of the pro-inflammatory cytokines IL-1β and IL-18), as well as to gasdermin D-mediated pyroptotic cell death." (Swanson et al. 2019)

- Modulates receptor-mediated effects
    - Estrogen Receptor (ER)
        - Low (0.05 micromolar (μM)) but not high (50 μM) concentrations of chlorpyrifos induced ER activation (*p*-Y537) when serum-starved estrogen-dependent MCF-7 breast cancer cells were exposed for 15 minutes to the pesticide (87% over control; $p < 0.001$) (Ventura et al. 2012)
            - ERα phosphorylation in tyrosine 537 (Y537) is required to stimulate the Src/Shc/Ras/Erk pathway in MCF-7 cells
            - Chlorpyrifos at 0.05 μM increased the proliferation of MCF-7 cells. ER inhibitor ICI182780 completely abolished the proliferation induced by 0.05 μM chlorpyrifos. This indicates that the induction of cell proliferation was mediated by the estrogenic effect of chlorpyrifos.

        - An interaction between chlorpyrifos and estradiol (E2) has been reported in the digestive gland of the marine mussel, based on the finding that pre-exposure to sublethal concentrations of chlorpyrifos affects the transcriptomic fingerprint that is induced in response to E2 (Canesi et al., 2011, as cited by Ventura et al 2012).
        - Chlorpyrifos weakly increased mRNA level of ERβ in MCF-7BUS cells (fibroblast). (Grünfeld and Bonefeld-Jorgensen 2004)
        - ToxCast/Tox21: Chlorpyrifos was active in the following assays measuring ER expression or activity. ATG_ERE_CIS_up, ATG_ERa_TRANS_up, OT_ER_ERaERa_0480, OT_ER_ERaERa_1440, OT_ER_ERaERb_0480, OT_ER_ERaERb_1440, OT_ER_ERbERb_0480
    - Androgen Receptor (AR)
        - Chlorpyrifos was screened with the NIH3T3 cell line (NIH3T3-hAR-Luc) stably expressing human androgen receptor (hAR) and luciferase reporter gene for the ability to stimulate luciferase activity or inhibit the response that was evoked by 0.4 nanomolar (nM) testosterone. Chlorpyrifos was one of the most potent anti-androgenic compounds identified in the experiments reported in this paper. (Viswanath et al. 2010)

- Alters cell proliferation, cell death or nutrient supply
    - Induced cell proliferation in estrogen-dependent MCF-7 breast cancer cell line at 0.05 μM and decreased proliferation at 50 μM. In the estrogen-independent MDA-MB231 breast cancer cell line, chlorpyrifos decreased cell proliferation at all doses from 0.05 to 50 μM. (Ventura et al. 2012) [See also *Modulates receptor mediated effects*]
    - Modulated cell cycle progression and cyclin E and D1 expression (Ventura et al., 2012)
    - Stimulated growth of colorectal adenocarcinoma H508 cells (Suriyo et al. 2015)

References cited in "Chlorpyrifos"

Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF, et al. 1996. The Agricultural Health Study. Environmental Health Perspectives 104:362-369.

Alavanja MC, Samanic C, Dosemeci M, Lubin J, Tarone R, Lynch CF, et al. 2003. Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. Am J Epidemiol 157:800-814.

Alavanja MC, Dosemeci M, Samanic C, Lubin J, Lynch CF, Knott C, et al. 2004. Pesticides and lung cancer risk in the agricultural health study cohort. Am J Epidemiol 160:876-885.

Andreotti G, Freeman LEB, Hou L, Coble J, Rusiecki J, Hoppin JA, et al. 2009. Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort. International journal of cancer 124:2495-2500.

Andreotti G, Beane Freeman Laura E, Shearer Joseph J, Lerro Catherine C, Koutros S, Parks Christine G, et al. 2020. Occupational Pesticide Use and Risk of Renal Cell Carcinoma in the Agricultural Health Study. Environmental Health Perspectives 128:067011.

Bhinder P, Chaudhry A. 2013. Evaluation of toxic potential of acephate and chlorpyrifos by dominant lethal test on Culex quinquefasciatus. J Environ Biol 34:573-577.

Engel LS, Hill DA, Hoppin JA, Lubin JH, Lynch CF, Pierce J, et al. 2005. Pesticide use and breast cancer risk among farmers' wives in the agricultural health study. Am J Epidemiol 161:121-135.

Engel LS, Werder E, Satagopan J, Blair A, Hoppin JA, Koutros S, et al. 2017. Insecticide Use and Breast Cancer Risk among Farmers' Wives in the Agricultural Health Study. Environ Health Perspect 125:097002.

Ezzi L, Belhadj Salah I, Haouas Z, Sakly A, Grissa I, Chakroun S, et al. 2016. Histopathological and genotoxic effects of chlorpyrifos in rats. Environ Sci Pollut Res Int 23:4859-4867.

Grünfeld HT, Bonefeld-Jorgensen EC. 2004. Effect of in vitro estrogenic pesticides on human oestrogen receptor alpha and beta mRNA levels. Toxicology letters 151:467-480.

Jang Y, Lee AY, Jeong SH, Park KH, Paik MK, Cho NJ, et al. 2015. Chlorpyrifos induces NLRP3 inflammasome and pyroptosis/apoptosis via mitochondrial oxidative stress in human keratinocyte HaCaT cells. Toxicology 338:37-46.

Lee WJ, Blair A, Hoppin JA, Lubin JH, Rusiecki JA, Sandler DP, et al. 2004. Cancer Incidence Among Pesticide Applicators Exposed to Chlorpyrifos in the Agricultural Health Study. JNCI: Journal of the National Cancer Institute 96:1781-1789.

Lee WJ, Colt JS, Heineman EF, McComb R, Weisenburger DD, Lijinsky W, et al. 2005. Agricultural pesticide use and risk of glioma in Nebraska, United States. Occupational and environmental medicine 62:786-792.

Lee WJ, Sandler DP, Blair A, Samanic C, Cross AJ, Alavanja MC. 2007. Pesticide use and colorectal cancer risk in the Agricultural Health Study. Int J Cancer 121:339-346.

Leon ME, Schinasi LH, Lebailly P, Beane Freeman LE, Nordby KC, Ferro G, et al. 2019. Pesticide use and risk of non-Hodgkin lymphoid malignancies in agricultural cohorts from France, Norway and the USA: a pooled analysis from the AGRICOH consortium. Int J Epidemiol 48:1519-1535.

Lerro CC, Koutros S, Andreotti G, Friesen MC, Alavanja MC, Blair A, et al. 2015. Organophosphate insecticide use and cancer incidence among spouses of pesticide applicators in the Agricultural Health Study. Occupational and environmental medicine 72:736-744.

Mehta A, Verma RS, Srivastava N. 2008. Chlorpyrifos-induced DNA damage in rat liver and brain. Environ Mol Mutagen 49:426-433.

Mužinić V, Ramić S, Želježić D. 2019. Chromosome Missegregation and Aneuploidy Induction in Human Peripheral Blood Lymphocytes In vitro by Low Concentrations of Chlorpyrifos, Imidacloprid and α-Cypermethrin. Environ Mol Mutagen 60:72-84.

Ojha A, Srivastava N. 2014. In vitro studies on organophosphate pesticides induced oxidative DNA damage in rat lymphocytes. Mutat Res Genet Toxicol Environ Mutagen 761:10-17.

Rahman MF, Mahboob M, Danadevi K, Saleha Banu B, Grover P. 2002. Assessment of genotoxic effects of chloropyriphos and acephate by the comet assay in mice leucocytes. Mutat Res 516:139-147.

Sandhu MA, Saeed AA, Khilji MS, Ahmed A, Latif MS, Khalid N. 2013. Genotoxicity evaluation of chlorpyrifos: a gender related approach in regular toxicity testing. J Toxicol Sci 38:237-244.

Simmon VF, Mitchell AD, Jorgenson TA. 1977. Evaluation of selected pesticides as chemical mutagens in vitro and in vivo studies. Contract No. 68-01-2458. EPA/600/1-77/028. (Environmental Health Effect Research Series).

Sultana Shaik A, Shaik AP, Jamil K, Alsaeed AH. 2016. Evaluation of cytotoxicity and genotoxicity of pesticide mixtures on lymphocytes. Toxicol Mech Methods 26:588-594.

Suriyo T, Tachachartvanich P, Visitnonthachai D, Watcharasit P, Satayavivad J. 2015. Chlorpyrifos promotes colorectal adenocarcinoma H508 cell growth through the activation of EGFR/ERK1/2 signaling pathway but not cholinergic pathway. Toxicology 338:117-129.

Swanson KV, Deng M, Ting JP. 2019. The NLRP3 inflammasome: molecular activation and regulation to therapeutics. Nature reviews Immunology 19:477-489.

Tayour C, Ritz B, Langholz B, Mills PK, Wu A, Wilson JP, et al. 2019. A case–control study of breast cancer risk and ambient exposure to pesticides. Environmental Epidemiology 3.

US EPA. 1980. Results of a two-year toxicity and oncogenic study of chlorpyrifos administered to CD-mice in the diet. Acc #242059. Available: https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/059101/059101-051.pdf.

US EPA. 1984. Data Evaluation Record. Chlorpyrifos. 2-year chronic toxicity/oncogenicity feeding study–rats. Office of Pesticides and Toxic Substances. Available: https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/059101/059101-151.pdf.

US EPA. 1989. Chlorpyrifos–2-year dietary chronic toxicity/oncogenicity study–rats. Office of Pesticides and Toxic Substances. Available: https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/059101/059101.htm.

US EPA. 2000. Memorandum. Toxicology Chapter for Chlorpyrifos. DP Barcode D263892, Case 818975, Submission S576466, PC Code 059101.: Office of Prevention, Pesticides and Toxic Substances. Available: https://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/059101/059101-2000-04-18a.pdf.

US EPA. 2006. Memorandum. Finalization of Interim Reregistration Eligibility Decisions (IREDs) and Interim Tolerance Reassessment and Risk Management Decisions (TREDs) for the Organophosphate Pesticides, and Completion of the Tolerance Reassessment and Reregistration Eligibility Process for the Organophosphate Pesticides.: Office of Prevention, Pesticides and Toxic Substances. Available: https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/ired_PC-059101_28-Sep-01.pdf.

Ventura C, Núñez M, Miret N, Martinel Lamas D, Randi A, Venturino A, et al. 2012. Differential mechanisms of action are involved in chlorpyrifos effects in estrogen-dependent or -independent breast cancer cells exposed to low or high concentrations of the pesticide. Toxicol Lett 213:184-193.

Ventura C, Zappia CD, Lasagna M, Pavicic W, Richard S, Bolzan AD, et al. 2019. Effects of the pesticide chlorpyrifos on breast cancer disease. Implication of epigenetic mechanisms. J Steroid Biochem Mol Biol 186:96-104.

Viswanath G, Chatterjee S, Dabral S, Nanguneri SR, Divya G, Roy P. 2010. Anti-androgenic endocrine disrupting activities of chlorpyrifos and piperophos. J Steroid Biochem Mol Biol 120:22-29.

Waddell BL, Zahm SH, Baris D, Weisenburger DD, Holmes F, Burmeister LF, et al. 2001. Agricultural use of organophosphate pesticides and the risk of non-Hodgkin's lymphoma among male farmers (United States). Cancer Causes Control 12:509-517.

Yano BL, Young JT, Mattsson JL. 2000. Lack of carcinogenicity of chlorpyrifos insecticide in a high-dose, 2-year dietary toxicity study in Fischer 344 rats. Toxicol Sci 53:135-144.

Coal dust

Coal dust is a complex mixture containing more than 50 different compounds. Exposure to coal dust in California may occur in occupational settings associated with rail transport to ports for export, and power generation (there is one coal-powered plant in the state). Lower-level exposure to residents in communities close to ports or railways with open-top coal cars is also likely.

Coal dust passed the human data and animal data screens, underwent a preliminary toxicological evaluation, and is being brought to the Carcinogen Identification Committee for consultation. The International Agency for Research on Cancer (IARC) classified coal dust in Group 3 in 1997. IARC's evaluations of the evidence from studies in humans and animals are presented below, followed by a summary of relevant studies[6] identified during the preliminary toxicological evaluation that were published after the IARC (1997) review.

IARC (1997) states the following regarding the carcinogenicity of coal dust:

- Human evidence: "The evidence from occupational cohort studies for an association between coal mine dust and lung cancer has not been consistent; some studies revealed excess risks, whereas others indicated cohort-wide lung cancer deficits. There is no consistent evidence supporting an exposure-response relation for lung cancer with any of the customary dose surrogates, including duration of exposure, cumulative exposure or radiographic evidence of pneumoconiosis. In contrast to the lung cancer findings, there have been reasonably consistent indications of stomach cancer excess among coal miners, detected both in occupational cohort studies and in community-based case-control studies. However, there is no consistent evidence supporting an exposure-response gradient for coal mine dust and stomach cancer."
- Animal evidence: "Coal dust was tested for carcinogenicity both separately and in combination with diesel particle aerosols by inhalation in one adequate experiment in rats. The incidence of tumours was not increased compared to controls. In one study in rats, single intrapleural injection of coal dust did not increase the incidence of thoracic tumours."

[6] Studies on coal miners that did not use "coal dust" explicitly as the exposure matrix were excluded as there could be co-exposure to other potential carcinogens. Exposure to coal dust in epidemiological studies is often assessed by job title or questionnaire.

Epidemiological data

Lung cancer

- Case-control study of lung cancer in US: Muscat et al. (1998)
  - 550 cases of lung cancer and 386 age-matched controls from 1978 to 1996.
  - Exposure to coal dust assessed by job title.
  - Men exposed to coal dust: Odds ratio (OR), 2.8; 95% confidence interval (CI), 1.1–7.0; adjusted for age, education and pack-years of smoking. (OR for diesel exhaust, 0.9; 95% CI, 0.3–2.6).
  - Women exposed to coal dust: OR, 0.5; 95% CI, 0.1–4.8.
- Case-control study of lung cancer in Polish women: Rachtan (2002)
  - 242 cases with primary carcinoma of the lung and 352 healthy controls between 1991 to 1997.
  - Exposure to coal dust assessed by questionnaire interview.
  - OR for coal dust, 3.25; 95% CI, 0.98–10.8, adjusted for age and pack-years of smoking.
  - OR for coal dust, 6.37; 95% CI, 1.49–27.26, from a multivariate analysis of all significant risk factors for lung cancer in a univariate model, e.g., smoking and vodka consumption.
- Cohort study of US underground coal miners: Attfield and Kuempel (2008)
  - 23 year follow-up of 8,899 coal miners started in 1969–1971 in 31 US coal mines.
  - Cumulative exposure to respirable coal mine dust computed by job title and duration of exposure in the job, and stratified into six exposure categories.
  - Standardized mortality ratios (SMRs) for the respiratory system cancers are not significantly associated with cumulative coal dust exposure in all six exposure categories.
- Cohort study of US underground coal miners: Graber et al. (2014)
  - 9,033 coal miners from 31 US mines enrolled between 1969–1971 and followed-up for about 37 years, an extended follow-up from Attfield and Kuempel (2008).
  - Cumulative exposure of coal mine dust for each mine was compiled by the job-specific dust concentration and the duration of time worked at that job. Respirable quartz was estimated using the average percent of silica in the coal dust from the compliance data. Analyses were stratified to three time periods: 1970–1989, 1990–1999, 2000–2007.

    - Lung cancer mortality with coal mine dust exposure: Hazard ratio (HR), 1.70; 95% CI, 1.02–2.83.
        - Respirable silica: HR, 1.05; 95% CI, 0.90–1.23, controlled for age at the study entry, race and year of birth and other covariates.
    - In the most recent follow-up period (2000–2007), coal mine dust exposures were positively associated with lung cancer mortality, HR, 1.55; 95% CI, 1.19–1.67.
        - HR, 1.21; 95% CI, 0.89–1.66 for respirable silica during the same period.
- Cohort study of British coal miners: Miller and MacCalman (2010)
    - 17,820 men followed-up from 1959 to 2005 (analyzed by three time periods: 1959–1974, 1975–1989, 1990–2005).
    - Lifetime cumulative respirable dust exposures to coal miners were compiled by extensive sampling data from different surveys and job deployment. Quartz exposures were estimated based on the percentage of quartz from a compositional analysis of the dust samples in surveys.
    - Relative risk (RR), 1.02; 95% CI, 0.95–1.09 for lung cancer mortality and lifetime cumulative exposure of 100 gram-hour per cubic meter (g-h/m^3) "dust exposure" (assumed to be coal dust), adjusted for age, smoking, cohort entry date and regional difference in population mortality rate. RR, 1.07; 95% CI, 1.01–1.13 for lung cancer mortality and 15-year lag of lifetime cumulative exposure of 5 g-h/m^3 quartz exposure.

Cancers of the larynx, hypopharynx, oropharynx

- Case-control study of squamous cell carcinomas of the larynx and hypopharynx cancer in male workers from 1989–1991 in France: Laforest et al. (2000)
    - 497 cases (201 hypopharyngeal cancers and 296 laryngeal cancers) and 296 controls (patients with other tumor sites).
    - Exposure to coal dust was assessed with a job exposure matrix. Exposure variables used in the analysis included probability, duration, and cumulative level of exposure.
    - Hypopharyngeal cancer and ever coal-dust exposure: OR, 2.31; 95% CI, 1.21–4.4, adjusted for age, smoking, alcohol and exposure to formaldehyde; significant increase in risk with probability ($p < 0.005$ for trend) and cumulative level of exposure ($p < 0.007$ for trend) but not with duration of exposure.
    - Laryngeal cancer and ever coal-dust exposure: OR, 1.67; 95% CI, 0.92–3.02; no dose-response relationship observed with duration of exposure or cumulative level of exposure.

    - After exposed subjects with a low probability of exposure (< 10%) were excluded, the ORs associated with exposure to coal dust increased for both hypopharyngeal and laryngeal cancers.
- Multi-center case-control study of laryngeal and hypopharyngeal cancer in male workers in four European countries (Poland, Romania, Russia, and Slovakia): Shangina et al. (2006)
    - 350 cancer cases (34 hypopharyngeal, 316 laryngeal) and 728 hospital controls.
    - Coal dust exposure evaluated by industrial hygienists based on occupational history.
    - Hypopharyngeal cancer and ever exposure to coal dust: OR, 4.19; 95% CI, 1.18–14.89; 4 cases, adjusted for age, country, smoking and alcohol consumption.
        - Clear dose-response patterns were observed by duration or cumulative exposure. Inclusion of a 20-year lag in the analysis strengthened these associations.
    - Laryngeal cancer and ever exposure to coal dust (all countries combined): OR, 1.81; 95% CI, 0.94–3.47; 26 cases
        - OR, 4.09, 95% CI, 1.59–10.52 for workers in Poland (accounting for the majority of cases), adjusted for age, country, smoking and alcohol consumption. Clear dose-response patterns for duration, weighted duration and cumulative exposure were also observed in this subgroup.
- Case-control study of oropharyngeal cancer in Belgrade, Serbia: Vlajinac et al. (2006)
    - 100 cases and 100 controls (non-malignant diseases of head and neck)
    - Exposure to coal dust assessed by interview.
    - Coal dust: OR, 0.88, 95% CI, 0.36–2.14; 16 cases and 14 controls; adjusted for smoking, body mass index) and other confounders.

Hematopoietic system cancers

- Population-based case-control study of multiple myeloma (MM) in Canadian men: Ghosh et al. (2011)
    - 342 cases and 1506 controls.
    - Exposure to coal dust collected by postal questionnaire, including a list of all full time jobs held for at least one year.
    - Coal dust and MM in men: OR, 1.7; 95% CI, 1.2–2.4; 62 cases and 149 controls; adjusted for age and residence.

- Population-based case-control study of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) in Minnesota: Poynter et al. (2017)
    - 420 AML and 265 MDS cases, and 1388 controls.
    - Exposure to coal dust by self-report.
    - Significantly increased OR (adjusted for age, sex, income, smoking, exposure to chemotherapy and residence type) for AML, not MDS:
        - AML OR, 4.03; 95% CI, 1.79–9.06 for 5 or more years of coal dust exposure.
        - MDS OR, 1.68; 95% CI, 0.63–4.50 for 5 or more years of coal dust exposure.
        - Statistically significant dose-response trend was observed with the number of years working as coal miners.

Stomach cancer

- Cohort study of US underground coal miners: Attfield and Kuempel (2008)
    - 23 year follow-up of 8,899 coal miners started in 1969–1971 in 31 US coal mines.
    - Cumulative exposure to respirable coal mine dust computed by job title and duration of exposure in the job, stratified into six exposure categories.
    - SMRs for stomach cancer were not significantly associated with cumulative coal dust exposure in any exposure category.
- Cohort study of British coal miners: Miller and MacCalman (2010)
    - 17,820 men followed-up from 1959 to 2005 (analyzed by three time periods: 1959–1974, 1975–1989, 1990–2005).
    - RR, 0.96; 95% CI, 0.87–1.07 for lifetime cumulative exposure of 100 g-h/m^3 "dust exposure" (assumed to be coal dust), adjusted for age, smoking, cohort entry date and regional difference in population mortality rate.

Animal carcinogenicity data

Intratracheal instillation

- Repeated intratracheal instillation (6 milligrams (mg) per instillation, 10–20 weekly instillations) of five different coal dusts (different particle size and SiO_2 content) in 7-week-old female SPF Wistar rats: (Pott and Roller 2005)
    - Animals were observed for up to 30 months.

- Significant increases in benign and malignant lung tumors were observed in all exposed groups (vs. none in the controls) (Table 9).

Table 9. Lung tumor incidence in female SPF Wistar rats exposed to coal dust via intratracheal instillation (Pott and Roller 2005)

Coal dust type	Dose: No. of instillation x mg coal dust/ instillation	Rats at start/ at risk[1]	Survival 50% (wk)[2]	Lung tumor[3] (%)		
				Benign	Malignant	Total
Carrier fluid[4] (Control)	20 x 0	48/47	110	0	0	0
Lean coal, <0.1% SiO_2	11 x 6	48/47	109	8.5	48.9	57.4
	20 x 6	48/48	101	2.1	62.5	64.6
Lower rich coal, <0.1% SiO_2	10 x 6	48/48	108	20.8	33.3	54.2
	20 x 6	48/44	106	4.5	72.7	77.3
Rich coal, 1.3% SiO_2	10 x 6	48/48	106	10.4	45.8	56.3
	20 x 6	48/45	99	22.2	57.8	80.0
Steam coal, 9% SiO_2	10 x 6	48/43	108	11.6	60.5	72.1
	20 x 6	48/45	95	17.8	66.7	84.4
Rock coal, 16.7% SiO_2	10 x 6	48/47	102	6.4	27.7	34.0
	20 x 6	48/45	105	11.1	46.7	57.8

[1] Number of sufficiently examined rats which survived at least 26 weeks after first instillation.
[2] Period after first instillation in which 50% of the animals died excluding rats which died immediately after anesthesia.
[3] The authors only reported percentage of animals with lung tumors, instead of tumor count. Thus no statistical tests were performed.
[4] 0.9% NaCl solution, phosphate buffered, with 1% Tween 80

- Repeated intratracheal instillation (10 weekly instillations X 1 mg dose) in 50 female Wistar rats: Kolling et al. (2011)
  - Coal dust*:* Milled lean coal with crystalline SiO_2 < 0.1%, ash 5%, density 1.4 mg/milliliter (ml), particle size 50% < 4 micrometers (µm) in diameter.
  - Number of rats examined: 55 rats in control and 51 in coal dust group.

        - Animals were observed for up to 125 weeks.
        - No treatment-related tumors were observed.

Other relevant data

Key characteristics of carcinogens

- Is genotoxic
    - As reviewed by IARC (1997):
        - Induced chromosomal aberrations and sister chromatid exchange (SCE) in human lymphocyte cultures.
        - Induced SCE in Chinese Hamster Ovary (CHO) cells.
        - Mutagenicity assays
            - Non-nitrosated extracts: negative or borderline with or without exogenous activation for TA98, TA100, YG1024.
            - Nitrosated extracts: positive for TA98, TA100 and YG1024 with or without exogenous activation; negative for TA1535.
- Induces oxidative stress
    - As reviewed by IARC (1997):
        - Long-lived radicals in coal dust recovered from coal miners' lungs and lymph nodes (Dalal et al. 1991, as reviewed by IARC 1997).
        - The ratio of 7-hydro-8-oxo-2'-deoxyguanosine to deoxyguanosine, a marker of oxidative DNA damage, in peripheral blood lymphocytes of retired miners was significantly higher than in age-matched controls (Schins et al. 1995, as reviewed by IARC 1997).
    - Increased reactive nitrogen or oxygen intermediates and tumor necrosis factor (TNF-α) in bronchioalveolar lavage fluid-derived cells (Ernst et al. 2002).
- Induces chronic inflammation
    - An epithelial rearrangement, hyperplastic (metaplastic) goblet cells, and scattered massive inflammatory cells were observed in bronchioalveolar epithelium of male Wistar rats exposed to coal dust (at three concentrations: 6.25, 12.5, and 25 mg/m^3) for 14 to 28 days by inhalation (Kania et al. 2014).
- Causes immortalization
    - Induced cell transformation in BALB/c-3T3 cell line (Wu et al., 1990, as reviewed by IARC 1997).

References cited in "Coal dust"

Attfield MD, Kuempel ED. 2008. Mortality among U.S. underground coal miners: a 23-year follow-up. American journal of industrial medicine 51:231-245.

Ernst H, Rittinghausen S, Bartsch W, Creutzenberg O, Dasenbrock C, Görlitz B-D, et al. 2002. Pulmonary inflammation in rats after intratracheal instillation of quartz, amorphous SiO2, carbon black, and coal dust and the influence of poly-2-vinylpyridine-N-oxide (PVNO). Experimental and Toxicologic Pathology 54:109-126.

Ghosh S, McLaughlin JR, Spinelli JJ, Dosman JA, McDuffie HH, Pahwa P. 2011. Multiple myeloma and occupational exposures: a population-based case-control study. Journal of occupational and environmental medicine 53:641-646.

Graber JM, Stayner LT, Cohen RA, Conroy LM, Attfield MD. 2014. Respiratory disease mortality among US coal miners; results after 37 years of follow-up. Occupational and environmental medicine 71:30-39.

IARC. 1997. Coal Dust. Silica, Some Silicates, Coal Dust and para-Aramid Fibrils. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 68. Available: https://publications.iarc.fr/86.

Kolling A, Ernst H, Rittinghausen S, Heinrich U. 2011. Relationship of pulmonary toxicity and carcinogenicity of fine and ultrafine granular dusts in a rat bioassay. Inhal Toxicol 23:544-554.

Laforest L, Luce D, Goldberg P, Bégin D, Gérin M, Demers PA, et al. 2000. Laryngeal and hypopharyngeal cancers and occupational exposure to formaldehyde and various dusts: a case-control study in France. Occupational and environmental medicine 57:767-773.

Miller BG, MacCalman L. 2010. Cause-specific mortality in British coal workers and exposure to respirable dust and quartz. Occupational and environmental medicine 67:270-276.

Muscat JE, Stellman SD, Richie JP, Wynder EL. 1998. Lung cancer risk and workplace exposures in black men and women. Environ Res 76:78-84.

Pott F, Roller M. 2005. Carcinogenicity study with nineteen granular dusts in rats. European Journal of Oncology 10:249-281.

Poynter JN, Richardson M, Roesler M, Blair CK, Hirsch B, Nguyen P, et al. 2017. Chemical exposures and risk of acute myeloid leukemia and myelodysplastic syndromes in a population-based study. International journal of cancer 140:23-33.

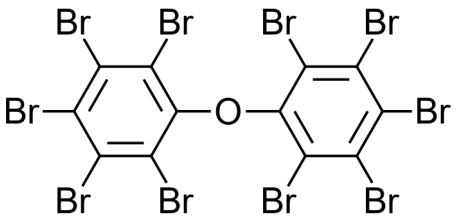
Rachtan J. 2002. A case-control study of lung cancer in Polish women. Neoplasma 49:75-80.

Shangina O, Brennan P, Szeszenia-Dabrowska N, Mates D, Fabiánová E, Fletcher T, et al. 2006. Occupational exposure and laryngeal and hypopharyngeal cancer risk in central and eastern Europe. American journal of epidemiology 164:367-375.

Vlajinac HD, Marinkovic JM, Sipetic SB, Andrejic DM, Adanja BJ, Stosic-Divjak SL. 2006. Case-control study of oropharyngeal cancer. Cancer detection and prevention 30:152-157.

Decabromodiphenyl ether

(Decabromobiphenyl oxide, DecaBDE, BDE-209, CAS No. 1163-19-5)



DecaBDE is a brominated flame retardant. The primary use of decaBDE is in high impact polystyrene-based products, and in the manufacture of rubber and plastics. The three major product categories in which decaBDE has been used as a flame retardant are textiles, electronic equipment, and building and construction materials (US EPA 2017). DecaBDE has been found in household and office dust, and in sewage sludge (CalEPA 2006; Dodson et al. 2012; Ward et al. 2014). It is also detected in human milk, serum and other tissues (Deziel et al. 2019; He et al. 2018; US EPA 2008).

Two US producers of decaBDE announced commitments in 2009 to voluntarily phase out decaBDE in the US by the end of 2013. Nevertheless, the US EPA Toxics Release Inventory reported total releases of more than 200,000 pounds in 2015, from 29 US sites. Three of those sites reported production or import of decaBDE, and 23 sites reported processing of decaBDE (US EPA 2017). As of 2018, there is one site in California that reported total releases of decaBDE amounting to 163 pounds[7]. In 2019, US EPA proposed a rule, which has not yet been adopted, to restrict or prohibit the manufacture (including import), processing, and distribution of decaBDE in commerce (US EPA 2019).

DecaBDE passed the animal data screen in 2010 and was brought to the Carcinogen Identification Committee (CIC) for consultation. At that time, the CIC recommended that decaBDE be placed in the 'medium' priority group for development of hazard identification materials. Since 2010, additional epidemiology data and mechanistic data have become available. In 2020, decaBDE passed both the human and the animal data screens, underwent a preliminary toxicological evaluation, and is being brought again to the CIC for consultation. This is a summary of the relevant studies identified during the preliminary toxicological evaluation. Studies identified since consultation with the CIC in 2010 are marked with an asterisk (*).

[7] US EPA (2020). Toxics Release Inventory (TRI) Program. TRI basic data files: calendar years 1987–2018. https://www.epa.gov/toxics-release-inventory-tri-program/tri-basic-data-files-calendar-years-1987-2018 (last updated on 7/31/2020; accessed on 8/6/2020)

Epidemiological data

- Papillary thyroid cancer (PTC)
  - * Case-control study in patients at the Duke Cancer Institute: Hoffman et al. (2017)
    - Controls were non-cancer hospital controls or members of the community who responded to flyers in Duke University medical facilities.
    - DecaBDE (BDE-209) was measured in household dust samples.
    - Those with decaBDE dust levels above the median were more likely to be cases compared to those with levels below the median (OR, 2.29; 95% CI, 1.09–5.08, 116 cases).
    - Association of PTC depends on the presence of the BRAF V600E mutation[8]. When PTC cases were stratified by BRAF status, increased decaBDE was more strongly associated with wild-type BRAF.
      - BRAF V600E: OR, 1.84; 95% CI, 0.66–5.15
      - BRAF wild-type: OR, 14.2; 95% CI, 1.63–123
      - BRAF not assessed: OR, 1.42; 95% CI, 0.47–4.28
  - * Population-based case-control study in women (250 cases, 250 controls) in Connecticut: Deziel et al. (2019)
    - Levels of decaBDE were measured in serum samples.
    - A decreased risk of PTC was observed with the highest (>90 percentile) category of decaBDE concentrations compared to the lowest (<median) category.
- Breast cancer
  - * Hospital-based case-control study of breast cancer risk among women in a Chinese population (209 cases, 165 controls): He et al. (2018)
    - Controls were patients with histopathologically confirmed benign breast disease or non-breast-related disease who underwent surgery.
    - Concentrations of decaBDE were measured in adipose tissue obtained from the breast for cases and the abdomen or breast for controls.

[8] BRAF gene mutation testing has emerged as an important tool for diagnosis, prognosis, treatment, and predicting patient outcome in response to targeted therapy for multiple cancer types. The BRAF V600E mutation is a driver mutation in multiple tumors.

        - Increased risks of breast cancer in 2nd tertile (OR, 2.48; 95% CI, 1.30–4.73; 67 cases) and 3rd tertile of decaBDE adipose concentrations (OR, 4.72; 95% CI, 2.52–8.83; 124 cases), compared with the 1st tertile ($p_{trend} < 0.001$, 18 cases).
        - When breast cancer cases were stratified by ER expression, increased decaBDE concentrations were associated with increased risk of breast cancer for both ER-negative and ER-positive cases.
- Acute lymphoblastic leukemia (ALL)
    - * Population-based case-control study in children 0-7 years of age (167 cases, 214 controls) in the Northern California Childhood Leukemia Study: Ward et al. (2014)
        - DecaBDE measured in carpet dust.
        - No association of decaBDE with ALL.

Animal carcinogenicity data

- 103-week feeding studies in male and female F344/N rats: NTP (1986)
    - Males: Increases in hepatocellular adenomas, and in pancreatic acinar cell adenomas in the high dose group, by pairwise comparison, and by trend (Table 10).
    - Females: Increases in hepatocellular adenomas, and adenoma and carcinoma combined in the high-dose group by pairwise comparison, and by trend (Table 11).

Table 10. Tumor incidence in male F344/N rats exposed to decaBDE in feed for 103 weeks (NTP 1986)

Tumor type, first appearance (week)	Control	25,000 ppm (1,120 mg/kg/day)	50,000 ppm (2,240 mg/kg/day)	Exact trend test *p*-value
Liver neoplastic nodules[1] (hepatocellular adenomas), 87 weeks	1/45	7/38*	15/44***	$p < 0.001$
Hepatocellular carcinomas, 97 weeks	1/41	1/33	1/33	NS
Pancreatic acinar cell adenomas, 97 weeks	0/40	0/33	4/33*	$p < 0.01$

Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, *** $p < 0.001$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

[1] Neoplastic nodule is an older term used for hepatocellular adenoma, although now the term hepatocellular adenoma is preferred (Bannasch and Zerban 1990; Maronpot et al. 1986).

Table 11. Tumor incidence in female F344/N rats exposed to decaBDE in feed for 103 weeks (NTP 1986)

Tumor type, first appearance (week)	Control	25,000 ppm (1,200 mg/kg/day)	50,000 ppm (2,500 mg/kg/day)	Exact trend test *p*-value
Liver neoplastic nodules[1] (hepatocellular adenomas), 87 weeks	1/47	3/43	9/44**	$p < 0.01$
Hepatocellular carcinomas, 104 weeks	0/40	2/33	0/34	NS
Hepatocellular adenoma and carcinoma combined, 87 weeks	1/47	5/43	9/44**	$p < 0.01$

Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): ** $p < 0.01$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

[1] Neoplastic nodule is an older term used for hepatocellular adenoma, although now the term hepatocellular adenoma is preferred (Bannasch and Zerban 1990; Maronpot et al. 1986).

- 103-week feeding studies in male and female B6C3F1 mice: NTP (1986)
  - Males: Increase in hepatocellular adenoma and carcinoma combined in the low dose group by pairwise comparison (Table 12).
  - Females: No treatment-related tumors were observed.

Table 12. Tumor incidence in male B6C3F1 mice exposed to decaBDE in feed for 103 weeks (NTP 1986)

Tumor type, first appearance (week)	Control	25,000ppm (3,200 mg/kg/day)	25,000ppm (6,650 mg/kg/day)	Exact trend test *p*-value
Hepatocellular adenomas, 60 weeks	4/31	12/43	12/43	NS
Hepatocellular carcinomas, 72 weeks	5/26	14/40	8/38	NS
Hepatocellular adenomas and carcinomas combined[1], 60 weeks	8/31	22/43*	18/43	NS

Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

[1] The loss of control mice (due to fighting) was significant during first part of the study. All male mice were caged individually after 15 months. Thereafter survival of control and dosed male mice was comparable. No significant differences in survival were observed between any groups of either sex (NTP 1986).

Reviews of above animal studies:

- US EPA (2008): Group C (possible human carcinogen) because of "suggestive evidence of carcinogenic potential" based on neoplastic nodules in male and female rats and hepatocellular adenomas and carcinomas (combined) in male mice.
- IARC (1999): There is limited evidence in experimental animals.

Other relevant data

Key characteristics of carcinogens

- Is genotoxic
  In vivo
  - * Negative in micronucleus tests and reporter gene mutation assays in B6C3F$_1$ *gpt* delta mice (Takasu et al. 2017).

  In vitro

- Negative in genotoxicity tests (Sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells) (IARC 1999).
- * Induced DNA damage using the Comet assay) in human neuroblastoma cells (Pellacani et al. 2012).
- Not mutagenic in mouse lymphoma cells (IARC 1999).
- * Modulated expression of histone gene clusters that may alter nucleosome organization in human embryonic kidney cells (HEK293T) in gene expression profiling study. Gene sets of cancer-related modules (nucleotide metabolism and nuclear pore complex regulation) were positively correlated with decaBDE exposure (Li et al. 2014).

In bacteria

- Not mutagenic in *Salmonella typhimurium* (IARC 1999).

- Induces oxidative stress

  In vitro

  - * Induced the production of ROS and endoplasmic reticulum stress, activates autophagy through IRE1α/AKT/mTOR signaling pathway and ultimately induces apoptosis in human umbilical vein endothelial cells (HUVECs) (Hou et al. 2019).
  - * Induced oxidative DNA damage measured by oxidized purine in human neuroblastoma cells in the presence of the bacterial repair enzyme formamidopyrimidine glycosylase (Pellacani et al. 2012).

- Modulates receptor-mediated effects

  In vivo

  - * Induced the expression of hepatic CYP3a11 in mice *in vivo*, indicating activation of pregnane X receptor (PXR). PXR is a ligand-activated transcription factor (Pacyniak et al. 2007).
  - * Reduced thyroid hormone triiodothyronine (T3), thyroid hormone thyroxine (T4) and testosterone levels and caused partial impairment of testicular steroidogenesis in male mice (Sarkar et al. 2016).
  - * Reduced T3, leading to perturbations of hypothalamic-pituitary-thyroid (HPT) axis and induced oxidative damage in the thyroid gland in male rats (Wang et al. 2019).
  - * DecaBDE treatment of pregnant female F_0 mice led to increased expression of ERα in male F_1 mice and impaired the structure and function of blood-testis-barrier, leading to spermatogenesis dysfunction (Zhai et al. 2019).

In vitro

- * Activation of Aryl Hydrocarbon Receptor (AhR) in rat H4IIE cells using a luciferase reporter assay (Alonso et al. 2008).
- * Activation of rodent PXR and its human counterpart, the steroid X receptor (SXR) in a luciferase reporter gene assay. In this study, decaBDE did not activate the AhR (Pacyniak et al. 2007).

- Alteration of cell proliferation and/or cell death

  In vitro

  - * Increased cell proliferation in human tumor cell lines (MCF-7 human breast cancer cells, multidrug-resistant MCF-7/ADR cells, and OVCAR-3 human ovarian cancer cells) and in Chinese hamster ovary (CHO) cells (Li et al. 2012).
  - * Decreased cell proliferation in MCF-7 cells (Llabjani et al. 2011)
  - * DecaBDE did not affect basal cell proliferation but significantly decreased basal caspase-9 activity in MCF-7 cells. DecaBDE exhibited an additive anti-apoptotic activity and ability to induce cell proliferation in the presence of 17β-estradiol (Kwiecińska et al. 2011).

Pharmacokinetics and metabolism

- DecaBDE does not accumulate in rats (US EPA 2008). In humans, the half-life was reported as 15 days (Thuresson et al. 2006).
- DecaBDE is mainly excreted as the parent compound but may also be excreted in the form of metabolites (US EPA 2008).
- Debromination may be the first step in decaBDE metabolism to form lower brominated BDE congeners (a range of penta- to nona-BDEs), followed by oxidation to form phenolic metabolites (US EPA 2008).

Structure-activity relationships

- DecaBDE shows broad structural similarities to other polyhalogenated persistent organic pollutants, including the Proposition 65 carcinogens polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Hooper and McDonald 2000).
- Pijnenburg et al. (1995) concluded that the main environmental properties and mechanisms of toxicity of PBDEs are similar to those of the structurally-related carcinogens polybrominated biphenyls (PBBs), PCBs, and dibenzodioxins. The

primary tumors observed from rodent studies of PBBs were also liver tumors, and dioxin-like compounds disrupt thyroid hormone balance.

- DecaBDE is structurally similar to pentabromodiphenyl ether mixture [DE-71 (technical grade)], which was listed under Proposition 65 in 2017 (OEHHA 2017) based on NTP (2016) studies. Positive findings for the pentabromodiphenyl ether mixture included liver tumors in male and female rats and mice, thyroid gland and pituitary gland tumors in male rats, and uterine tumors in female rats.

Reviews

- IARC (1999)
- US EPA (2008)

References cited in "DecaBDE"

Alonso M, Casado S, Miranda C, Tarazona JV, Navas JM, Herradón B. 2008. Decabromobiphenyl (PBB-209) Activates the Aryl Hydrocarbon Receptor While Decachlorobiphenyl (PCB-209) Is Inactive: Experimental Evidence and Computational Rationalization of the Different Behavior of Some Halogenated Biphenyls. Chemical Research in Toxicology 21:643-658.

Bannasch P, Zerban H. 1990. Tumours of the Liver. In: Pathology of tumours in laboratory animals Tumours of the rat, Part 2nd (Turusov V, Mohr U, eds). Lyon, France:International Agency For Research On Cancer (publication No. 99).

CalEPA. 2006. Polybrominated Diphenyl Ethers: Recommendations to Reduce Exposure in California. A Report of the Cal/EPA PBDE Workgroup.: California Environmental Protection Agency. Available: https://oehha.ca.gov/media/downloads/risk-assessment/report/pbdewrkgrprptfeb06.pdf.

Deziel NC, Alfonso-Garrido J, Warren JL, Huang H, Sjodin A, Zhang Y. 2019. Exposure to Polybrominated Diphenyl Ethers and a Polybrominated Biphenyl and Risk of Thyroid Cancer in Women: Single and Multi-Pollutant Approaches. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology 28:1755-1764.

Dodson RE, Perovich LJ, Covaci A, Van den Eede N, Ionas AC, Dirtu AC, et al. 2012. After the PBDE Phase-Out: A Broad Suite of Flame Retardants in Repeat House Dust Samples from California. Environmental Science & Technology 46:13056-13066.

He Y, Peng L, Zhang W, Liu C, Yang Q, Zheng S, et al. 2018. Adipose tissue levels of polybrominated diphenyl ethers and breast cancer risk in Chinese women: A case–control study. Environmental Research 167:160-168.

Hoffman K, Lorenzo A, Butt CM, Hammel SC, Henderson BB, Roman SA, et al. 2017. Exposure to flame retardant chemicals and occurrence and severity of papillary thyroid cancer: A case-control study. Environment international 107:235-242.

Hooper K, McDonald TA. 2000. The PBDEs: an emerging environmental challenge and another reason for breast-milk monitoring programs. Environ Health Perspect 108:387-392.

Hou Y, Fu J, Sun S, Jin Y, Wang X, Zhang L. 2019. BDE-209 induces autophagy and apoptosis via IRE1α/Akt/mTOR signaling pathway in human umbilical vein endothelial cells. Environmental Pollution 253:429-438.

IARC. 1999. Decabromodipheny Oxide. IARC Monographs on the evaluation of carcinogenic risks to humans. Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. Available: https://publications.iarc.fr/89.

Kwiecińska P, Wróbel A, Gregoraszczuk E. 2011. Combinatory effects of PBDEs and 17β-estradiol on MCF-7 cell proliferation and apoptosis. Pharmacological reports : PR 63:189-194.

Li M, Liu Z, Gu L, Yin R, Li H, Zhang X, et al. 2014. Toxic effects of decabromodiphenyl ether (BDE-209) on human embryonic kidney cells. Front Genet 5:118-118.

Li Z-H, Liu X-Y, Wang N, Chen J-S, Chen Y-H, Huang J-T, et al. 2012. Effects of decabrominated diphenyl ether (PBDE-209) in regulation of growth and apoptosis of breast, ovarian, and cervical cancer cells. Environ Health Perspect 120:541-546.

Llabjani V, Trevisan J, Jones KC, Shore RF, Martin FL. 2011. Derivation by Infrared Spectroscopy with Multivariate Analysis of Bimodal Contaminant-Induced Dose-Response Effects in MCF-7 Cells. Environmental Science & Technology 45:6129-6135.

Maronpot RR, Montgomery CA, Boorman GA, McConnell EE. 1986. National Toxicology Program nomenclature for hepatoproliferative lesions of rats. Toxicol Pathol 14:263-273.

NTP. 1986. Toxicology and Carcinogenesis Studies of Decabromodiphenyl Oxide (CAS No: 1163-19-5) In F344/N Rats and B6C3F1 Mice (Feed Studies). (National Toxicology Program Technical Report Series No 309). Available: https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr309.pdf.

NTP. 2016. Toxicology Studies of a Pentabromodiphenyl Ether Mixture [DE-71 (Technical Grade)] in F344/N Rats and B6C3F1/N Mice and Toxicology and Carcinogenesis Studies of a Pentabromodiphenyl Ether Mixture [DE-71 (Technical Grade)] in Wistar Han [Crl:WI(Han)] Rats and B6C3F1/N Mice (Gavage Studies). (National Toxicology Program Technical Report Series No 589). Available: https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr589_508.pdf.

OEHHA. 2017. Chemical Listed Effective July 7, 2017 as Known to the State of California to Cause Cancer: Pentabromodiphenyl Ether Mixture [DE-71 (Technical Grade)] Available: https://oehha.ca.gov/proposition-65/crnr/chemical-listed-effective-july-7-2017-known-state-california-cause-cancer [accessed on July 24, 2020].

Pacyniak EK, Cheng X, Cunningham ML, Crofton K, Klaassen CD, Guo GL. 2007. The Flame Retardants, Polybrominated Diphenyl Ethers, Are Pregnane X Receptor Activators. Toxicological Sciences 97:94-102.

Pellacani C, Buschini A, Galati S, Mussi F, Franzoni S, Costa LG. 2012. Evaluation of DNA damage induced by 2 polybrominated diphenyl ether flame retardants (BDE-47 and BDE-209) in SK-N-MC cells. International journal of toxicology 31:372-379.

Pijnenburg AMCM, Everts JW, de Boer J, Boon JP. 1995. Polybrominated Biphenyl and Diphenylether Flame Retardants: Analysis, Toxicity, and Environmental Occurrence. In:

Reviews of Environmental Contamination and Toxicology: Continuation of Residue Reviews, (Ware GW, Gunther FA, eds). New York, NY:Springer New York, 1-26.

Sarkar D, Chowdhury JP, Singh SK. 2016. Effect of polybrominated diphenyl ether (BDE-209) on testicular steroidogenesis and spermatogenesis through altered thyroid status in adult mice. General and comparative endocrinology 239:50-61.

Takasu S, Ishii Y, Yokoo Y, Tsuchiya T, Kijima A, Kodama Y, et al. 2017. In vivo reporter gene mutation and micronucleus assays in gpt delta mice treated with a flame retardant decabromodiphenyl ether. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 816-817:7-11.

Thuresson K, Höglund P, Hagmar L, Sjödin A, Bergman A, Jakobsson K. 2006. Apparent half-lives of hepta- to decabrominated diphenyl ethers in human serum as determined in occupationally exposed workers. Environ Health Perspect 114:176-181.

US EPA. 2008. Toxicological Review of Decabromodiphenyl Ether (BDE-209) (CAS No. 1163-19-5) In Support of Summary Information on the Integrated Risk Information System (IRIS). Available: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/toxreviews/0035tr.pdf.

US EPA. 2017. Preliminary Information on Manufacturing, Processing, Distribution, Use, and Disposal: Decabromodiphenyl Ether (CASRN: 1163-19-5). Office of Chemical Safety and Pollution Prevention. Available: https://www.epa.gov/sites/production/files/2017-08/documents/decabde_-_use_information_-_8-7-17-clean.pdf.

US EPA. 2019. Regulation of Persistent, Bioaccumulative, and Toxic Chemicals under Section 6(h) of the Toxic Substances Control Act. Document ID EPA-HQ-OPPT-2019-0080-0001. Federal Registration Number 2019-14022. 40 CFR Part 751. Available: https://beta.regulations.gov/document/EPA-HQ-OPPT-2019-0080-0001 [accessed on August 4, 2020].

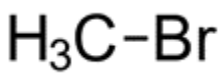
Wang Y, Chen T, Sun Y, Zhao X, Zheng D, Jing L, et al. 2019. A comparison of the thyroid disruption induced by decabrominated diphenyl ethers (BDE-209) and decabromodiphenyl ethane (DBDPE) in rats. Ecotoxicology and environmental safety 174:224-235.

Ward MH, Colt JS, Deziel NC, Whitehead TP, Reynolds P, Gunier RB, et al. 2014. Residential levels of polybrominated diphenyl ethers and risk of childhood acute lymphoblastic leukemia in California. Environ Health Perspect 122:1110-1116.

Zhai J, Geng X, Ding T, Li J, Tang J, Chen D, et al. 2019. An increase of estrogen receptor α protein level regulates BDE-209-mediated blood-testis barrier disruption during spermatogenesis in F1 mice. Environmental science and pollution research international 26:4801-4820.

Methyl bromide

(Bromomethane; 74-83-9)



Methyl bromide, also known as bromomethane, is an odorless gas with uses as a fumigant pesticide. The Montreal Protocol originally outlined the phase out of methyl bromide in most countries by the early 2000s due to its ozone-depleting actions, with the United States adopting an incremental phase out by 2005. However, through allowable exemptions, some uses of this pesticide are permitted. In California, approximately 1.8 million pounds were used in 2017, the most recent year for which data are available[9]. Specific uses included pre-planting soil fumigation, treatment of certain plants and trees, and post-harvest fumigation of commodities such as dried beans, dried fruit, and nuts[10]. These uses have occurred under two types of exemptions, critical uses and quarantine and pre-shipment uses[11].

Methyl bromide passed the human data screen, underwent a preliminary toxicological evaluation, and is being brought to the Carcinogen Identification Committee for consultation. This is a summary of the relevant studies identified during the preliminary toxicological evaluation.

Epidemiological data

- Stomach cancer
    - Prospective cohort study (Agricultural Health Study, AHS)
        - Cohort of more than 80,000 people: farmers and pesticide applicators (n>54,000) in Iowa and North Carolina and their spouses (n>30,000)
        - Enrollment period 1993 to 1997 (Alavanja et al. 1996)
        - Followed up until 2015 in Iowa and 2014 in North Carolina
        - Incident cancers identified through linkage to state cancer registries

[9] US EPA. Methyl Bromide. https://www.epa.gov/ods-phaseout/methyl-bromide (last updated on 5/28/2020; accessed on 8/3/2020)

[10] California Pesticide Information Portal (CalPIP) Application. Version 2019.04 (2017 PUR Data Update). Available at: https://calpip.cdpr.ca.gov/main.cfm (accessed on 8/4/2020).

[11] US EPA. Methyl Bromide. Full citation provided in footnote 9.

        - Exposure data collected prospectively through self-administered questionnaire at enrollment, additional take-home questionnaires, and follow-up telephone interview. At enrollment, participants reported ever/never use of 50 pesticides; further details gathered for 22 of the pesticides (i.e., years and days per year each pesticide was applied, use of personal protective equipment, pesticide application method).
        - Barry et al. (2012)
            - 14.6% of the applicators used methyl bromide, predominantly before enrollment.
            - Low methyl bromide use, unlagged exposure: relative risk (RR), 1.42; 95% confidence interval (CI), 1.51–3.95; 5 exposed cases
            - High methyl bromide use, unlagged exposure: RR, 3.13; 95% CI, 1.25–7.80; 10 exposed cases
            - Significant exposure-response association (*p*-value for trend = 0.02)
            - Similar results with exposure lagged 15 years
    - Nested case-control study conducted in California Hispanic farm workers within the United Farm Workers of America (UFW) cohort: (Mills and Yang 2007)
        - Study period: 1998–2003
        - 100 cases, 210 controls
        - Ever vs never methyl bromide use: Odds Ratio (OR), 1.01; 95% CI, 0.59–1.74
        - Lowest (1–133 pounds [lbs]) vs no use: OR, 0.56; 95% CI, 0.25–1.24
        - Middle (134–4856 lbs) vs no use: OR, 0.99; 95% CI, 0.50–1.99
        - Highest (4857–280,130 lbs) vs no use: OR, 1.33; 95% CI, 0.67–2.67
- Prostate cancer
    - Prospective cohort study (AHS) [see above for details]
        - Barry et al. (2012)
            - Follow-up through 2007, 280 methyl bromide exposed cases
            - No suggestion of increasing risk of prostate cancer with increasing methyl bromide use (*p*-value for trend = 0.90)
            - Non-significant elevated risk of prostate cancer with methyl bromide use among those with a family history of prostate

cancer, but the interaction with a family history did not achieve statistical significance.

- Alavanja et al. (2003)
  - 54,766 non-cases, 566 prostate cancer cases (approximately 1% of cohort)
  - Follow-up through 1999
  - ORs were computed for individual pesticides and for pesticide use patterns identified by factor analysis
  - Methyl bromide was used by approximately 12% of the cohort
  - Methyl bromide showed no association with prostate cancer in crude ever vs never use analysis: OR, 1.10; 95% CI, 0.85–1.36; 84 exposed cases.
  - Elevated risks were present in the two highest cumulative exposure score categories compared with the reference category (no exposure) (*p*-value for trend = 0.004)
    - Category I (0.1–33.3 percentile of use): OR, 1.01; 95% CI, 0.66–1.56; 23 cases
    - Category II (33.4–66.7 percentile of use): OR, 0.76, 95% CI, 0.47–1.25; 22 cases
    - Category III (66.8–83.3 percentile of use): OR, 0.70, 95% CI, 0.38–1.28; 11 cases
    - Category IV (83.4–91.6 percentile of use): OR, 2.73; 95% CI, 1.18–6.33; 6 cases
    - Category V (> 91.6 percentile of use): OR, 3.47; 95% CI, 1.37–8.76; 5 cases
  - Significant associations were unchanged when other pesticides were added to the statistical model (data not shown)
  - Methyl bromide was the only pesticide from the enrollment questionnaire that showed a significant linear trend (p = 0.004) with prostate cancer risk.
    - This pattern was consistent regardless of type of applicator, state, exposure metric, or tumor grade (p-values for linear trend presented):
      - Private applicators in both states (p = 0.05 in North Carolina; p = 0.04 in Iowa)
      - Commercial applicators in Iowa (p = 0.01)
      - Frequency of use (p = 0.02)
      - Lifetime application days (p = 0.02)

- Tumor grade (p = 0.03 for well-differentiated; p = 0.04 for poorly differentiated tumors) (data not shown).
- This significant exposure-response trend was almost entirely due to the elevated risk in the two highest methyl bromide exposure categories.

- Nested case-control study: Mills and Yang (2003)
  - Conducted in California within a large predominantly Hispanic cohort, the UFW labor union (1988–1999)
  - 222 cases, 1110 age-matched controls
  - High vs low exposure to methyl bromide: OR, 1.16; 95% CI, 0.77–1.75
    - Similar associations when analyzed by quartiles of methyl bromide exposure
    - No evidence of an exposure-response association (p-value for trend = 0.25)
- Population-based case-control study: Cockburn et al. (2011)
  - Conducted in the Central Valley, California (2005–2006)
  - 173 cases, 162 controls
  - Past ambient exposures to pesticides/fungicides were derived from residential history and independently recorded pesticide and land-use data, using geographic information systems (GIS) model
  - ORs calculated for all of the originally selected participants (e.g., “population”) based on diagnosis address/tax assessor parcel centroids and for subjects participating in the study based on their residential diagnosis/contact address only (e.g., “sample”)
  - Ever exposed to methyl bromide: OR, 1.62; 95% CI, 1.02–2.59
    - Population: OR, 1.47; 95% CI, 1.22–1.7
    - Sample: OR, 1.44; 95% CI, 0.93–2.22
    - Low exposure: OR, 1.81; 95% CI, 1.03–3.18
    - High exposure: OR, 1.45; 95% CI, 0.82–2.57
    - No evidence of an exposure-response association (p-value for trend = 0.10)
  - Methyl bromide exposure at the diagnosis address: OR, 3.60; 95% CI, 1.62–8.20
    - There was evidence of exposure-response: OR, 2.75 for ‘‘low’’ exposure; OR, 4.01 for ‘‘high’’ exposure; p = 0.009 for the difference

- Kidney cancer
    - Prospective cohort study (AHS) [see above for details]
        - Andreotti et al. (2020)
            - 308 renal cell carcinoma cases
            - No significant association with methyl bromide exposure and no significant exposure-response association
            - Associations were similar whether the exposure was unlagged or lagged 10 or 20 years. For the highest category of methyl bromide exposure:
                - Unlagged exposure: RR, 1.26; 95% CI, 0.74–2.14
                - 10-year lagged exposure: RR, 1.27; 95% CI, 0.74–2.18
                - 20-year lagged exposure: RR, 1.28; 95% CI, 0.74–2.23
        - Barry et al. (2012)
            - 25 methyl bromide exposed cases
            - No association between intensity weighted lifetime days and kidney cancer risk, regardless of whether methyl bromide exposure was unlagged or lagged 15 or 20 years
            - No exposure response trend (*p*-value for trend > 0.05)
- Breast cancer
    - Population-based case-control study: Mills et al. (2019)
        - Enrolled Latina residents of the San Joaquin Valley, California (2008-2009)
        - 101 breast cancer cases, 88 controls
        - Exposure to methyl bromide assessed using the California Department of Pesticide Regulation (DPR) Pesticide Usage Database and self-reported residence and work history in agricultural areas obtained from the telephone interview.
        - A total of 19 agro-chemicals were included in the multivariate adjusted odds ratio.
        - High exposure to methyl bromide: OR, 2.06; 95% CI, 0.53–8.06
- Other adult cancers
    - Prospective cohort study (AHS) [see above for details]: Barry et al. (2012)
        - No association between methyl bromide use and cancers of the lymphohematopoietic system (non-Hodgkin’s lymphoma, leukemia,

Hodgkin's lymphoma, and multiple myeloma), oral cavity, colon, rectum, lung, bladder, or melanoma
        - No significant exposure-response relationships

- Childhood cancer
    - Population-based case-control study: Reynolds et al. (2005)
        - Enrolled cases of early childhood cancer (age 0–4 years) among California children born between 1990 and 1997
        - 2189 case children, 4335 controls matched for birth date and sex
        - Estimated the *in utero* exposure potential from methyl bromide use in the 9 months before birth within a half mile of the maternal residence and mother's residential proximity to agricultural applicators of pesticide at the time of the child's birth
        - Reported on "all sites combined", leukemias, and central nervous system tumors
            - No associations with methyl bromide exposure
    - Ecologic study: Reynolds et al. (2002)
        - Enrolled children <15 years old diagnosed with invasive cancer in California 1988–1994
        - California DPR GIS information was used to assign summary population, exposure, and outcome attributes at the block group level
        - Reported on "all childhood cancers", leukemia, and glioma
            - No associations with methyl bromide exposure

Animal carcinogenicity data

- Short-term gavage studies in rats:
    - 90-day gavage studies in male and female Wistar rats: Danse et al. (1984)
    - 10 rats/sex/dose; exposure beginning at weaning (i.e., postnatal day 21) (40-60 grams bodyweight [g bw]); 0, 0.4, 2, 10, or 50 milligrams per kilogram (mg/kg) bodyweight in peanut oil administered for 5 days per week (d/wk) for 90 days
    - Male rat results:
        - No statistically significant difference in mortality rates compared to controls.
        - Statistically significant decrease in body weight in the 50 mg/kg-bw treatment group compared to control.

- Reported by original authors: Increase in forestomach squamous cell carcinoma (Table 13)
- The forestomach tumors reported by Danse et al. (1984) were questioned by others (NTP 1992; Boorman et al. 1986). According to US EPA (1989), "A panel of NTP *[National Toxicology Program]* scientists reevaluated the histological slides and concluded that the lesions were hyperplasia and inflammation rather than neoplasia."
- Danse et al. (1984) also reported a dose-related increase in squamous cell hyperplasia of the forestomach.

Table 13. Tumor incidence in male Wistar rats exposed to methyl bromide via gavage in a 90-day study (Danse et al. 1984)

Organ	Tumor type[1]	Gavage Dose (mg/kg bw)					Exact trend test *p*-value
		0	0.4	2	10	50	
Forestomach	Papilloma	0/10	0/10	0/10	0/10	2/10	NS
	Squamous cell carcinoma	0/10	0/10	0/10	0/10	7/10**	$p < 0.001$

Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): ** $p < 0.01$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

[1] These tumor findings were later questioned by others. "A panel of NTP scientists reevaluated the histological slides and concluded that the lesions were hyperplasia and inflammation rather than neoplasia." (US EPA 1989)

- Female rat results:
  - No statistically significant difference in mortality rates or body weight compared to controls
  - Increase in squamous cell forestomach carcinoma (Table 14)
  - The forestomach tumors reported by Danse et al. (1984) were questioned by others (NTP 1992; Boorman et al. 1986). According to US EPA (1989), "A panel of NTP scientists reevaluated the histological slides and concluded that the lesions were hyperplasia and inflammation rather than neoplasia."
  - Danse et al. (1984) also reported a dose-related increase in squamous cell hyperplasia of the forestomach

Table 14. Tumor incidence in female Wistar rats exposed to methyl bromide via gavage in a 90-day study (Danse et al. 1984)

Organ	Tumor type[1]	Gavage Dose (mg/kg bw)					Exact trend test *p*-value
		0	0.4	2	10	50	
Forestomach	Squamous cell carcinoma	0/10	0/10	0/10	0/10	6/10**	$p < 0.001$

Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): ** $p < 0.01$. Exact trend test conducted by OEHHA.
[1] These tumor findings were later questioned by others. "A panel of NTP scientists reevaluated the histological slides and concluded that the lesions were hyperplasia and inflammation rather than neoplasia." (US EPA 1989)

- 25-week gavage study with interim sacrifices at 13, 17, or 21 weeks in male Wistar rats: Boorman et al. (1986)
  - 15 rats/dose; exposure beginning at 6 weeks old (105–120 g bw); 0 or 50 mg/kg-bw/day in peanut oil
    - One out of eleven treated rats examined at 25 weeks presented with forestomach squamous cell carcinoma.
    - By 21 weeks, all treated rats had pseudoepitheliomatous hyperplasia characterized by hyperkeratosis, acanthosis, and epithelial peg formation.
- 120-day gavage study with interim sacrifices at 30, 60, or 90 days in male Wistar rats: Hubbs (1986), as reported by US EPA (2007)
  - 10 rats/dose/time point; 0, 25, or 50 mg/kg in peanut oil; 5 days/week; age at start of study unknown
    - Additional groups of animals were treated with 50 mg/kg methyl bromide 5 days/week for 90 days, followed by a recovery period of 30 or 60 days before sacrifice.
  - Statistically significant decrease in body weight in the treated mice compared to controls.
  - No treatment-related tumor findings were reported.

- Long-term inhalation studies in rats:
  - 29-month inhalation studies in male and female Wistar rats: Reuzel et al. (1991)
    - 90 rats/sex/dose; 0, 3, 30, or 90 ppm methyl bromide for 6h/d for 5d/wk for 29 months

        - Statistically significant decrease in mortality (males only) and body weight in rats treated with 90 ppm methyl bromide.
    - No treatment-related tumor findings in male or female rats.

- Long-term inhalation studies in mice:
    - 103-week inhalation studies in male and female B6C3F1 mice: NTP (1992)
        - 70 mice/sex/dose; 0, 10, 33, or 100 ppm for 6 h/d, 5 d/wk for up to 103 weeks
            - High-dose groups in the male and female studies were discontinued after 29 weeks due to mortality issues.
            - No treatment-related tumor findings in male or female mice.

Other Relevant Data

Key characteristics of carcinogens

- Is electrophilic
    - DNA-alkylation: [C^{14}]methyl bromide binds to DNA in rat liver, lung, stomach and forestomach after both oral and inhalation exposure (Gansewendt et al. 1991)
        - Adenine and guanine adducts were measured using HPLC or GC-MS.
        - The highest levels of methylated guanines were found in the stomach and forestomach.
- Is genotoxic
    - Mutations
        - Induces forward mutations at the TK and HPRT loci in L5178Y mouse lymphoma cells (Kramers et al. 1985).
        - Negative in SA7 adenovirus transformation assay in Syrian hamster embryo cells (Hatch et al. 1983).
        - Active in the fluctuation test using the bacteria *Klebsiella pneumoniae*, indicative of a frameshift mutation (Kramers et al. 1985).
        - Mutagenic in *Salmonella typhimurium* TA100 (base-pair substitution) (Kramers et al. 1985; Moriya et al. 1983; NTP 1992), and TA1535 (base-pair substitution) (Moriya et al. 1983); not mutagenic in TA98 (frameshift mutation) (Kramers et al. 1985;

Moriya et al. 1983; NTP 1992), TA1537 or TA1538 (frameshift mutation) (Moriya et al. 1983).
        - Mutagenic in *E. coli* WP2 hcr (Moriya et al. 1983) and *E. coli* Sd4 (Djalali-Behzad et al. 1981).
        - Active in the sex-linked recessive lethal test (Kramers et al., 1985) and somatic recombination (Katz 1987), and gives a high hypermutability response in *Drosophila melanogaster* (Ballering et al. 1994).
    - Chromosomal damage
        - Increased micronuclei (MN) formation in lymphocytes and oropharyngeal cells of fumigation workers (Calvert et al. 1998) and in peripheral erythrocytes of mice by inhalation (NTP 1992).
        - Induced sister chromatid exchange (SCE) in human lymphocytes (Garry et al. 1990).
        - Induced chromosomal aberrations in human lymphocytes (Garry et al. 1990).
        - Induced SCE in bone marrow cells and MN *in vivo* in peripheral erythrocytes of female mice after 14 days of inhalation exposure (negative in 12-week studies) (NTP 1992).
- Alters DNA repair or causes genomic instability
    - Decreased O^6-alkylguanine-DNA alkyltransferase activity in rats *in vivo* (Pletsa et al. 1998).
- Induces epigenetic alterations
    - Increased DNA methylation in liver, lung, stomach and forestomach tissues from rats *in vivo* (Gansewendt et al. 1991).
- Induces oxidative stress
    - Inhibition of glutathione-s-transferase (GST) activities and decreased glutathione levels in different regions of the brain in methyl bromide-treated rats (Davenport et al. 1992).
- Is immunosuppressive
    - Decrease in white blood cell count of mice fed methyl bromide at 30 ppm for four weeks (Mostafa et al. 1992).

Structure-activity Considerations

- Structurally similar to two Proposition 65 carcinogens, methyl iodide and dichloromethane.

Reviews

- US EPA (1989)
- IARC (1999)
- US EPA (2007)

References cited in "Methyl bromide"

Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF, et al. 1996. The Agricultural Health Study. Environmental Health Perspectives 104:362-369.

Alavanja MC, Samanic C, Dosemeci M, Lubin J, Tarone R, Lynch CF, et al. 2003. Use of agricultural pesticides and prostate cancer risk in the Agricultural Health Study cohort. American journal of epidemiology 157:800-814.

Andreotti G, Beane Freeman Laura E, Shearer Joseph J, Lerro Catherine C, Koutros S, Parks Christine G, et al. 2020. Occupational Pesticide Use and Risk of Renal Cell Carcinoma in the Agricultural Health Study. Environmental Health Perspectives 128:067011.

Ballering LAP, Nivard MJM, Vogel EW. 1994. A deficiency for nucleotide excision repair strongly potentiates the mutagenic effectiveness of methyl bromide in Drosophila. Mutagenesis 9:387-389.

Barry KH, Koutros S, Lubin JH, Coble JB, Barone-Adesi F, Beane Freeman LE, et al. 2012. Methyl bromide exposure and cancer risk in the Agricultural Health Study. Cancer Causes Control 23:807-818.

Boorman GA, Hong HL, Jameson CW, Yoshitomi K, Maronpot RR. 1986. Regression of methyl bromide-induced forestomach lesions in the rat. Toxicology and applied pharmacology 86:131-139.

Calvert GM, Talaska G, Mueller CA, Ammenheuser MM, Au WW, Fajen JM, et al. 1998. Genotoxicity in workers exposed to methyl bromide. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 417:115-128.

Cockburn M, Mills P, Zhang X, Zadnick J, Goldberg D, Ritz B. 2011. Prostate cancer and ambient pesticide exposure in agriculturally intensive areas in California. American journal of epidemiology 173:1280-1288.

Danse LH, van Velsen FL, van der Heijden CA. 1984. Methylbromide: carcinogenic effects in the rat forestomach. Toxicology and applied pharmacology 72:262-271.

Davenport CJ, Ali SF, Miller FJ, Lipe GW, Morgan KT, Bonnefoi MS. 1992. Effect of methyl bromide on regional brain glutathione, glutathione-S-transferases, monoamines, and amino acids in F344 rats. Toxicology and applied pharmacology 112:120-127.

Djalali-Behzad G, Hussain S, Osterman-Golkar S, Segerbäck D. 1981. Estimation of genetic risks of alkylating agents: VI. Exposure of mice and bacteria to methyl bromide. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 84:1-9.

Gansewendt B, Foest U, Xu D, Hallier E, Bolt HM, Peter H. 1991. Formation of DNA adducts in F-344 rats after oral administration or inhalation of [14C]methyl bromide. Food and Chemical Toxicology 29:557-563.

Garry VF, Nelson RL, Griffith J, Harkins M. 1990. Preparation for Human Study of Pesticide Applicators: Sister Chromatid Exchanges and Chromosome Aberrations in Cultured Human Lymphocytes Exposed to Selected Fumigants. Teratogenesis, Carcinogenesis, and Mutagenesis 10:21-29.

IARC. 1999. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 71. Re-evaluation of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide (Part 1, Part 2, Part 3). Methyl Bromide. Available: https://publications.iarc.fr/_publications/media/download/2311/b814f78f6b80a455b26f7341d3e9d118ab13fb8b.pdf.

Katz AJ. 1987. Inhalation of methyl bromide gas induces mitotic recombination in somatic cells of Drosophila melanogaster. Mutation Research Letters 192:131-135.

Kramers P, Voogd C, Knaap A, Van Der Heijden C. 1985. Mutagenicity of methyl bromide in a series of short-term tests. Mutation Research/Genetic Toxicology 155:41-47.

Mills PK, Yang R. 2003. Prostate cancer risk in California farm workers. Journal of occupational and environmental medicine 45:249-258.

Mills PK, Yang RC. 2007. Agricultural exposures and gastric cancer risk in Hispanic farm workers in California. Environmental research 104:282-289.

Mills PK, Dodge JL, Bush J, Thompson Y, Shah P. 2019. Agricultural Exposures and Breast Cancer Among Latina in the San Joaquin Valley of California. Journal of occupational and environmental medicine 61:552-558.

Moriya M, Ohta T, Watanabe K, Miyazawa T, Kato K, Shirasu Y. 1983. Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutation Research/Genetic Toxicology 116:185-216.

Mostafa IY, Zayed SMAD, Hazzaa NI, Hegazi B. 1992. Bioavailability to rats and toxicity of bound residues in bean seeds fumigated with 14C-methyl bromide. Journal of Environmental Science and Health, Part B 27:407-417.

NTP. 1992. Toxicology and Carcinogenesis Studies of Methyl Bromide (CAS NO. 74-83-9) in B6C3F1 Mice (Inhalation Studies) (Technical Report Series No 385). US Department of Health and Human Services, NTP, Research Triangle Park, NC. Available: https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr385.pdf.

Pletsa V, Steenwinkel M-JST, van Delft JHM, Baan RA, Kyrtopoulos SA. 1998. Methyl bromide causes DNA methylation in rats and mice but fails to induce somatic mutations in λlacZ transgenic mice. Cancer Letters 135:21-27.

Reuzel PGJ, Dreef-van der Meulen HC, Hollanders VMH, Kuper CF, Feron VJ, van der Heijden CA. 1991. Chronic inhalation toxicity and carcinogenicity study of methyl bromide in wistar rats. Food and Chemical Toxicology 29:31-39.

Reynolds P, Von Behren J, Gunier RB, Goldberg DE, Hertz A, Harnly ME. 2002. Childhood cancer and agricultural pesticide use: an ecologic study in California. Environ Health Perspect 110:319-324.

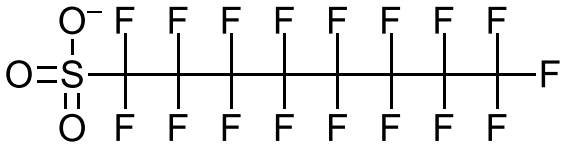
Reynolds P, Von Behren J, Gunier RB, Goldberg DE, Harnly M, Hertz A. 2005. Agricultural pesticide use and childhood cancer in California. Epidemiology 16:93-100.

US EPA. 1989. Bromomethane; CASRN 74-83-9. (Chemical Assessment Summary). Integrated Risk Information System (IRIS). Available: https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0015_summary.pdf.

US EPA. 2007. Provisional Peer Reviewed Toxicity Values for Bromomethane (CASRN 74-83-9). Cincinnati, OH: Superfund Health Risk Technical Support Center, National Center for Environmental Assessment, Office of Research and Development. Available: https://cfpub.epa.gov/ncea/pprtv/documents/Bromomethane.pdf.

Perfluorooctane sulfonate (PFOS) and its salts and transformation and degradation precursors

(Perfluorooctane sulfonic acid; CAS No. 1763-23-1)



PFOS and its salts are perfluorinated organic compounds with surfactant properties. Common salts of PFOS include the ammonium, diethanolamine, potassium, and lithium forms. PFOS can be released from several fluorochemicals, such as perfluorooctane sulfonamide (PFOSA), N-ethyl perfluorooctane sulfonamidoethanol (N-EtFOSE), N-ethylperfluorooctane sulfonamido acetic acid (EtFOSAA), and N-ethylperfluorooctane sulfonamide (N-EtFOSA) by transformation or degradation processes.

PFOS and its salts and precursors have been used in the manufacture of a wide array of industrial and household products, including firefighting foams, and stain- or water-resistant coatings for cookware, fabrics, leather, food packaging and paper products. The principal US manufacturer of PFOS phased out its production of the chemical in the early 2000s; however, PFOS and PFOS commercial products are still manufactured in some parts of the world and may be imported to California. There is also continued production and use of chemicals that can be transformed or degraded to release PFOS.

PFOS is resistant to thermal, chemical and biological degradation and is persistent in the environment. It is readily absorbed into biota and has a tendency to accumulate with repeated exposure. It is present in fish and other foods, and has been found in drinking water supplies in California and other parts of the US. Biomonitoring California studies indicate widespread exposure of the population to PFOS. For example, PFOS was detected in 98% of blood samples analyzed from 425 participants in the 2018 California Regional Exposure Study, Los Angeles County (CARE-LA)[12].

"PFOS and its salts and transformation and degradation precursors" passed the animal data screen in 2010 and was brought to the Carcinogen Identification Committee (CIC) for consultation. At that time, the CIC recommended that PFOS and its salts and transformation and degradation precursors be placed in the 'medium' priority group for development of hazard identification materials. Since 2010, additional epidemiology

[12] From Biomonitoring California, Results for Perfluorochemicals (PFCs) (accessed June 11, 2020)

data, animal carcinogenicity data, and mechanistic data have become available. In 2020, "PFOS and its salts and transformation and degradation precursors" passed the human and animal data screens, underwent a preliminary toxicological evaluation, and are being brought again to the CIC for consultation. This is a summary of the relevant studies identified during the preliminary toxicological evaluation. Studies identified since consultation with the CIC in 2010 are marked with an asterisk (*).

Epidemiological data

- Breast cancer
  - *Nested case-control study of breast cancer risk in the French E3N cohort: Mancini et al. (2020)
    - E3N (Etude Epidémiologique auprès de femmes de l'Education Nationale) is a prospective cohort study of French women insured by a national health insurance program covering workers from the French National Education System.
    - 281 breast cancer cases were identified for which at least three aliquots of serum were available in the biobank. PFOS was measured in these serum samples.
    - Increased risk of estrogen receptor positive (ER^+) breast cancer for the 3rd quartile (OR, 2.22; 95% CI, 1.05–4.69) and 4th quartile (OR, 2.33; 95% CI, 1.11–4.90) of PFOS levels compared to 1^{st} quartile, $p_{trend} = 0.04$.
    - Increased risk of progesterone receptor $(PR)^+$ breast cancer for the 3rd quartile (OR, 2.47; 95% CI, 1.07–5.65) and 4th quartile (OR, 2.76; 95% CI, 1.21–6.30), $p_{trend} = 0.02$.
    - When considering receptor-negative breast cancer, only the 2nd quartile of PFOS was associated with increased risk (ER^-: OR, 15.40; 95% CI, 1.84–129.19; PR^-: OR, 3.47; 95% CI, 1.29–9.15).
  - *Hospital-based case-control study and the risk of breast cancer in Taiwanese women from 2013 to 2015: Tsai et al. (2020)
    - PFOS was measured in the plasma samples collected from 120 breast cancer patients and 119 controls.
    - Increased risk of breast cancer in women ≤50 years old (OR, 2.34; 95% CI, 1.02–5.38) per natural log unit increase PFOS.
    - After stratifying the ER status and age group, a positive association for PFOS concentrations with respect to the risk of ER^+ tumors for ≤50 years age group was observed (ER^+: OR, 3.25; 95% CI, 1.29–8.23).

- *Nested case-control study of the risk of breast cancer in women exposed to perfluoroalkyl and polyfluoroalkyl substances (PFASs) *in utero* in the California Child Health and Development Studies pregnancy cohort: Cohn et al. (2019)
    - PFOS and EtFOSAA (a PFOS precursor) measured in archived maternal perinatal blood samples in 102 daughter breast cancer cases diagnosed by age 52 (daughters born 1959-1967; 54-year follow-up).
    - PFOS was associated with reduced risk of breast cancer (OR, 0.3; 95% CI, 0.1–0.9).
    - Daughters were at increased risk of breast cancer when their mothers had both higher levels of EtFOSAA (OR, 3.3; 95% CI, 1.2–8.8) and higher total cholesterol (OR, 3.6; 95% CI, 1.1–11.6); interaction was statistically significant ($p < 0.05$).
- *Nested case-control study in the Danish National Birth Cohort to investigate the effect of polymorphisms in xenobiotic and estrogen metabolizing genes on the risk of breast cancer: Ghisari et al. (2017)
    - PFOS and PFOSA (a PFOS precursor) measured in blood samples of 178 cases and 233 controls.
    - Positive association between PFOS and risk of breast cancer observed for wild-type aromatase (*CYP19*) genotype (RR, 6.42; 95% CI, 1.08–38.3; 36 cases) but not observed with any genotypes of the other genes studied (*CYP1A1, CYP1B1, COMT, or CYP17*).
    - Positive association between PFOSA and risk of breast cancer overall (RR, 1.25; 95% CI, 1.10–1.56; 158 cases)
        - Risk varied between different genotypes, with significantly increased risk confined to carriers of the following genotypes:
            - COMT (catechol-O-methyltransferase) (homozygous Val158Met) (RR, 2.04; 95% CI, 1.27–3.28; 45 cases).
            - CYP17 (homozygous A1 alleles) (RR, 2.02; 95% CI, 1.29–3.16; 44 cases).
            - CYP19 (wild-type) (RR, 2.08; 95% CI, 1.06–4.09; 35 cases).
- *Case-control study of breast cancer in Inuit women from Greenland during 2000-2003 and 2011-2014: Wielsoe et al. (2017)
    - Participants were recruited during 2000–2003 and 2011–2014. The participants recruited during 2000-2003 were included in Bonefeld-Jørgensen et al. (2011). Controls recruited 2011-2014 were

hospital-based. Altogether, this study included 77 breast cancer patients and 84 matched controls.
        - PFOS measured in serum.
        - Increase in breast cancer risk with increasing PFOS
            - Continuous: OR, 1.02; 95% CI, 1.01–1.03; 77 cases.
            - 2nd tertile compared to 1st: OR, 3.13; 95% CI, 1.20–8.15; 25 cases.
            - 3rd tertile compared to 1st: OR, 5.50; 95% CI, 2.19–13.84; 44 cases.
    - *Nested case-control study of breast cancer in the Danish National Birth Cohort: Bonefeld-Jorgensen et al. (2014)
        - Cases were 250 women diagnosed after recruitment.
        - Controls taken at random from entire cohort.
        - PFOS and PFOSA (a PFOS precursor) measured in blood samples drawn in early pregnancy among women (mean age ~30 years) participating in 1996–2002.
        - No clear association with breast cancer incidence and any level of PFOS exposure (RR, 0.99; 95% CI, 0.98–1.01; 221 cases).
        - Significant elevated breast cancer risk for PFOSA in the 5th quintile compared to the lowest quintile (RR, 1.89; 95% CI, 1.01–3.54; 51 cases).
    - *Population-based case-control study of breast cancer in the Inuit female population of Greenland during 2000-2003: Bonefeld-Jorgensen et al. (2011)
        - PFOS measured in the serum of 31 breast cancer cases and 115 controls.
        - Breast cancer risk associated with serum levels of PFOS (OR, 1.03; 95% CI, 1.001–1.07; p = 0.05; 9 cases) (median 45.6 ng/ml).

- Bladder cancer:
    - *Prospective cohort study of cancer risk in the general Danish population: Eriksen et al. (2009)
        - Cases ascertained through the Danish Cancer Registry and the Danish Pathology Data Bank.
        - 680 men and 92 women randomly selected as comparison group
        - PFOS measured in serum samples.
        - No increase in incidence rate ratios (IRR) for bladder cancer related to PFOS exposure.
    - Retrospective occupational cohort study of bladder cancer incidence in workers in Decatur, Alabama: Alexander and Olsen (2007); EFSA (2008) p. 77

        - Same population and exposure assessment as Alexander et al. (2003) [see below].
        - Cases were ascertained by questionnaire and verified by physician.
        - No increased risk of bladder cancer associated with PFOS exposure compared to expected cancer rates in US population for those exposed ≥10 years (Standardized incidence ratio [SIR], 1.43; 95% confidence interval [CI], 0.16–5.15; 2 cases) or using the cohort as an internal referent population for those exposed ≥10 years (Risk ratio [RR], 1.52; 95% CI, 0.21–10.99; 2 cases) (Alexander and Olsen 2007).
        - No increased risk of bladder cancer in workers ever employed in a high-exposure job (SIR, 1.74; 95% CI, 0.64-3.79; 6 cases) compared to expected cancer rates in the state of Alabama.
    - Retrospective occupational cohort study of cancer mortality in workers at a perfluorooctanesulphonyl fluoride-based fluorochemicals production facility in Decatur, Alabama: Alexander et al. (2003); EFSA (2008) p. 77
        - Cohort enumerated from work history records. Eligible subjects were followed from the day they accrued 365 days of employment until death or 12/31/1998.
        - Exposure assessed through a job exposure matrix based on work history records of the study cohort. Matrix was constructed by taking PFOS serum measurements from a random sample of participants.
        - Outcome was ascertained by death certificates coded by licensed oncologist using International Statistical Classification of Diseases and Related Health Problems (ICD) codes.
        - Increased risk of death from bladder cancer in workers ever employed in a high-exposure job (Standardized mortality ratio [SMR], 12.77; 95% CI, 2.63-37.35; 3 cases) compared to expected cancer rates in the state of Alabama.
- Prostate cancer
    - *Population-based case-control study and risk of prostate cancer in Sweden during 2007-2011: Hardell et al. (2014)
        - PFOS measured in the blood samples from 201 prostate cancer patients and 186 matched controls.
        - No association with prostate cancer overall (Odds ratio [OR], 1.0; 95% CI, 0.6–1.5; 109 cases).
        - Increased risk in cases with a first degree relative reporting prostate cancer and PFOS > median levels compared to cases with no

heredity and PFOS ≤ median (OR, 2.7; 95% CI, 1.04–6.8; 20 cases).

- *Prospective cohort study of cancer risk in the general Danish population: Eriksen et al. (2009) [see above for details]
  - Non-significant increase in risk of prostate cancer in the upper quartiles (Q2, Q3, and Q4) of PFOS compared to the lowest quartile (Q1), with very similar IRRs.
    - IRR for Q2 compared to Q1, 1.35; 95% CI, 0.97–1.87.
    - IRR for Q3 compared to Q1, 1.31; 95% CI, 0.94–1.82.
    - IRR for Q4 compared to Q1, 1.38; 95% CI, 0.99–1.93.

- Liver cancer
  - *Prospective cohort study of cancer risk in the general Danish population: Eriksen et al. (2009) [see above for details]
    - No increase in IRR for liver cancer related to PFOS exposure.
  - Retrospective occupational cohort study of cancer mortality in Decatur, Alabama: Alexander et al. (2003); EFSA (2008) p. 77 [see above for details]
    - No associations with death from cancer of the biliary passages and liver.

- Other cancers
  - *Cross-sectional study of colorectal cancer in an Appalachian population during 2005-2006: Innes et al. (2014)
    - PFOS measured in the blood samples from C8 Health project participants, along with a comprehensive health survey.
    - Inverse (i.e., negative) association was found between PFOS blood levels (in quartiles) and colorectal cancer incidence.
      - Q2 vs Q1 (i.e., the lowest quartile) (OR, 0.38; 95% CI, 0.25–0.59)
      - Q3 vs Q1 (OR, 0.27; 95% CI, 0.17–0.42)
      - Q4 vs Q1 (OR, 0.24; 95% CI, 0.16–0.37)
  - *Cross-sectional study of malignant cancers [type not specified] in adults living in Greece: Vassiliadou et al. (2010)
    - PFOS measured in 182 individuals in blood samples collected at three different locations (40 cancer patients).
    - No significant difference in PFOS values found between cancer and non-cancer groups.
  - *Prospective cohort study of cancer risk in the general Danish population: Eriksen et al. (2009) [see above for details]
    - No increase in IRR for pancreatic cancer related to PFOS exposure.

- Retrospective occupational cohort study of cancer mortality in Decatur, Alabama: Alexander et al. (2003); EFSA (2008) p. 77 [see above for details]
    - No associations with death from all malignant neoplasms; or cancers of the digestive organs and peritoneum; esophagus; respiratory system; bronchus, trachea, lung; other urinary organs; malignant melanoma; or the lymphatic and hematopoietic systems.

Animal carcinogenicity data

- Long-term feeding studies in rats
    - 104-week studies of potassium PFOS (K-PFOS) in male and female Sprague-Dawley-derived Crl:CD (SD) IGS BR rats: Butenhoff et al. (2012); Thomford (2002)
        - Increases in hepatocellular adenoma (by pairwise comparison and trend) and pancreatic islet cell carcinoma (by trend) in male rats (Table 15).
        - Increases in hepatocellular adenoma, and adenoma and carcinoma combined (by pairwise comparison and trend), and non-significant increases in rare thyroid follicular cell adenoma and carcinoma combined (Dinse et al. 2010; NTP 2019a), and mammary fibroadenoma (by pairwise comparison; low-dose only) in females (Table 16).

Table 15. Tumor incidence in male Crl:CD (SD) IGS BR rats exposed to K-PFOS in feed for 104 weeks (Thomford 2002)

Organ	Tumor type (day of occurrence of first tumor)	Concentration in feed (ppm)					Exact trend test *p*-value
		0	0.5	2	5	20	
Liver	Adenoma (day 506)	0/41	3/44	3/47	1/44	7/43**	$p < 0.01$
Pancreas	Islet cell adenoma (day 513)	4/41	4/42	4/46	4/44	4/42	NS
	Islet cell carcinoma (day 542)	1/38	2/42	2/44	5/44	5/40	$p < 0.05$
	Adenoma or carcinoma (day 513)	5/41	6/42	6/46	8/44	9/42	NS

Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): ** $p < 0.01$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

Table 16. Tumor incidence in female Crl:CD (SD) IGS BR rats exposed to K-PFOS in feed for 104 weeks (Thomford 2002)

Organ	Tumor type (day of occurrence of first tumor)	Concentration in feed (ppm)					Exact trend test *p*-value
		0	0.5	2	5	20	
Liver	Adenoma (day 653)	0/28	1/29	1/16	1/31	5/32*	$p < 0.01$
	Carcinoma (day 653)	0/28	0/29	0/16	0/31	1/32	NS
	Adenoma or carcinoma (day 653)	0/28	1/29	1/16	1/31	6/32*	$p < 0.01$
Thyroid[1]	Follicular cell adenoma (day 671)	0/26	0/25	0/14	2/26	1/30	NS
	Follicular cell carcinoma (day 671)	0/26	0/25	0/14	1/26	0/30	NS
	Adenoma or carcinoma (day 671)	0/26	0/25	0/14	3/26	1/30	NS
Mammary	Fibroadenoma (day 229)	20/60	27/50*	19/49	24/49	11/60	NS

Tumor incidence is expressed as the number of tumor-bearing animals over the number of animals alive at the time of first occurrence of the tumor. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).
[1]Thyroid follicular cell adenomas and carcinomas are both rare in female SD rats (adenoma: 0.21% and carcinoma: 0.43% (Dinse et al. 2010); adenoma: 0%, carcinoma: 0.45% (NTP 2019a)).

- Studies with 52-week exposure and additional observation until week 104 in male and female Crl:CD (SD) IGS BR rats: Butenhoff et al. (2012); Thomford (2002)
    - Increase in thyroid follicular cell adenomas in males (by pairwise comparison) (Table 17).
    - No treatment-related tumor findings in females.

Table 17. Tumor incidence in male Crl:CD (SD) IGS BR rats exposed to K-PFOS in feed for 52 weeks and additional observation until week 104 (Butenhoff et al. 2012)

Organ	Tumor type	Concentration in feed (ppm)	
		0	20
Thyroid	Follicular cell adenoma	3/60	9/39**

Tumor incidence is expressed as the number of rats with the specified neoplastic lesion over the number of rats examined at the site. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): ** $p < 0.01$.

- Tumor promotion study
    - *6-month study in Rainbow trout: Benninghoff et al. (2012)
        - Trout were initiated with 10 ppb aflatoxin B1 (AFB1) for 30 minutes at 15 weeks of age. After initiation, trout were fed 100 ppm PFOS 5 days per week for 6 months. The histological evaluation of tumors in trout was conducted at 12.5-months of age.
        - In the group initiated with AFB1, dietary PFOS increased liver adenomas and carcinomas combined to 13% vs the control rate of 1% ($p = 0.0014$). No liver tumors were observed in the group treated with sham initiation and PFOS.

Other relevant data

Key characteristics of carcinogens

There are numerous mechanistic studies examining the cancer-related effects of PFOS and its salts and transformation and degradation precursors. In this document, findings related to the key characteristics of carcinogens from a selection of studies of PFOS and its salts are summarized.

- Is genotoxic

    In vivo

    - *Mutation frequencies in livers of *gpt* delta transgenic mice exposed to PFOS by gavage (positive): Wang et al. (2015)
    - *Micronucleus (MN) in peripheral blood erythrocytes of rats exposed to PFOS by gavage for 28 days; males (negative), females (positive): NTP (2019b); Appendix B

- MN in mouse bone marrow assay (negative): reviewed by EFSA (2008, p. 73)
- *MN and DNA strand breaks (Comet assays) in bone marrow of rats exposed to PFOS by gavage (positive): Celik et al. (2013)
- *MN and DNA strand breaks (Comet assays) in peripheral blood cells of male rats exposed to PFOS by gavage (positive): Eke and Celik (2016)
- *MN and DNA strand breaks (Comet assays) in male rat livers (positive): Eke et al. (2017)
- *Non-significant increase of MN in the livers of *gpt* delta transgenic mice: Wang et al. (2015)

In vitro

- *Mutation frequencies and γ-H2AX foci (a marker of DNA double strand breaks) in embryonic fibroblasts of *gpt* delta transgenic mice (positive): Wang et al. (2015)
- Chromosome aberrations (CA) in human peripheral blood lymphocytes (negative): reviewed by EFSA (2008, p. 73)
- *MN and DNA strand breaks (Comet assays) in human HepG2 cells (negative): Florentin et al. (2011)
- *DNA strand breaks (Comet assays) and FPG-sensitive sites (modified version of comet assay) in human HepG2 cells (negative): Eriksen et al. (2010)
- *DNA strand breaks (Comet assays) in human HepG2 cells (positive): Wielsoe et al. (2015)
- *DNA strand breaks (Comet assays) in Syrian hamster embryo (SHE) cells (negative): Jacquet et al. (2012)
- Unscheduled DNA synthesis (UDS) in rat liver primary cultures (negative): reviewed by EFSA (2008, p. 73)

Non-mammalian systems

- Mutagenicity in Salmonella typhimurium, Saccharomyces cerevisiae and E. coli (negative): reviewed by EFSA (2008, p. 73)
- *Mutagenicity in *Salmonella typhimurium* and *E. coli* (negative): NTP (2019b); Appendix B
- *Mutations in transgenic Medaka fish liver *in vivo* (positive): Chen et al. (2016)
- *MN and DNA strand breaks (Comet assays) in peripheral blood cells in Zebrafish *in vivo* (positive): Du et al. (2016)
- *DNA damage in germ cell nuclei in *Caenorhabditis elegans* (positive): Guo et al. (2016)

- *DNA strand breaks (Comet assays) in green mussels and earthworms (positive): Liu et al. (2014); Xu et al. (2013)
- *DNA strand breaks (Comet assays) in *Paramecium caudatum* and *Larus michahellis* (Gull) eggs (negative): Kawamoto et al. (2010); Parolini et al. (2016)
- *DNA adducts and DNA damage in calf thymus DNA (positive): Lu et al. (2012)

- Induces epigenetic alterations

  - *Increased PFOS in cord blood was associated with global hypomethylation of the Alu elements and Long interspersed element-1 (LINE-1) in 363 cord blood DNA samples from Taiwan: Liu et al. (2018)
  - *Prenatal PFOS exposure was associated with several differentially methylated regions indicating global methylation shifts in cord blood DNA samples in a Japanese cohort study: (Miura et al. 2018)
  - *Serum PFOS concentrations were associated with global DNA methylation LINE-1 in peripheral blood leukocytes from the adults in the C8 cohort Health Project (positive): Leung et al. (2018)
  - *No association between PFOS levels and global hypomethylation were found in human umbilical cord serum DNA: Guerrero-Preston et al. (2010)
  - *Hypomethylation at several sites by genome-wide DNA methylation analysis in human adipocytes *in vitro* (positive): van den Dungen et al. (2017)
  - *S-D rats exposed to PFOS during gestation exhibited decreased global DNA methylation in the liver, as well as increased methylation of the glutathione-S-transferase Pi (GSTp) promoter, and no effect on the methylation of the p16 gene promoter: Wan et al. (2010)
  - *No effect on global DNA methylation status in human and murine neuroblastoma cells *in vitro*: Bastos Sales et al. (2013)

- Induces oxidative stress

  - *Generation of reactive oxygen species (ROS) in human HepG2 cells *in vitro* (positive): Eriksen et al. (2010); Hu and Hu (2009); Wielsoe et al. (2015)
  - *Oral PFOS exposure significantly increased intracellular ROS and nitric oxide (NO) production, inhibited catalase and superoxide dismutase activities, and decreased the ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) in male S-D rats *in vivo*: Han et al. (2018)
  - *Increases in ROS formation, lipid peroxidation, and GSH depletion in isolated rat hepatocytes *in vitro* (positive): Khansari et al. (2017)

- *Increases in ROS levels in PFOS-treated *Caenorhabditis elegans* (positive): Guo et al. (2016)
- *Increases in ROS levels and 8-hydroxydeoxyguanosine (8-OHdG) in PFOS-treated *Paramecium caudatum* (negative): Kawamoto et al. (2010)
- *PFOS lowered total antioxidant capacity in human HepG2 cells *in vitro*: Wielsoe et al. (2015)
- *PFOS modulates the activities of superoxide dismutase, catalase, glutathione reductase, glutathione-S-transferase, and glutathione peroxidase in human HepG2 cells *in vitro*: Hu and Hu (2009)
- *PFOS modulates the activities of superoxide dismutase, catalase, and glutathione peroxidase; decreases GSH levels; and increases malondialdehyde (a marker of lipid peroxidation) production in human erythrocytes *in vitro*: Pan et al. (2018)
- *PFOS increased malondialdehyde production in undifferentiated and differentiating PC12 rat neurological cells *in vitro*: Slotkin et al. (2008)
- *In a cohort of Chinese adult males, serum levels of PFOS were associated with modified serum metabolome shown by differential levels of biomarkers related to oxidative/nitrosative stress pathways: Wang et al. (2017)
- *PFOS did not increase 8-OHdG levels in human HepG2 cells *in vitro* (negative): Eriksen et al. (2010)

- Induces chronic inflammation

    - 10-day dietary exposure in C57BL/6 male mice: Qazi et al. (2009a); Qazi et al. (2009b)
        - Thymus and spleen atrophy, decreased CD45+CD8+ thymocyte and splenic B lymphocyte counts, increased production of tumor necrosis factor-alpha (TNF-α) and interleukin (IL) 6 (IL-6) by peritoneal macrophages treated with lipopolysaccharide (LPS) *in vitro* (positive)
    - *PFOS increased the releases of pro-inflammatory cytokines, IL-1α and IL-1β; moderately suppressed the release of IL-8 and IL-10 in human bronchial epithelial cells *in vitro*: Sorli et al. (2020)
    - *PFOS modulated processes associated with intestinal inflammation such as cell proliferation and IL-6 production in human colon myofibroblasts *in vitro*: Giménez-Bastida et al. (2015)
    - *PFOS increased the release of the cytokine IL-6 in human leukocytes *in vitro*: Brieger et al. (2011)

- Is immunosuppressive
    - Seven-day gavage exposure in male C57BL/6 mice: Zheng et al. (2009)
        - Decreased lymphocyte counts, decreased plaque forming cell (PFC) response, decreased natural killer (NK) cell activity, and decreased splenic lymphocyte proliferation response.
    - Gestational exposure on days 1-17 in $B6C3F_1$ mice: Keil et al. (2008)
        - Decreased NK cell function and Immunoglobulin M (IgM) antibody production.
    - 28-day gavage exposure in male and female $B6C3F_1$ mice: Peden-Adams et al. (2008)
        - Reduced NK cell activity in males.
        - Altered T cell subpopulations, reduced sheep red blood cells, PFC response and trinitrophenylated derivatives of lipopolysaccharide (TNP-LPS) IgM titer in males and females.
    - *Reduced NK cell activity and LPS-induced release of the pro-inflammatory cytokine TNFα in human leukocytes *in vitro*: Brieger et al. (2011)
    - *Reduced NK cell activity and LPS-induced TNF-α release in human promyelocytic cell line (THP-1) *in vitro*: Corsini et al. (2011)
    - *Reduced NK cell activity, LPS-induced TNF-α release, and T-cell derived phytohemagglutinin (PHA)-induced IL-10 release in human peripheral blood leukocytes *in vitro*: Corsini et al. (2012)
    - *Reduced NK cell activity, phagocytosis, and antibody response in mice *in vivo*: Vetvicka and Vetvickova (2013)

- Modulates receptor-mediated effects
    - Two-week intraperitoneal exposure study in female S-D rats: Austin et al. (2003)
        - Altered estrous cyclicity (reduced regular cyclers and increased irregular cyclers and persistent diestrus).
        - Increased serum corticosterone.
        - Decreased serum leptin.
        - Increased norepinephrine concentrations in hypothalamus; no change on dopamine concentrations.
    - *Increased activation of peroxisome proliferator-activated receptor α (PPARα) and constitutive androstane receptor (CAR)/pregnane X receptor (PXR) in S-D rat liver *in vivo*: Elcombe et al. (2012a); Elcombe et al. (2012b)

- *Significantly induced estrogen receptor (ER) transactivity, and significantly antagonized androgen receptor (AR) activity in a concentration-dependent manner in human MVLN (breast carcinoma) cell lines and Chinese hamster ovary (CHO-K1) cells *in vitro*: Kjeldsen and Bonefeld-Jorgensen (2013)
- *Increased the gene expression of PPARα-independent transcriptional targets, CAR, ERα and PPARγ in microarray analysis in mice exposed to PFOS by gavage *in vivo*: Rosen et al. (2017)
- *Male and female S-D rats exposed to PFOS by gavage for 28 days exhibited significant increases in expression of *Acox1*, *Cyp4a1*, *Cyp2b1*, and *Cyp2b2* compared to controls, indicating significantly increased PPARα and CAR activity *in vivo*: NTP (2019b)
- *PFOS promotes liver tumors in rainbow trout via an estrogen-like mechanism, a PPARα-independent mechanism: Benninghoff et al. (2012)
- *PFOS did not affect estrogen receptor or androgen receptor activity nor steroidogenesis in several human cell lines *in vitro*: Behr et al. (2018).
- *No estrogenic effects on human ERα or ERβ in a yeast two-hybrid assay : Ishibashi et al. (2007)
- *Serum PFOS level was associated with a significant increase in serum thyroxine (T4) and a significant reduction in triiodothyronine (T3) uptake in all participants in the C8 Health Project, a cross-sectional study: Knox et al. (2011)
- *Serum levels of T4 and T3 in PFOS-treated rat dams were significantly reduced as early as one week after PFOS exposure; no feedback response of thyroid-stimulating hormone (TSH) was observed: Thibodeaux et al. (2003)
- *No effect on expression of ER alpha or beta in MCF-10A cells. An ER blocker was able to partial block the effect of PFOS-induced proliferation, indicating that PFOS can activate ER rather than increase expression of ER: Pierozan and Karlsson (2018)

- Causes immortalization (cell transformation)

    - *Increased cell transformation frequency in SHE cells at non-cytotoxic concentrations, analogous to the ones found in human serum of PFOS-exposed workers: Jacquet et al. (2011); Jacquet et al. (2012)
    - *Highest serum concentration of PFOS levels was associated with and elongated telomeres in both sexes of wild Arctic seabirds: Blévin et al. (2017)

- Alters cell proliferation, cell death or nutrient supply

    - *Increased cell proliferation, cell-cycle progression, and malignant phenotypes, e.g. cell migration and invasion, in human breast epithelial cells (MCF-10A cells). ER blocker ICI 182, 780 partially blocked PFOS-induced cell proliferation, indicating stimulation of proliferation was at least in part driven by ER activation [see above under receptor-mediated effects]: Pierozan and Karlsson (2018)
    - *Increased S-D rat liver proliferative index and decreased liver apoptotic index *in vivo*: Elcombe et al. (2012a); Elcombe et al. (2012b)
    - *PFOS was estrogenic via the E-SCREEN assay, an assay designed to use the estrogen sensitivity of MCF-7 cells to determine effects of exogenous agents on cell proliferation *in vitro*: Henry and Fair (2013)
    - *PFOS treatment at low dose stimulated cell proliferation of a human hepatic cell line (HL-7702). PFOS treatment resulted in differential expression of 27 proteins associated with cell proliferation, such as hepatoma-derived growth factor (HdGF), proliferation biomarkers (Ki67), Cyclin D1, Cyclin E2, Cyclin A2, Cyclin B1, c-Myc, and p53 in *in vitro*: Cui et al. (2015)
    - *PFOS increased cell proliferation in two human granulosa cell tumor cell lines, COV434 and KGN *in vitro*: Gogola et al. (2019)

Reviews

- EFSA (2008)
- US EPA (2016)
- EFSA (2018)

References cited in "PFOS"

Alexander BH, Olsen GW, Burris JM, Mandel JH, Mandel JS. 2003. Mortality of employees of a perfluorooctanesulphonyl fluoride manufacturing facility. Occupational and environmental medicine 60:722-729.

Alexander BH, Olsen GW. 2007. Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers. Annals of epidemiology 17:471-478.

Austin ME, Kasturi BS, Barber M, Kannan K, MohanKumar PS, MohanKumar SM. 2003. Neuroendocrine effects of perfluorooctane sulfonate in rats. Environmental health perspectives 111:1485-1489.

Bastos Sales L, Kamstra JH, Cenijn PH, van Rijt LS, Hamers T, Legler J. 2013. Effects of endocrine disrupting chemicals on in vitro global DNA methylation and adipocyte differentiation. Toxicology in vitro : an international journal published in association with BIBRA 27:1634-1643.

Behr AC, Lichtenstein D, Braeuning A, Lampen A, Buhrke T. 2018. Perfluoroalkylated substances (PFAS) affect neither estrogen and androgen receptor activity nor steroidogenesis in human cells in vitro. Toxicol Lett 291:51-60.

Benninghoff AD, Orner GA, Buchner CH, Hendricks JD, Duffy AM, Williams DE. 2012. Promotion of hepatocarcinogenesis by perfluoroalkyl acids in rainbow trout. Toxicol Sci 125:69-78.

Blévin P, Angelier F, Tartu S, Bustamante P, Herzke D, Moe B, et al. 2017. Perfluorinated substances and telomeres in an arctic seabird: Cross-sectional and longitudinal approaches. Environmental pollution (Barking, Essex : 1987) 230:360-367.

Bonefeld-Jorgensen EC, Long M, Bossi R, Ayotte P, Asmund G, Kruger T, et al. 2011. Perfluorinated compounds are related to breast cancer risk in greenlandic inuit: A case control study. Environmental health : a global access science source 10:88.

Bonefeld-Jorgensen EC, Long M, Fredslund SO, Bossi R, Olsen J. 2014. Breast cancer risk after exposure to perfluorinated compounds in danish women: A case-control study nested in the danish national birth cohort. Cancer Causes Control 25:1439-1448.

Brieger A, Bienefeld N, Hasan R, Goerlich R, Haase H. 2011. Impact of perfluorooctanesulfonate and perfluorooctanoic acid on human peripheral leukocytes. Toxicol In Vitro 25:960-968.

Butenhoff JL, Chang SC, Olsen GW, Thomford PJ. 2012. Chronic dietary toxicity and carcinogenicity study with potassium perfluorooctanesulfonate in sprague dawley rats. Toxicology 293:1-15.

Celik A, Eke D, Ekinci SY, Yildirim S. 2013. The protective role of curcumin on perfluorooctane sulfonate-induced genotoxicity: Single cell gel electrophoresis and micronucleus test. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 53:249-255.

Chen Y, Hu W, Huang C, Hua S, Wei Q, Bai C, et al. 2016. Subchronic perfluorooctanesulfonate (PFOS) exposure induces elevated mutant frequency in an in vivo lambda transgenic medaka mutation assay. Scientific reports 6:38466.

Cohn BA, La Merrill MA, Krigbaum NY, Wang M, Park JS, Petreas M, et al. 2019. In utero exposure to poly- and perfluoroalkyl substances (PFASS) and subsequent breast cancer. Reproductive toxicology (Elmsford, NY).

Corsini E, Avogadro A, Galbiati V, dell'Agli M, Marinovich M, Galli CL, et al. 2011. In vitro evaluation of the immunotoxic potential of perfluorinated compounds (PFCS). Toxicol Appl Pharmacol 250:108-116.

Corsini E, Sangiovanni E, Avogadro A, Galbiati V, Viviani B, Marinovich M, et al. 2012. In vitro characterization of the immunotoxic potential of several perfluorinated compounds (PFCS). Toxicology and applied pharmacology 258:248-255.

Cui R, Zhang H, Guo X, Cui Q, Wang J, Dai J. 2015. Proteomic analysis of cell proliferation in a human hepatic cell line (HI-7702) induced by perfluorooctane sulfonate using itraq. J Hazard Mater 299:361-370.

Dinse GE, Peddada SD, Harris SF, Elmore SA. 2010. Comparison of NTP historical control tumor incidence rates in female harlan sprague dawley and fischer 344/N rats. Toxicologic pathology 38:765-775.

Du J, Wang S, You H, Jiang R, Zhuang C, Zhang X. 2016. Developmental toxicity and DNA damage to zebrafish induced by perfluorooctane sulfonate in the presence of ZNO nanoparticles. Environmental toxicology 31:360-371.

EFSA. 2008. Opinion of the scientific panel on contaminants in the food chain on perfluorooctane sulfonate (PFOS), perfluorooctanoic acid and their salts. The EFSA Journal 653:1-131.

EFSA. 2018. Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA Journal 16:e05194.

Eke D, Celik A. 2016. Curcumin prevents perfluorooctane sulfonate-induced genotoxicity and oxidative DNA damage in rat peripheral blood. Drug and chemical toxicology 39:97-103.

Eke D, Celik A, Yilmaz MB, Aras N, Kocaturk Sel S, Alptekin D. 2017. Apoptotic gene expression profiles and DNA damage levels in rat liver treated with perfluorooctane sulfonate and protective role of curcumin. International journal of biological macromolecules 104:515-520.

Elcombe CR, Elcombe BM, Foster JR, Chang SC, Ehresman DJ, Butenhoff JL. 2012a. Hepatocellular hypertrophy and cell proliferation in sprague-dawley rats from dietary exposure to potassium perfluorooctanesulfonate results from increased expression of xenosensor nuclear receptors PPARalpha and CAR/PXR. Toxicology 293:16-29.

Elcombe CR, Elcombe BM, Foster JR, Chang SC, Ehresman DJ, Noker PE, et al. 2012b. Evaluation of hepatic and thyroid responses in male sprague dawley rats for up to eighty-four days following seven days of dietary exposure to potassium perfluorooctanesulfonate. Toxicology 293:30-40.

Eriksen KT, Sorensen M, McLaughlin JK, Lipworth L, Tjonneland A, Overvad K, et al. 2009. Perfluorooctanoate and perfluorooctanesulfonate plasma levels and risk of cancer in the general danish population. Journal of the National Cancer Institute 101:605-609.

Eriksen KT, Raaschou-Nielsen O, Sorensen M, Roursgaard M, Loft S, Moller P. 2010. Genotoxic potential of the perfluorinated chemicals PFOA, PFOS, PFBS, PFNA and PFHXA in human HepG2 cells. Mutation research 700:39-43.

Florentin A, Deblonde T, Diguio N, Hautemaniere A, Hartemann P. 2011. Impacts of two perfluorinated compounds (PFOS and PFOA) on human hepatoma cells: Cytotoxicity but no genotoxicity? International journal of hygiene and environmental health 214:493-499.

Ghisari M, Long M, Roge DM, Olsen J, Bonefeld-Jorgensen EC. 2017. Polymorphism in xenobiotic and estrogen metabolizing genes, exposure to perfluorinated compounds and subsequent breast cancer risk: A nested case-control study in the danish national birth cohort. Environmental research 154:325-333.

Giménez-Bastida JA, Surma M, Zieliński H. 2015. In vitro evaluation of the cytotoxicity and modulation of mechanisms associated with inflammation induced by perfluorooctanesulfonate and perfluorooctanoic acid in human colon myofibroblasts CCD-18CO. Toxicol In Vitro 29:1683-1691.

Gogola J, Hoffmann M, Ptak A. 2019. Persistent endocrine-disrupting chemicals found in human follicular fluid stimulate the proliferation of granulosa tumor spheroids via GPR30 and IGF1R but not via the classic estrogen receptors. Chemosphere 217:100-110.

Guerrero-Preston R, Goldman LR, Brebi-Mieville P, Ili-Gangas C, Lebron C, Witter FR, et al. 2010. Global DNA hypomethylation is associated with in utero exposure to cotinine and perfluorinated alkyl compounds. Epigenetics 5:539-546.

Guo X, Li Q, Shi J, Shi L, Li B, Xu A, et al. 2016. Perfluorooctane sulfonate exposure causes gonadal developmental toxicity in Caenorhabditis elegans through ROS-induced DNA damage. Chemosphere 155:115-126.

Han R, Hu M, Zhong Q, Wan C, Liu L, Li F, et al. 2018. Perfluorooctane sulphonate induces oxidative hepatic damage via mitochondria-dependent and NF-kappaB/TNF-alpha-mediated pathway. Chemosphere 191:1056-1064.

Hardell E, Karrman A, van Bavel B, Bao J, Carlberg M, Hardell L. 2014. Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. Environment international 63:35-39.

Henry ND, Fair PA. 2013. Comparison of in vitro cytotoxicity, estrogenicity and anti-estrogenicity of triclosan, perfluorooctane sulfonate and perfluorooctanoic acid. Journal of applied toxicology : JAT 33:265-272.

Hu XZ, Hu DC. 2009. Effects of perfluorooctanoate and perfluorooctane sulfonate exposure on hepatoma HepG2 cells. Archives of toxicology 83:851-861.

Innes KE, Wimsatt JH, Frisbee S, Ducatman AM. 2014. Inverse association of colorectal cancer prevalence to serum levels of perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) in a large appalachian population. BMC cancer 14:45.

Ishibashi H, Ishida H, Matsuoka M, Tominaga N, Arizono K. 2007. Estrogenic effects of fluorotelomer alcohols for human estrogen receptor isoforms alpha and beta in vitro. Biol Pharm Bull 30:1358-1359.

Jacquet N, Maire MA, Rast C, Bonnard M, Vasseur P. 2011. Perfluorooctanoic acid (PFOA) acts as a tumor promoter on syrian hamster embryo (SHE) cells. Environmental science and pollution research international 19:2537-2549.

Jacquet N, Maire MA, Landkocz Y, Vasseur P. 2012. Carcinogenic potency of perfluorooctane sulfonate (PFOS) on syrian hamster embryo (SHE) cells. Archives of toxicology 86:305-314.

Kawamoto K, Oashi T, Oami K, Liu W, Jin Y, Saito N, et al. 2010. Perfluorooctanoic acid (PFOA) but not perfluorooctane sulfonate (PFOS) showed DNA damage in comet assay on paramecium caudatum. J Toxicol Sci 35:835-841.

Keil DE, Mehlmann T, Butterworth L, Peden-Adams MM. 2008. Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. Toxicological sciences : an official journal of the Society of Toxicology 103:77-85.

Khansari MR, Yousefsani BS, Kobarfard F, Faizi M, Pourahmad J. 2017. In vitro toxicity of perfluorooctane sulfonate on rat liver hepatocytes: Probability of distructive binding to CYP 2E1 and involvement of cellular proteolysis. Environmental science and pollution research international 24:23382-23388.

Kjeldsen LS, Bonefeld-Jorgensen EC. 2013. Perfluorinated compounds affect the function of sex hormone receptors. Environmental science and pollution research international 20:8031-8044.

Knox SS, Jackson T, Frisbee SJ, Javins B, Ducatman AM. 2011. Perfluorocarbon exposure, gender and thyroid function in the C8 health project. The Journal of toxicological sciences 36:403-410.

Leung YK, Ouyang B, Niu L, Xie C, Ying J, Medvedovic M, et al. 2018. Identification of sex-specific DNA methylation changes driven by specific chemicals in cord blood in a faroese birth cohort. Epigenetics 13:290-300.

Liu C, Chang VW, Gin KY, Nguyen VT. 2014. Genotoxicity of perfluorinated chemicals (PFCS) to the green mussel (Perna viridis). The Science of the total environment 487:117-122.

Liu CY, Chen PC, Lien PC, Liao YP. 2018. Prenatal perfluorooctyl sulfonate exposure and alu DNA hypomethylation in cord blood. Int J Environ Res Public Health 15.

Lu L, Xu L, Kang T, Cheng S. 2012. Investigation of DNA damage treated with perfluorooctane sulfonate (PFOS) on ZRO2/DDAB active nano-order film. Biosens Bioelectron 35:180-185.

Mancini FR, Cano-Sancho G, Gambaretti J, Marchand P, Boutron-Ruault MC, Severi G, et al. 2020. Perfluorinated alkylated substances serum concentration and breast cancer risk: Evidence from a nested case-control study in the french E3N cohort. International journal of cancer 146:917-928.

Miura R, Araki A, Miyashita C, Kobayashi S, Kobayashi S, Wang SL, et al. 2018. An epigenome-wide study of cord blood DNA methylations in relation to prenatal perfluoroalkyl substance exposure: The hokkaido study. Environ Int 115:21-28.

NTP. 2019a. NTP historical controls report: All routes and vehicles Harlan Sprague-Dawley rats, april 2019. Available: https://ntp.niehs.nih.gov/ntp/historical_controls/ntp2000_2019/r_hcrpt_allrte20190400.pdf.

NTP. 2019b. NTP technical report on the toxicity studies of perfluoroalkyl sulfonates (perfluorobutane sulfonic acid, perfluorohexane sulfonate potassium salt, and perfluorooctane sulfonic acid) administered by gavage to Sprague Dawley (HSD:Sprague Dawley SD) rats. Available: https://ntp.niehs.nih.gov/ntp/htdocs/st_rpts/tox096_508.pdf?utm_source=direct&utm_medium=prod&utm_campaign=ntpgolinks&utm_term=tox096.

Pan X, Qin P, Liu R, Yu W, Dong X. 2018. Effects of carbon chain length on the perfluoroalkyl acids-induced oxidative stress of erythrocytes in vitro. J Agric Food Chem 66:6414-6420.

Parolini M, Colombo G, Valsecchi S, Mazzoni M, Possenti CD, Caprioli M, et al. 2016. Potential toxicity of environmentally relevant perfluorooctane sulfonate (PFOS)

concentrations to yellow-legged gull larus michahellis embryos. Environmental Science and Pollution Research 23:426-437.

Peden-Adams MM, Keller JM, Eudaly JG, Berger J, Gilkeson GS, Keil DE. 2008. Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. Toxicological sciences : an official journal of the Society of Toxicology 104:144-154.

Pierozan P, Karlsson O. 2018. PFOS induces proliferation, cell-cycle progression, and malignant phenotype in human breast epithelial cells. Archives of toxicology 92:705-716.

Qazi MR, Bogdanska J, Butenhoff JL, Nelson BD, DePierre JW, Abedi-Valugerdi M. 2009a. High-dose, short-term exposure of mice to perfluorooctanesulfonate (PFOS) or perfluorooctanoate (PFOA) affects the number of circulating neutrophils differently, but enhances the inflammatory responses of macrophages to lipopolysaccharide (LPS) in a similar fashion. Toxicology 262:207-214.

Qazi MR, Xia Z, Bogdanska J, Chang SC, Ehresman DJ, Butenhoff JL, et al. 2009b. The atrophy and changes in the cellular compositions of the thymus and spleen observed in mice subjected to short-term exposure to perfluorooctanesulfonate are high-dose phenomena mediated in part by peroxisome proliferator-activated receptor-alpha (PPARalpha). Toxicology 260:68-76.

Rosen MB, Das KP, Rooney J, Abbott B, Lau C, Corton JC. 2017. PPARalpha-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. Toxicology 387:95-107.

Slotkin TA, MacKillop EA, Melnick RL, Thayer KA, Seidler FJ. 2008. Developmental neurotoxicity of perfluorinated chemicals modeled in vitro. Environ Health Perspect 116:716-722.

Sorli JB, Lag M, Ekeren L, Perez-Gil J, Haug LS, Da Silva E, et al. 2020. Per- and polyfluoroalkyl substances (PFASS) modify lung surfactant function and pro-inflammatory responses in human bronchial epithelial cells. Toxicology in vitro : an international journal published in association with BIBRA 62:104656.

Thibodeaux JR, Hanson RG, Rogers JM, Grey BE, Barbee BD, Richards JH, et al. 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. I: Maternal and prenatal evaluations. Toxicological sciences : an official journal of the Society of Toxicology 74:369-381.

Thomford PJ. 2002. 104-week dietary chronic toxicity and carcinogenicity study with perfluorooctane sulfonic acid potassium salt (PFOS; T-6295) in rats. Covance Laboratories, Madison, Wisconsin.

Tsai M-s, Chang S-H, Kuo W-H, Kuo C-H, Li S-Y, Wang M-Y, et al. 2020. A case-control study of perfluoroalkyl substances and the risk of breast cancer in Taiwanese women. Environment international 142:105850.

USEPA. 2016. Drinking water health advisory for perfluorooctane sulfonate (PFOS). Available: https://www.epa.gov/sites/production/files/2016-05/documents/pfos_health_advisory_final_508.pdf.

van den Dungen MW, Murk AJ, Kok DE, Steegenga WT. 2017. Persistent organic pollutants alter DNA methylation during human adipocyte differentiation. Toxicology in vitro : an international journal published in association with BIBRA 40:79-87.

Vassiliadou I, Costopoulou D, Ferderigou A, Leondiadis L. 2010. Levels of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) in blood samples from different groups of adults living in greece. Chemosphere 80:1199-1206.

Vetvicka V, Vetvickova J. 2013. Reversal of perfluorooctanesulfonate-induced immunotoxicity by a glucan-resveratrol-vitamin c combination. Oriental Pharmacy and Experimental Medicine 13:77-84.

Wan YJ, Li YY, Xia W, Chen J, Lv ZQ, Zeng HC, et al. 2010. Alterations in tumor biomarker GSTp gene methylation patterns induced by prenatal exposure to PFOS. Toxicology 274:57-64.

Wang X, Liu L, Zhang W, Zhang J, Du X, Huang Q, et al. 2017. Serum metabolome biomarkers associate low-level environmental perfluorinated compound exposure with oxidative /nitrosative stress in humans. Environ Pollut 229:168-176.

Wang Y, Zhang X, Wang M, Cao Y, Wang X, Liu Y, et al. 2015. Mutagenic effects of perfluorooctanesulfonic acid in gpt delta transgenic system are mediated by hydrogen peroxide. Environmental science & technology 49:6294-6303.

Wielsoe M, Long M, Ghisari M, Bonefeld-Jorgensen EC. 2015. Perfluoroalkylated substances (PFAS) affect oxidative stress biomarkers in vitro. Chemosphere 129:239-245.

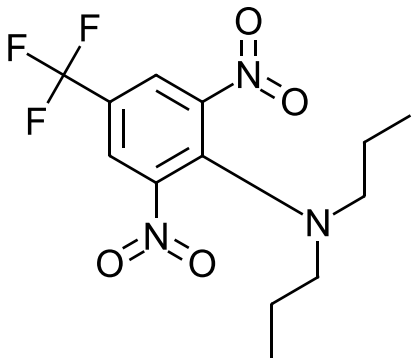
Wielsoe M, Kern P, Bonefeld-Jorgensen EC. 2017. Serum levels of environmental pollutants is a risk factor for breast cancer in inuit: A case control study. Environmental health : a global access science source 16:56.

Xu D, Li C, Wen Y, Liu W. 2013. Antioxidant defense system responses and DNA damage of earthworms exposed to perfluorooctane sulfonate (PFOS). Environmental pollution (Barking, Essex : 1987) 174:121-127.

Zheng L, Dong GH, Jin YH, He QC. 2009. Immunotoxic changes associated with a 7-day oral exposure to perfluorooctanesulfonate (PFOS) in adult male C57BL/6 mice. Archives of toxicology 83:679-689.

Trifluralin

(CAS No. 1582-09-8)



Trifluralin is an herbicide used on a variety of crops, shrubs, and flowers to control annual grasses and some broadleaf annual weeds. It is used mostly on cotton, as well as on soybeans and some fruits and vegetables. According to the California Department of Pesticide Regulation (DPR), approximately 347,000 pounds of trifluralin were used in California in 2017[13].

Trifluralin was previously brought to the Carcinogen Identification Committee (CIC) in 2011 as an individual chemical, as well as part of the "dinitroaniline pesticides and prodiamine and trifluralin" group, which also includes benfluralin, ethalfuralin, pendimethalin, and oryzalin (which is on the Proposition 65 list as causing cancer). At that time, the CIC recommended that trifluralin as well as the "dinitroaniline pesticides and prodiamine and trifluralin" group be placed in the 'medium' priority group for development of hazard identification materials. Since 2011, additional epidemiology data and mechanistic data on trifluralin have been identified. In 2020, trifluralin passed the human and animal data screens, underwent a preliminary toxicological evaluation, and is being brought again to the CIC for consultation. This is a summary of the relevant studies identified during the preliminary toxicological evaluation. Studies identified since consultation with the CIC in 2011 are marked with an asterisk (*).

[13] California Pesticide Information Portal (CalPIP) Application. Version 2019.04 (2017 PUR Data Update). Available at: https://calpip.cdpr.ca.gov/main.cfm (accessed on 8/4/2020).

Epidemiological data

- Adult lymphohematopoietic cancers
    - Prospective cohort study (Agricultural Health Study, AHS): Kang et al. (2008)
        - Cohort of more than 80,000 people: farmers and pesticide applicators (n>54,000) in Iowa and North Carolina and their spouses (n>30,000).
        - Enrollment period 1993 to 1997 (Alavanja et al. 1996).
        - Followed up until 2015 in Iowa and 2014 in North Carolina.
        - Incident cancers identified through linkage to state cancer registries
        - Exposure data collected prospectively through self-administered questionnaire at enrollment, additional take-home questionnaires, and follow-up telephone interview. At enrollment, participants reported ever/never use of 50 pesticides; further details gathered for 22 of the pesticides (i.e., years and days per year each pesticide was applied, use of personal protective equipment, pesticide application method).
        - No association with lymphatic-hematopoietic cancers, non-Hodgkin lymphoma (NHL), or leukemia.
    - * Population-based case-control study of NHL in white male farmers in Iowa and Minnesota: Cantor et al. (1992)
        - Cases ascertained from Iowa State Health Registry records (1981-83) and Minnesota hospital and pathology records (1980-82).
        - Exposure assessed through interviews of subjects or close relative/friend.
        - Non-significantly increased risk for farmers who ever handled trifluralin (Odds ratio [OR], 1.2; 95% confidence interval [CI], 0.8–1.8; 45 cases) and who handled trifluralin prior to 1965 (OR, 1.5; 95% CI, 0.8–3.1; 14 cases).
    - Population-based case-control study in male farmers in Iowa and Minnesota: Brown et al. (1990) as reviewed in IARC [International Agency for Research on Cancer] (1991)
        - No association with leukemia (OR, 1.0; 95% CI, 0.7–1.6; 32 cases).
        - Exposure to other pesticides could not be excluded.
    - Population-based case-control study of soft-tissue sarcoma, Hodgkin lymphoma, and NHL in Kansas: Hoar et al. (1986) as reviewed in IARC (1991)
        - Occupational exposure through insecticide application.

        - Increased risk of NHL (OR, 12.5; 95% CI, 1.6–116.1; 3 cases).
        - Exposure to other pesticides could not be excluded.

- Childhood leukemia

    - * Population-based case-control study of early childhood cancer among California children born between 1990-1997 and mother's residential proximity to agriculture applications of pesticides at the time of the child's birth: Reynolds et al. (2005)
        - 2189 cases identified from California cancer registry.
        - Exposure assessed by estimating pesticide use in proximity to residence.
        - Non-significantly increased risk of childhood acute lymphoid leukemia in the high-use areas (OR, 1.35; 95% CI, 0.71–2.56) compared to low-use areas.
        - No associations of trifluralin exposure with leukemias combined.
    - * Ecologic study of childhood cancers and agricultural pesticide use based on California's population-based cancer registry 1988-1994: Reynolds et al. (2002)
        - Used geographic information system (GIS) and DPR's pesticide use reporting database to identify pesticide uses for each census block group (quantified as pounds per square mile (lb/mi^2)).
        - No association between trifluralin and leukemias.
        - Did not evaluate residential/garden use, which the authors acknowledged may be significant.

- Esophageal and stomach cancers

    - * Population-based case-control study on stomach and esophageal cancer risks from farming and agricultural pesticide use in eastern Nebraska: Lee et al. (2004)
        - Trifluralin was one of the "Nitrosatable pesticides with experimental evidence of carcinogenicity or were likely to be carcinogenic". "Nitrosatable" was explained as "able to form N-nitroso compounds on reaction with nitrite" by the study authors.
        - Cases were diagnosed 1988-1993.
        - Exposure assessed through telephone interviews during 1992-94
        - No association of ever use of trifluralin with stomach cancer (OR, 0.8; 95% CI, 0.3–2.1; 6 exposed cases) or esophageal cancer (OR, 0.7; 95% CI, 0.2–1.9; 5 exposed cases).

- Colorectal cancer
    - * Prospective cohort study (AHS): Kang et al. (2008) [see above for details]
        - 57,311 pesticide applicators; 170 colon cancer cases; 77 rectum cancer cases.
        - Followed up through 2002 (mean 7.43 years).
        - Increased risk of colon cancer for highest tertile of intensity-weighted lifetime days compared to non-exposed (RR, 1.76; 95% CI, 1.05–2.95; 23 cases; p_{trend}=0.036) and for highest exposed compared to lowest exposed tertile (RR, 1.93; 95% CI, 1.08–3.45; 23 cases; p_{trend}=0.037).
        - Risks were not statistically significant for lifetime days as the exposure metric or for right-sided or left-sided colon cancers.
        - No significantly increased risks observed for cancer of the rectum.
- Urinary tract cancers
    - * Prospective cohort study (AHS) of renal cell carcinoma (RCC): Andreotti et al. (2020) [see above for details]
        - Borderline increased risk of RCC in the 3rd quartile of exposure (20-year lagged intensity-weighted lifetime days) vs never users (p_{trend} = 0.42).
            - Quartile 1: RR, 0.89; 95% CI, 0.59–1.33; 29 cases
            - Quartile 2: RR, 1.25, 95% CI, 0.83–1.88; 29 cases
            - Quartile 3: RR, 1.51, 95% CI, 1.00–2.27; 29 cases
            - Quartile 4: RR, 1.10, 95% CI, 0.73–1.64; 30 cases
    - * Prospective cohort study (AHS): Kang et al. (2008) [see above for details]
        - Non-significantly elevated risk for kidney cancer in highest tertile of intensity-weighted lifetime days compared to non-exposed (RR, 1.51; 95% CI, 0.59–3.89; 7 cases).
        - Non-significantly elevated risk for bladder cancer in highest tertile of intensity-weighted lifetime days compared to non-exposed (RR, 1.21; 95% CI, 0.46–3.22; 7 cases).
- Pancreatic cancer
    - * Nested case-control study (AHS): Andreotti et al. (2009) [see above for details]
        - Follow-up through 2004.

        - Non-significantly elevated risk for pancreatic cancer for ever exposure in applicators and spouses (OR, 1.4; 95% CI, 0.8–2.4; 32 exposed cases) or intensity-weighted exposure days in applicators (p_{trend} = 0.70).

- Brain and central nervous system cancers

    - * Population-based case-control study on risk of brain cancer from farming and agricultural pesticide use in eastern Nebraska: Lee et al. (2005)
        - Trifluralin was tested because it is one of the "Nitrosatable pesticides with experimental evidence of carcinogenicity or were likely to be carcinogenic".
        - Due to the severity of the disease, exposure was assessed through interviews with proxies for 76% of cases. Most proxy respondents were either spouses (62%) or other first-degree relatives (33%).
        - Increased risk of glioma for ever-use of pesticides for overall responses (OR, 3.2; 95% CI, 1.4–7.3; 17 cases) and proxy responses (OR, 5.9; 95% CI, 1.9–18.2; 12 cases), but not significant for self-responses (OR, 1.2; 95% CI 0.3–4.3; 5 cases).
        - Regarding the use of proxy response, the authors noted "Use of proxy respondents may introduce non-differential misclassification if proxies are less knowledgeable and tend to underreport use. In our study, proxies of cases and controls were more likely to provide ''don't know'' responses and were less likely to report use of specific pesticides than subjects themselves. However, this would lead to ORs biased towards the null among proxy respondents, which was the opposite of what we observed".
    - * Population-based case-control study of childhood cancers: Reynolds et al. (2005) [see above for details]
        - No associations of trifluralin with central nervous system tumors.
    - * Ecologic study of childhood cancers: Reynolds et al. (2002) [see above for details]
        - No association between trifluralin and gliomas.

- Ovarian cancer

    - Population-based case-control study in farmers in northern Italy: Donna et al. (1989) as reviewed in IARC (1991)
        - No association with ovarian epithelial cancer (OR, 0.64; 95% CI, 0.1–6.5; 1 case).
        - These individuals were also exposed to triazine herbicides.

- Prostate cancer
    - * Prospective cohort study (AHS): Kang et al. (2008) [see above for details]
        - No association with prostate cancer.
- Lung cancer
    - * Prospective cohort study (AHS): Kang et al. (2008) [see above for details]
        - No association with lung cancer.
- All cancers combined
    - * Prospective cohort study (AHS): Kang et al. (2008) [see above for details]
        - No association with all cancers combined.
    - * Population-based case-control study of childhood cancer: Reynolds et al. (2005) [see above for details]
        - No association between trifluralin and all cancer sites.
    - * Ecologic study of childhood cancers: Reynolds et al. (2002) [see above for details]
        - No association between trifluralin and all cancer sites.

Animal carcinogenicity data

- Long-term feeding studies in rats
    - 78-week exposure and additional 33-week observation studies in male and female Osborne-Mendel rats: NCI (1978)
        - No treatment-related tumor findings in males or females.
        - 50 per sex per group; 0, 3250, 6500 part per million (ppm) trifluralin in the diet.
        - Technical grade trifluralin was purchased from Eli Lilly Company.
        - Three years after completion of the bioassays, the trifluralin sample used was tested and found to be contaminated with the carcinogen N-nitrosodi-*n*-propylamine (NDPA) (84-88 ppm). Subsequently in 1982, following a special review, US EPA set the allowable nitrosamine level to be 0.5 ppm in all registered trifluralin technical products (US EPA 1996).
        - Hypotheses for source of NDPA in trifluralin:

        - Formation during trifluralin synthesis: “NDPA is present in trifluralin by virtue of a side-reaction between nitrosating agents and dipropylamine during the amination step of the manufacturing process”. “Several dinitroaniline herbicides and other compounds that utilize secondary amines for the manufacturing process have been shown to contain nitrosoamines.” (Ambrus et al. 2003; West and Day 1979)
        - Possible dealkylative nitrosation of trifluralin:
            - Trifluralin is a tertiary amine. Tertiary amines have been shown to undergo nitrosative cleavage to form carcinogenic nitrosamines (Sun et al. 2010).
            - Nitrosamine formation may happen *in vitro*, as demonstrated by the enhanced mutagenicity of secondary and tertiary amines after treatment with nitrite in acetic acid (Andrews et al. 1980).
            - The formation of nitroso compounds from agricultural chemicals in the presence of nitrite is expected to be significant at 37ºC and at low concentrations of the tertiary amine (Elespuru and Lijinsky 1973). Studies have shown that the formation can happen in an environment resembling that of the human stomach (at pH 3, 37ºC, reacted for 4 hours) (Egert and Greim 1976).
- Two-year studies in male and female Sprague-Dawley rats: Eli Lilly Company (1966), as reviewed in US EPA (1986) (p. 4)
    - 25 per sex per group; 0, 200, 1000, 2000 ppm trifluralin in the diet.
    - No treatment-related tumor findings in males or females.
- Two-year dietary studies in male and female Fischer 344 rats: Eli Lilly Company (1980), as reviewed in US EPA (1986) (pp. 6-10) and US EPA (1988)
    - The level of NDPA present was determined to be <0.01 ppm.
    - Increases in renal pelvis carcinoma (by pairwise comparison and trend) and thyroid follicular cell adenoma and combined thyroid follicular cell tumors (by pairwise comparison) in males (Table 18).
    - Increase in bladder papilloma and carcinoma combined (by pairwise comparison and trend) in females (Table 19).

Table 18. Tumor incidence in male F344 rats exposed to trifluralin in feed for 2 years: Eli Lilly Company (1980), as reviewed by US EPA (1986; 1988)

Organ	Tumor type	Concentration in feed (ppm)				Exact trend test *p*-value
		0	813	3250	6500	
Urinary tract (transitional epithelium)	Bladder papilloma	0/60	1/59	1/60	1/60	NS
	Bladder carcinoma	0/60	0/59	0/60	0/60	NS
	Renal pelvis carcinoma	0/60	2/59	3/60	6/60*	$p < 0.01$
	Combined urinary tract tumors	0/60	3/59	4/60	7/60**	$p < 0.01$
Thyroid	Follicular cell adenoma	1/60	0/59	3/59	10/60**	$p < 0.001$
	Follicular cell papillary adenoma	2/60	0/59	2/59	0/60	NS
	Follicular cell cystadenoma	0/60	0/59	0/59	2/60	NS
	Follicular cell carcinoma	2/60	1/59	3/59	1/60	NS
	Combined follicular cell tumors	5/60	1/59	8/59	13/60*	$p < 0.001$

Tumor incidence is expressed as the number of rats with the specified neoplastic lesion over the number of rats examined at the site. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p < 0.01$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

Table 19. Tumor incidence in female F344 rats exposed to trifluralin in feed for 2 years: Eli Lilly Company (1980), as reviewed by US EPA (1986; 1988)

Organ	Tumor type	Concentration in feed (ppm)				Exact trend test *p*-value
		0	813	3250	6500	
Urinary bladder (transitional epithelium)	Papilloma	0/60	0/60	1/60	3/60	$p = 0.02$
	Carcinoma	0/60	0/60	0/60	2/60	NS
	Combined papilloma and carcinoma	0/60	0/60	1/60	5/60*	$p = 0.002$

Tumor incidence is expressed as the number of rats with the specified neoplastic lesion over the number of rats examined at the site. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

- 28-month studies in male and female Wistar rats: as reviewed in US EPA (1987) (pp. 2, 29, 55-57, 60)
  - Increase in benign granular cell meningioma of the brain (by pairwise comparison and trend) and benign liver tumors (by trend) in males (Table 20). US EPA deemed the tumors to be "incidental age-related" and not due to treatment.

Table 20. Tumor incidence in male Wistar rats treated with trifluralin in feed for 28 months (US EPA 1987)

Organ	Tumor type	Concentration in feed (ppm)				Exact trend test *p*-value
		0	200	800	3200	
Brain	Benign granular cell meningioma	0/60	1/59	0/60	7/59**	$p < 0.001$
Liver	Benign tumors	0/60	0/60	0/60	3/60	$p < 0.05$

Tumor incidence is expressed as the number of rats with the specified neoplastic lesion over the number of rats examined at the site. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): ** $p < 0.01$. Exact trend test conducted by OEHHA.

- Long-term feeding studies in mice
  - 78-week exposure and additional 12-week observation studies in male and female B6C3F1 mice: NCI (1978)
    - Increase in hepatocellular carcinoma, and hepatocellular carcinoma and adenoma (combined) in females (by pairwise and trend when compared to matched or pooled controls) (Table 21).

- Increase in alveolar/bronchiolar adenoma, and adenoma and carcinoma (combined) (by pairwise comparison and trend when compared to pooled controls), and squamous-cell carcinoma of the forestomach, an uncommon tumor (by pairwise comparison with pooled controls) in females (Table 21).
- No treatment-related tumor findings in males.
- Technical grade trifluralin was purchased from Eli Lilly Company.
- Doses used were 0, 2000, and 3744 ppm in male mice, and 0, 2740, and 5192 ppm in female mice. These are time-weighted average concentrations, as the doses in females had to be decreased due to toxicity and the authors used a “cyclic dosing regimen” (1 week off, 4 weeks on) for both male and female high-dose groups starting week 57.
- Three years after completion of the bioassays, a sample of the trifluralin compound used was tested and found to be contaminated with the carcinogen N-nitrosodi-*n*-propylamine (NDPA) (84-88 ppm). In C57BL mice, animals receiving NDPA by gavage had higher incidences of forestomach papillomas, forestomach carcinomas, and pulmonary adenomas compared to the group receiving 40% ethanol by gavage. A vehicle control group was not included (Griciute et al. 1982, as reviewed by ATSDR 2019).

Table 21. Tumor incidence in female B6C3F1 mice treated with technical-grade trifluralin in feed for 78 weeks (with additional 12 weeks of observation) (NCI 1978)

Organ	Tumor type	Concentration in feed (ppm)				Exact trend test *p*-value
		0 Pooled control[1]	0 Matched control	2740	5192	
Lung	Alveolar/ bronchiolar adenoma	0/59	0/19	6/43** (pooled)	3/30* (pooled)	$p < 0.05$ (pooled)
	Alveolar/ bronchiolar adenoma or carcinoma combined	0/59	0/19	7/43** (pooled)	3/30* (pooled)	$p < 0.05$ (pooled)
Liver	Hepatocellular carcinoma	0/60	0/20	12/47*** (***pooled; **matched)	21/44*** (***pooled; ***matched)	$p < 0.001$ (pooled & matched)
	Hepatocellular adenoma or carcinoma	0/60	0/20	15/47*** (***pooled; **matched)	21/44*** (***pooled; ***matched)	$p < 0.001$ (pooled & matched)
Stomach	Squamous cell carcinoma (uncommon)	0/60	0/20	4/45* (pooled)	1/44	NS

Tumor incidence is expressed as the number of mice with the specified neoplastic lesion over the number of mice examined at the site. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

[1] Pooled control group combined controls from the studies of trifluralin, pentachloronitrobenzone, and p,p'-TDE. The control mice used for the pool were of the same strain, from the same supplier, housed in the same room, tested concurrently for more than 1 year, and were diagnosed by the same pathologists.

- Two-year studies in male and female B6C3F1 mice: Eli Lilly Company (1980) as reviewed in US EPA (1986) (p. 4); Francis et al. (1991)
  - In response to the NCI mouse studies, Eli Lilly conducted these studies using NDPA-free trifluralin.
  - Doses were 0, 563, 2250, and 4500 ppm in the feed.
  - No treatment-related tumor findings in males or females.
- Two-year studies in male and female NMRI mice: as reviewed in US EPA (1987) (pp. 1-2, 22-27)

- Test material: “Trifluralin active ingredient (technical)”, purity >99%. US EPA (1996) confirmed that this study used purified trifluralin.
- Increases in hepatocellular carcinoma and hepatocellular adenoma and carcinoma (combined), and in bronchioalveolar tumors in mid-dose males (by pairwise comparison) (all bronchioalveolar tumors were classified as malignant) (Table 22).
- US EPA did not consider liver tumors sufficient cause to return trifluralin to Peer Review group, and noted that data are within historic control range.
- No treatment-related tumor findings in females.

Table 22. Tumor incidence in male NMRI mice treated with trifluralin in feed for two years (US EPA 1987)

Organ	Tumor type	Concentration in feed (ppm)				Exact trend test *p*-value
		0	50	200	800	
Liver	Hepatocellular adenoma	5/50	8/50	7/50	6/50	NS
	Hepatocellular carcinoma	1/50	3/50	7/50*	4/50	NS
	Hepatocellular adenoma/ carcinoma combined	6/50	11/50	14/50*	10/50	NS
Lung	Bronchioalveolar tumor	12/50	19/50	25/50**	17/50	NS
	Metastasis/ carcinoma	0/50	0/50	0/50	1/50	NS

Tumor incidence is expressed as the number of mice with the specified neoplastic lesion over the number of mice examined at the site. Treatment group tumor incidences with asterisks indicate significant results from Fisher pairwise comparison with controls (conducted by OEHHA): * $p < 0.05$, ** $p < 0.01$. Exact trend test conducted by OEHHA. NS, not significant ($p \geq 0.05$).

Other relevant data

Key characteristics of carcinogens

- Is genotoxic

  In vivo

  - Micronucleus formation

        - * NMRI mice (positive in females; negative in males): Gebel et al. (1997) (99.5% purity)
        - * Fish (*O. niloticus*; tilapia) (positive): Konen and Cavas (2008) (98% purity)
        - * *Allium cepa* (onion) (positive): Mazzeo and Marin-Morales (2015)
    - Chromosomal abnormalities
        - Chromosomal aberrations in mouse bone marrow (positive and negative)*:* as reviewed in IARC (1991)
        - Chromosomal aberrations in mouse embryos (positive): as reviewed in IARC (1991)
        - Mouse spermatocyte mutation assay (positive): as reviewed in IARC (1991)
        - Sister chromatid exchange in Chinese hamster bone marrow (negative): Garriott et al. (1991)
        - * Chromosomal damage in *Allium cepa* (onion) (positive): Mazzeo and Marin-Morales (2015)
        - Mutations and chromosomal aberrations in plants (positive): as reviewed in IARC (1991)
        - X and Y chromosome loss in male larval-fed *Drosophila melanogaster* (positive): as reviewed in IARC (1991)
        - XXY nondisjunction in progeny of male larval-fed *Drosophila melanogaster* (positive): as reviewed in IARC (1991)
    - Other endpoints, reviewed in IARC (1991)
        - Sex-linked recessive lethal mutation in *Drosophila melanogaster* (negative)
        - Male rat dominant lethal test (negative)
        - Mouse dominant lethal test (positive)
        - Aneuploidy in *Drosophila melanogaster* (positive and negative)

In vitro

- Micronucleus formation
    - * Human peripheral blood lymphocytes (positive): Sarıgöl Kılıç et al. (2018) (98.8% purity)
    - * Human peripheral blood lymphocytes (negative): Ribas et al. (1996)
    - * Human HepG2 cells (negative): Franco-Bernardes et al. (2017)
    - * Chinese hamster lung fibroblast V79 cells (positive): Sarıgöl Kılıç et al. (2018)

- Chromosomal abnormalities
    - * Chromosomal aberrations in human peripheral blood lymphocytes (negative): Ribas et al. (1996) (99.4% purity)
    - Chromosomal aberrations in human lymphocytes (positive and negative): as reviewed in IARC (1991)
    - Chromosomal aberrations in Chinese hamster ovary cells (negative): Garriott et al. (1991)
    - * Sister chromatid exchange in human peripheral blood lymphocytes (slight positive): Ribas et al. (1996) (99.4% purity)
    - Sister chromatid exchange in human lymphocytes (positive): as reviewed in IARC (1991)
- DNA damage
    - * Human peripheral blood lymphocytes (positive): Sarıgöl Kılıç et al. (2018) (98.8% purity)
    - * DNA strand breaks (comet assay) in human lymphocytes (positive): Ribas et al. (1995) (99.4% purity)[14]
    - * DNA strand breaks (comet assay) in human HepG2 cells (negative): Franco-Bernardes et al. (2017)
    - * DNA strand breaks (comet assay) in Chinese hamster lung fibroblast V79 cells (positive): Sarıgöl Kılıç et al. (2018) (98.8% purity)
    - * DNA damage (genomic template stability) in maize (positive): Bozari and Aksakal (2013)
- Mutagenicity in L5178Y mouse lymphoma $TK^{+/-}$ assay (negative): Garriott et al. (1991)

In bacteria and fungi

- *Salmonella* reverse mutation assay (negative): as reviewed in IARC (1991)
- Bacteriophage mutation assays (negative): as reviewed in IARC (1991)
- *Escherichia coli* mutation assays (negative): as reviewed in IARC (1991)
- *Saccharomyces cerevisiae* mutation assays (*negative*): as reviewed in IARC (1991)
- *Aspergillus nidulans* mitotic recombination (positive): as reviewed in IARC (1991)
- *Aspergillus nidulans* nondisjunction (negative): as reviewed in IARC (1991)

[14] Reported purity 9.40%, which appears to be a typo as Ribas et al. (1996) used the same source of trifluralin and stated purity as 99.4%.

- Mitotic crossing-over in *Aspergillus nidulans* (positive): Cardoso et al. (2010)
- *Neurospora crassa* aneuploidy (positive): as reviewed in IARC (1991)

- Alters DNA repair or causes genomic instability

  In vivo

  - * Shortened relative telomere length in buccal DNA of exposed humans (male pesticide applicators in the AHS) (positive): Hou et al. (2013)

  In vitro

  - Unscheduled DNA synthesis in human EUE cells (negative): as reviewed in IARC (1991)
  - DNA repair in cultured human cells (negative): as reviewed in IARC (1991)

- Induces oxidative stress

  In vitro

  - * Reactive oxygen species in V79 cells (positive): Sarıgöl Kılıç et al. (2018) (98.8% purity)

- Modulate receptor-mediated effects

  In vivo

  - Effects on thyroid receptors
    - * Increase thyroid stimulating hormone and decrease T3 and T4 in rats *in vivo*: Saghir et al. (2008)

  In vitro

  - * ToxCast/Tox21: active in 88 of 833 assays tested, including many assays on nuclear receptors, including assays measuring estrogen receptor (ER) activity (ATG_ERa_TRANS_up), pregnane X receptor (PXR) activity (ATG_PXR_TRANS_up), and thyroid hormone agonist activity (TOX21_TSHR_HTRF_Agonist_ch1)

Structure-activity considerations

- Structurally related to the dinitroaniline compound oryzalin, a carcinogen listed under Proposition 65.
- Structurally related to the dinitroaniline compound ethalfluralin, which induces mammary fibroadenomas in female rats and is mutagenic in *Salmonella* and *E. coli*: US EPA (1986, pp.10-11).

Reviews

- IARC (1991)
- US EPA (1986, 1987, 1988)

References cited in "Trifluralin"

Alavanja MC, Sandler DP, McMaster SB, Zahm SH, McDonnell CJ, Lynch CF, et al. 1996. The agricultural health study. Environ Health Perspect 104:362-369.

Ambrus Á, Hamilton DJ, Kuiper HA, Racke KD. 2003. Significance of impurities in the safety evaluation of crop protection products (IUPAC Technical Report). Pure and Applied Chemistry 75:937.

Andreotti G, Freeman LE, Hou L, Coble J, Rusiecki J, Hoppin JA, et al. 2009. Agricultural pesticide use and pancreatic cancer risk in the Agricultural Health Study Cohort. Int J Cancer 124:2495-2500.

Andreotti G, Beane Freeman Laura E, Shearer Joseph J, Lerro Catherine C, Koutros S, Parks Christine G, et al. 2020. Occupational Pesticide Use and Risk of Renal Cell Carcinoma in the Agricultural Health Study. Environmental Health Perspectives 128:067011.

Andrews AW, Fornwald JA, Lijinsky W. 1980. Nitrosation and mutagenicity of some amine drugs. Toxicology and Applied Pharmacology 52:237-244.

ATSDR. 2019. Agency for Toxic Substances and Disease Registry. Toxicological profile for N-nitrosodi-N-propylamine. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Bozari S, Aksakal O. 2013. Application of random amplified polymorphic DNA (RAPD) to detect genotoxic effect of trifluralin on maize (Zea mays). Drug and chemical toxicology 36:163-169.

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM, et al. 1990. Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. Cancer research 50:6585-6591.

Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM, et al. 1992. Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. Cancer research 52:2447-2455.

Cardoso RA, Pires LT, Zucchi TD, Zucchi FD, Zucchi TM. 2010. Mitotic crossing-over induced by two commercial herbicides in diploid strains of the fungus Aspergillus nidulans. Genetics and molecular research : GMR 9:231-238.

Donna A, Crosignani P, Robutti F, Betta PG, Bocca R, Mariani N, et al. 1989. Triazine herbicides and ovarian epithelial neoplasms. Scand J Work Environ Health 15:47-53.

Egert G, Greim H. 1976. Formation of dimethylnitrosamine from pesticides carrying methylated tertiary aminogroups in the presence of nitrite at pH 3. Food and Cosmetics Toxicology 14:193-195.

Elespuru RK, Lijinsky W. 1973. The Formation of Carcinogenic Nitroso Compounds from Nitrite and Some Types of Agricultural Chemicals. Food and Cosmetics Toxicology 11:807-817.

Eli Lilly Company. 1966. Rat oncogenicity study of trifuralin. Cited in US EPA 1986.

Eli Lilly Company. 1980. Mouse oncogenicity study of trifuralin. Cited in US EPA 1986.

Francis PC, Emmerson JL, Adams ER, Owen NV. 1991. Oncogenicity study of trifluralin in B6C3F1 mice. Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association 29:549-555.

Franco-Bernardes MF, Rocha OP, Pereira LC, Tasso MJ, Meireles G, de Oliveira DP, et al. 2017. The herbicides trifluralin and tebuthiuron have no genotoxic or mutagenic potential as evidenced by genetic tests. Environmental science and pollution research international 24:24029-24037.

Garriott ML, Adams ER, Probst GS, Emmerson JL, Oberly TJ, Kindig DE, et al. 1991. Genotoxicity studies on the preemergence herbicide trifluralin. Mutation research 260:187-193.

Gebel T, Kevekordes S, Pav K, Edenharder R, Dunkelberg H. 1997. In vivo genotoxicity of selected herbicides in the mouse bone-marrow micronucleus test. Archives of Toxicology 71:193-197.

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R, et al. 1986. Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. JAMA 256:1141-1147.

Hou L, Andreotti G, Baccarelli AA, Savage S, Hoppin JA, Sandler DP, et al. 2013. Lifetime pesticide use and telomere shortening among male pesticide applicators in the Agricultural Health Study. Environ Health Perspect 121:919-924.

IARC. 1991. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 53. Occupational Exposures in Insecticide Application, and Some Pesticides. Trifluralin. Available: https://publications.iarc.fr/_publications/media/download/1864/08a9c846ccbf612b4e746dcfb2053d5e7ee0ebea.pdf.

Kang D, Park SK, Beane-Freeman L, Lynch CF, Knott CE, Sandler DP, et al. 2008. Cancer incidence among pesticide applicators exposed to trifluralin in the Agricultural Health Study. Environ Res 107:271-276.

Konen S, Cavas T. 2008. Genotoxicity testing of the herbicide trifluralin and its commercial formulation Treflan using the piscine micronucleus test. Environ Mol Mutagen 49:434-438.

Lee WJ, Lijinsky W, Heineman EF, Markin RS, Weisenburger DD, Ward MH. 2004. Agricultural pesticide use and adenocarcinomas of the stomach and oesophagus. Occup Environ Med 61:743-749.

Lee WJ, Colt JS, Heineman EF, McComb R, Weisenburger DD, Lijinsky W, et al. 2005. Agricultural pesticide use and risk of glioma in Nebraska, United States. Occupational and environmental medicine 62:786-792.

Mazzeo DEC, Marin-Morales MA. 2015. Genotoxicity evaluation of environmental pollutants using analysis of nucleolar alterations. Environmental Science and Pollution Research 22:9796-9806.

NCI. 1978. Bioassay of trifluralin for possible carcinogenicity. CAS 1582-09-8. (National Cancer Institute Carcinogenesis Technical Report Series No 34).

Reynolds P, Von Behren J, Gunier RB, Goldberg DE, Hertz A, Harnly ME. 2002. Childhood cancer and agricultural pesticide use: an ecologic study in California. Environ Health Perspect 110:319-324.

Reynolds P, Von Behren J, Gunier RB, Goldberg DE, Harnly M, Hertz A. 2005. Agricultural pesticide use and childhood cancer in California. Epidemiology 16:93-100.

Ribas G, Frenzilli G, Barale R, Marcos R. 1995. Herbicide-induced DNA damage in human lymphocytes evaluated by the single-cell gel electrophoresis (SCGE) assay. Mutation research 344:41-54.

Ribas G, Surrallés J, Carbonell E, Xamena N, Creus A, Marcos R. 1996. Genotoxic evaluation of the herbicide trifluralin on human lymphocytes exposed in vitro. Mutation research 371:15-21.

Saghir SA, Charles GD, Bartels MJ, Kan LH, Dryzga MD, Brzak KA, et al. 2008. Mechanism of trifluralin-induced thyroid tumors in rats. Toxicology letters 180:38-45.

Sarıgöl Kılıç Z, Aydın S, Ündeğer Bucurgat Ü, Başaran N. 2018. In vitro genotoxicity assessment of dinitroaniline herbicides pendimethalin and trifluralin. Food and Chemical Toxicology 113:90-98.

Sun Z, Liu YD, Zhong RG. 2010. Theoretical Investigation of N-Nitrosodimethylamine Formation from Nitrosation of Trimethylamine. The Journal of Physical Chemistry A 114:455-465.

US EPA. 1986. Toxicology Branch Peer Review Committee Memorandum on Trifluralin, April 11. Washington, DC: United States Environmental Protection Agency. Available: http://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-036101_11-Apr-86_066.pdf.

US EPA. 1987. Memorandum. Review of the chronic/oncogenicity studies in mice and rats on trifluralin. Washington, DC: United States Environmental Protection Agency. Available: http://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-036101_14-Jul-87_086.pdf.

US EPA. 1988. IRIS Chemical Assessment Summary for Trifluralin. Washington, DC: United States Environmental Protection Agency. Available: http://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0268_summary.pdf#nameddest=woe

US EPA. 1996. Reregistration Eligibility Decision for Trifluralin. Washington, DC: United States Environmental Protection Agency. Available: https://archive.epa.gov/pesticides/reregistration/web/pdf/0179.pdf.

West SD, Day EW. 1979. Determination of volatile nitrosamines in crops and soils treated with dinitroaniline herbicides. Journal of Agricultural and Food Chemistry 27:1075-1080.