OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Proposition 65

Prioritization: Chemicals Identified for Consultation with the Developmental and Reproductive Toxicant Identification Committee

October 2020

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

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Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is proposing 22 chemicals or chemical groups for prioritization review by the Developmental and Reproductive Toxicant Identification Committee (DARTIC), using the prioritization process endorsed by the DARTIC and adopted by OEHHA in 2004. These chemicals are not proposed for listing at this time. OEHHA is seeking public comment and the DARTIC'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 November 16, 2020.

After receiving advice on priority from the DARTIC, OEHHA will choose chemicals for consideration for potential listing by the DARTIC at future meetings.

Part I. 2020 Application of the Prioritization Process to Identify Chemicals for Consultation with the Developmental and Reproductive Toxicant 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 DARTIC 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 DARTIC, other state programs, the scientific community, or the general public) for developmental and reproductive toxicity (DART) evaluation.
- OEHHA identifies chemicals with some evidence of reproductive 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 reproductive hazard (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 DARTIC.

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 22 chemicals or chemical groups (see Table 1 below) for Committee discussion, advice, and consultation.

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

At its upcoming meeting, the DARTIC 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 chemicals that will be presented to the DARTIC.

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 reproductive toxicity 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. Of the chemicals screened as of July 2020, those meeting either the human epidemiology or animal data screen were 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 epidemiologic studies suggesting evidence of adverse developmental or reproductive effects. The screen involves finding relevant analytical epidemiology studies through a literature search and evaluating them to identify studies reporting an association between exposure to the chemical and

increased risk of adverse developmental or reproductive effects. For those chemicals with studies available, the abstracts were reviewed to determine whether there was a report of developmental or reproductive toxicity associated with exposure to the chemical and whether the effect might be attributed to the chemical with some confidence. Two or more acceptable[1] analytical studies reporting adverse effects for the same major DART endpoint (i.e., developmental, female reproductive, or male reproductive effects) were required for the chemical to pass the screen.

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 the chemical name and a string of DART-related search terms developed in consultation with the OEHHA librarian. 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 needed.
3. Epidemiologic studies were identified from the titles retrieved in the online search.
4. DART-related effects reported in epidemiologic studies were identified from the titles and abstracts retrieved in the online search; in some cases such identification required retrieval of the full article.
5. Articles identified as potentially relevant were considered in assessing whether they provide evidence of human developmental or reproductive toxicity that is related to exposure to the chemical.

Applying the Animal Data Screen

The animal data screen involves finding relevant animal studies examining possible DART effects through a literature search and evaluating study findings with regard to the screening criteria.

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[1] For the purposes of prioritization, acceptable analytical studies are defined as those with 1) exposed and non-exposed groups, or with groups that have different exposure levels, and 2) both participants with the DART outcome and participants without the DART outcome, and 3) a temporal component that establishes that exposure preceded the DART outcome.

To pass the animal screen at least one of the following criteria were met:

- A minimum of one *in vivo* DART study that meets guideline[2] (US EPA or OECD) standards for methodology and reporting, and which reports at least one statistically significant DART outcome.
- A minimum of one *in vivo*, non-DART, guideline-quality toxicity study[3] that provides statistically significant evidence of at least one DART outcome in accordance with US EPA Guidelines for Reproductive Toxicity Risk Assessment[4].
- A minimum of five *in vivo* studies that do not meet guideline standards but which taken together appear to support a relationship between exposure and one or more specific DART outcomes.
- Results from a minimum of one *in vitro*[5] or non-standard species[6] experiment reporting disruption of essential developmental or reproductive processes, combined with *in vivo* data indicating that the upstream effect would result in one or more DART outcomes.

For each chemical, the steps used in applying the animal 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 the chemical name and a string of DART-related search terms developed in consultation with the OEHHA librarian. 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 needed.
3. Animal studies were identified from the titles retrieved in the online search.

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[2] Guideline studies are typically conducted using a rodent or rabbit model, but other mammalian species may be acceptable.
[3] Examples of relevant study types include: chronic or subchronic toxicity studies, or cancer bioassays.
[4] US Environmental Protection Agency (US EPA, 1996). Guidelines for Reproductive Toxicity Risk Assessment. Federal Register 61(212): 56274-56322.
[5] Typically, but not limited to, primary cell or organ culture experimental systems focused on effects such as expression of specific genes, binding of specific receptors known to be necessary for normal developmental events to occur, disruption of biochemical events known to be necessary for normal developmental events to occur, disruption of cell differentiation, etc. Protocols may involve exposure *in vivo* or *in vitro*, with further experimental elements performed *in vitro*. Experimental focus should be validated by targeted *in vivo* experiments, ideally in mammalian species but not necessarily as part of a "Guideline" type protocol.
[6] Non-mammalian test species, such as zebrafish, which commonly serve as model systems for potential DART effects.

4. DART-related effects reported in animal studies were identified from the titles and abstracts retrieved in the online search; in some cases such identification required retrieval of the full article.
5. 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. Additional information relevant to DART, such as studies on mechanisms of action, metabolism, and pharmacokinetics were taken into consideration in the preliminary toxicological evaluation. Chemicals for which the overall evidence indicated that developmental or reproductive toxicity may be a concern are being proposed here for DARTIC consideration.

Chemicals Proposed for DARTIC Consideration

OEHHA identified the 22 chemicals or chemical groups listed in Table 1 below for possible preparation of hazard identification materials. At its next meeting, the DARTIC will provide OEHHA with advice on the prioritization of these chemicals for possible preparation of hazard identification materials.

For each of the chemicals, OEHHA has compiled a separate overview of the relevant findings from studies that were identified during the preliminary toxicological evaluation and these are presented later in this document.

An overview of the exposure characteristics for each of the chemicals is presented in Table 2, below.

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

Chemical	CAS Registry Number
Benzophenone-3	131-57-7
Bisphenol S (BPS)	80-09-1
Diazinon	333-41-5
Diethylphthalate (DEP)	84-66-2
Domoic acid	14277-97-5
Glyphosate and its salts	---
Manganese	7439-96-5
Neonicotinoid pesticides	
Acetamiprid	135410-20-7
Clothianidin	210880-92-5
Imidacloprid	138261-41-3
Thiamethoxam	153719-23-4
Parabens	
Butyl paraben	94-26-8
Isobutyl paraben	4247-02-3
Methyl paraben	99-76-3
Propyl paraben	94-13-3
Per- and polyfluorinated substances (PFASs)	
Perfluorodecanoic acid (PFDA)	335-76-2
Perfluorohexanesulfonic acid (PFHxS)	355-46-4
Perfluorononanoic acid (PFNA)	375-95-1
Perfluoroundecanoic acid (PFUnDA)	2058-94-8
Titanium dioxide nanoparticles	---
Vinpocetine	42971-09-5
Zearalenone	17924-92-4

Table 2. Exposure Characteristics of Chemicals for DARTIC Consultation

Chemical	Exposure			
	Widespread	High in frequent consumers	Limited / occupational	High in infrequent consumers
Benzophenone-3	✓	✓		
Bisphenol S (BPS)	✓			
Diazinon	✓		✓	
Diethylphthalate (DEP)	✓			
Domoic acid			✓	
Glyphosate and its salts	✓		✓	
Manganese	✓		✓	
Neonicotinoid pesticides				
Acetamiprid	✓		✓	
Clothianidin	✓		✓	
Imidacloprid	✓		✓	
Thiamethoxam	✓		✓	
Parabens				
Butyl paraben	✓	✓		
Isobutyl paraben	✓	✓		
Methyl paraben	✓	✓		
Propyl paraben	✓	✓		
PFASs				
PFDA	✓			
PFHxS	✓			
PFNA	✓			
PFUnDA	✓			
Titanium dioxide nanoparticles	✓	✓		
Vinpocetine		✓		✓
Zearalenone	✓	✓		

Next Steps

The DARTIC 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 DARTIC for consideration. The public is also given the opportunity at the DARTIC meeting to comment on the chemicals being proposed for hazard identification materials preparation.

The DARTIC may also suggest chemicals other than these 22 for which hazard identification materials should be prepared. The DARTIC 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' potential to cause developmental or reproductive toxicity following a comprehensive search and evaluation of the scientific literature. These materials will be provided to the DARTIC, and released for public comment, prior to the public meeting at which the DARTIC deliberates on a listing decision.

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

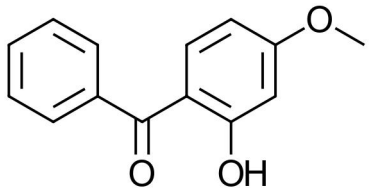
References cited in Part I

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

Part II. Chemicals Identified for Consultation with the Developmental and Reproductive Toxicant Identification Committee

Benzophenone-3

(Oxybenzone, BP-3, 4-Methoxy-2-hydroxybenzophenone, CAS No. 131-57-7)



Benzophenone-3 (BP-3) is used as a sunscreen agent because it absorbs ultraviolet (UV) light. Along with other UV-absorbing agents, it has been used in industry and medicine for more than 30 years. Benzophenone-3 is also used in cosmetic products such as lipsticks, hair sprays, hair dyes, shampoo, detergent bars and sunscreen lotions, and as such, millions of consumers are exposed to it on a daily basis. It is also found in plastic packaging for some foods, and in paint products, toys, and furniture finishes, to limit degradation from UV light.

BP-3 exposure is widespread, and it is readily absorbed when applied to the skin (Matta et al. 2020). In a study of 60 individuals living in Los Angeles, BP-3 was been measured with a detection frequency of 95% (Biomonitoring California, 2020). BP-3 has been detected in amniotic fluid (Philippat et al. 2013), breast milk (Hines et al. 2015), placental tissue (Philippat et al. 2019), and in the urine of 96.8% of individuals tested in the 2013-2014 National Health and Nutrition Examination Survey (NHANES). In this NHANES survey the geometric mean (and 95th percentile) of BP-3 in urine µg/g (creatinine-corrected) was higher in females, i.e., 38.9 (2550), than the values reported for the total population, i.e., 25.2 (1190) (CDC, 2019).

BP-3 passed the human and animal data screens, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of a number of relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Numerous human studies reporting developmental and reproductive toxicity (DART)-related effects associated with BP-3 were identified in the recent literature. DART findings reported in a number of epidemiologic studies published within the last 12 years are summarized here. The findings are organized by groups of outcomes.

Placental weight

- Positive association of maternal urine levels with placental weight (prospective cohort study) (Philippat et al. 2019).

Birth weight

- Higher birth weight associated with paternal preconception urine levels (prospective preconception cohort study of subfertile couples) (Messerlian et al. 2018).
- Higher birth weight associated with maternal urine levels in boys (nested case-control study) (Philippat et al. 2012).
- Higher birth weight associated with maternal urine levels (prospective cohort study) (Philippat et al. 2019).
- Higher birth weight associated with maternal urine levels in boys; sex x BP-3 interaction with decreased birth weight in girls (prospective cohort study) (Wolff et al. 2008).
- No association with BP-3; association of lower birth weight in boys with maternal serum 4-hydroxy-benzophenone, a metabolite of BP-3, for the middle exposure group, compared to the low exposure group (prospective cohort study) (Krause et al. 2018).

Gestation duration

- Shorter gestational age associated with maternal urine levels (prospective cohort study) (Aker et al. 2019).
- Shorter gestational age, associated with maternal urine sample at admission for delivery, stronger association observed in boys (approximately 1 week) (cross-sectional study) (Tang et al. 2013).

Congenital malformations

- Increased odd ratios for Hirschsprung's disease with increasing exposure levels, p-value for trend <0.05, (case-control study), and in an *in vitro* study, BP-3 influenced cell migration via SLIT2/ROBO1-miR-218-RET/PLAG1 pathway, a possible mechanism for Hirschsprung's disease (Huo et al. 2016).

Secondary sex ratio

- No association with BP-3; excess male births associated with maternal 4-hydroxybenzophenone, a metabolite of BP-3 (prospective cohort study) (Bae et al. 2016).

Thyroid hormones

- Lower maternal total triiodothyronine (T_3), total thyroxine (T_4), and T_3/T_4 ratio, and higher thyroid stimulating hormone levels observed at least once during pregnancy; urinary levels measured at four time points during pregnancy (nested case-control study) (Aker et al. 2018).
- Lower maternal free triiodothryronine (FT_3), measured in urine at two time points during pregnancy (prospective cohort study) (Aker et al. 2016).
- No association with BP-3, measured in urine at two time points during pregnancy, and maternal serum thyroid hormone levels; in sensitivity analysis restricted to women with no imputed specific gravity, maternal BP-3 was associated with lower neonatal serum thyroid stimulating hormone (prospective cohort study) (Berger et al. 2018).
- No association with BP-3, positive association between maternal serum levels of 4-hydroxy-benzophenone, a metabolite of BP-3, and T_3,T_4, insulin-like growth factor I (IGF-I) and its binding protein IGFBP3 in mothers carrying male fetuses (prospective cohort study) (Krause et al. 2018).

Neurodevelopment, prenatal exposure

- Poorer prosocial behaviors at 10 years of age associated with higher maternal urinary BP-3, no association observed between concurrent phenol exposures and children's neurobehavioral problems at 10 years of age (prospective cohort study) (Guo et al. 2020).

Childhood fat mass, prenatal exposure

- Lower fat mass in girls (at 4 and 9 years of age), not in boys, associated with third trimester maternal urine levels (prospective cohort study) (Buckley et al. 2016).

Age at menarche and pubertal development

- Earlier pubertal development, exposures quantified in urine collected prior to the onset of breast development, and during adolescence (prospective cohort study) (Binder et al. 2018).
- Later pubertal development, exposure measured in children at age 6-8, followed for 7 years (prospective cohort study) (Wolff et al. 2015).

- No difference in age of menarche (cross-sectional study, NHANES) (Buttke et al. 2012).
- No difference in pubertal timing, measured in maternal urine during pregnancy and in children at age 9 (prospective cohort study) (Harley et al. 2019).

Steroid hormone levels

- Higher serum testosterone and estradiol and lower serum follicle-stimulating hormone in young men, associated with higher BP-3 within the group of mutation carriers of the filaggrin gene (loss of function mutation carriers), i.e. the cases, compared to controls ("case-control"/cross-sectional study) (Joensen et al. 2018).
- Lower serum total testosterone in male adolescents, not in females or in children ages 6-11 years (cross-sectional study, NHANES) (Scinicariello and Buser 2016).

Animal studies

Findings reported in whole animal studies examining possible DART effects of exposure to BP-3 were identified. Findings from a number of studies published within the last 20 years are summarized here.

Developmental effects

- Mouse: Three groups of pregnant dams were exposed to a dermal dose of 50 mg/k/day BP-3 from gestation day (GD) 0 to 6. In the first group fetal parameters of these 'first pregnancies' were assessed between GD 5-14; in the second group the dams delivered, were mated a second time, and fetal parameters of these 'second pregnancies' were assessed between GD 10-14; in the third group dams delivered litters from the first and second pregnancies, and pup and early postnatal body weights were assessed. BP-3 treatment resulted in reduced fetal weight at GD 14, feto-placenta index, and weight at postnatal day (PND) 4 and then from PND10 onwards in males, and in females at PND1, PND10, and PND13 but recovered normal weight from PND16 onwards in the first pregnancy, and decreased placental weights in the second pregnancy. In addition, first and second progenies of exposed mothers showed a higher percentage of females (female sex ratio) (Santamaria et al. 2020).
- Zebrafish: Decreased number of hatched embryos in a concentration dependent manner, caused tail deformation, impaired development of jaw, and lack of swim bladder inflation in a concentration dependent manner (Balázs et al. 2016).

Neurodevelopmental effects

- Zebrafish: BP-3 exposure at 10 µg/L during 6-24 hours post fertilization (hpf), resulted in increased spontaneous movement at 21 and 24 hpf; decreased touch response at 27 hpf; heightened hyperactivity in locomotor response at 5 days post fertilization (dpf); decreased shoaling behavior at 11 dpf and decreased mirror attacks at 12 dpf. Additional effects included decreased axonal growth at 27 hpf; decreased cell proliferation and increased cell apoptosis in the head region of larval zebrafish immediately after BP-3 exposure at 24 hpf; and increased expression of retinoid X receptor gene rxrgb at 5 dpf. Rxrgb knockdown through morpholino injection largely blocked most of the BP-3-induced neurodevelopmental effects, (e.g., on axonal growth, cell proliferation and cell apoptosis) (Tao et al. 2020).

Mammary gland effects

- Mouse: In female offspring of dams exposed during pregnancy day zero until weaning, effects on the mammary gland included permanent changes to ductal density, an intermediate phenotype for expression of the progesterone receptor, a monotonic, dose-dependent increase in cell proliferation, and an intermediate phenotype for Esr1 expression (LaPlante et al. 2018).
- Mouse: Exposure *in utero* and during lactation reduced mammary cell proliferation, decreased the number of cells expressing estrogen receptor α, and altered mammary gland morphology in adulthood in females. In males, exposure reduced the size and growth of the mammary gland prior to and during puberty (Matouskova et al. 2019).

Uterine weight effect

- Rat: Acute (4-day) exposure slightly increased uterine weight in sexually immature females (Schlumpf et al. 2001).

Endocrine effects

- Zebrafish: Embryo exposure, decreases in whole-body T_4 and T_3 at day 6 post fertilization; up-regulation of *dio1* and *ugt1ab* genes (Lee et al. 2018).
- Zebrafish: Down-regulation of the *hsd3b*, *hsd17b3*, *hsd11b2* and *cyp11b2* transcripts in the testes, suggesting antiandrogenic activity in adult males and in eleuthero (free-floating) embryos, down-regulation of *esr1*, *ar* and *cyp19b* in the brain of adult males (Blüthgen et al. 2012).
- Japanese medaka: Plasma testosterone concentrations significantly increased in males; 17β-estradiol to 17β-estradiol/testosterone ratio showed significant decreases in both males and females; down-regulation of gonadal steroidogenic

genes such as *star*, *cyp11a*, *cyp17*, *hsd3b*, *hsd17b3*, and *cyp19a*; daily average egg reproduction per female significantly reduced. Hatchability of F1 eggs was not affected by continuous exposure; juvenile fish showed a concentration-dependent decrease in the condition factor (K = 100 [total weight (g)]/total length (cm3)]) but mortality was not affected in this two generation study (Kim et al. 2014).

- Japanese medaka: Exposure resulted in a lower number of eggs produced after 7 days, but returned to control values after 21 days; a lower percentage of fertilized eggs hatched, and a temporal effect of diminished egg viability 13-15 days after eggs were collected (Coronado et al. 2008).
- *Chironomus riparius* (Diptera) (harlequin fly, a species of non-biting midge): Decreased egg hatching, strong dose-response relationship observed for fertility with none of the egg ropes hatching at 8 mg BP-3/kg, emergence and development time were impaired in FI generation (whose parents were exposed) even when maintained in control/clean conditions (Campos et al. 2019).

Other DART effects

- Zebrafish: In the Fish Development Test[7], newly fertilized eggs were exposed until the completion of sexual differentiation at about 60 days post hatch, resulting in a monotone dose-dependent skewing of phenotypic sex ratio toward fewer males and more females; gonad maturation was also affected in both males and females (Kinnberg et al. 2015).
- Zebrafish: 12-day exposure of adult males resulted in a slight yet significant increase in the vitellogenin concentration at the middle exposure dose (Kinnberg et al. 2015).
- Rainbow trout: Significant induction of vitellogenin (Coronado et al. 2008).

Mechanistic, in vitro and other relevant data

- Human: In breast epithelial cell lines low concentrations of BP-3 resulted in increased formation of ERα-dependent R-loops (RNA:DNA triplex structures) and DNA damage (Majhi et al. 2020).
- Human: Increased cell proliferation and increased secretion of the estrogen-regulated protein pS2 in MCF-7cells, a breast cancer cell line (Schlumpf et al. 2001).
- Human: Adverse effect on the viability of neuroblastoma SH-SY5Y cells at 10^{-4}M, and enhanced activity of caspase-3 activity at much lower concentrations (from 10^{-8} to 10^{-7}M), indicating apoptosis. The authors stated that these effects were seen “at concentrations that may be reached *in vivo*” (Broniowska et al. 2016).

[7] https://www.oecd-ilibrary.org/environment/test-no-234-fish-sexual-development-test_9789264122369-en

- Rat: In whole ovary culture, BP-3 decreased the number of total oocytes, the number of nests per ovary, the population of early primary follicles, and the number of p27-positive oocytes; induced overexpression of *Foxl2* mRNA levels through ESR2; and increased *Fst* mRNA levels independently from ESR2 or Foxl2 (Santamaría et al. 2019).
- Rat: In a pituitary (GH3) cell line, observed down-regulation of *Tshβ*, *Trhr*, and *Trβ* genes and in a thyroid follicular cell (FRTL-5) line, observed up-regulation of *Nis* and *Tg* genes while down-regulating the *Tpo* gene (Lee et al. 2018).
- Rat: Exposure *in utero* and via lactation, and continuing up to 7 weeks of age, induced the mitochondrial apoptosis pathway in the 7-week old rat frontal cortex, increased caspase-9 and reduced levels of anti-apoptotic proteins in the hippocampus, reduced levels of ERβ in the nuclear fraction and GPR30 in the membrane fraction in both brain regions, and significantly increased AhR in the cytosol of the frontal cortex (Krzyżanowska et al. 2018).
- Mouse: Primary neocortical and hippocampal neuronal cell cultures prepared from mouse embryos on GD 15-17 were exposed to BP-3 for 6 or 24 hours. Treatment affected mRNA and protein expression levels of *Erα*, *Erβ*, *Gpr30*, and *Pparγ*, in parallel with BP-3-induced apoptosis and neurotoxicity, suggesting that "BP-3-evoked apoptosis of neuronal cells is mediated via attenuation of Erα/Pparγ and stimulation of Erβ/Gpr30 signaling" (Wnuk et al. 2018a).
- Mouse: Neocortical cells cultured from isolated neocortical brain tissue obtained from embryos of pregnant mice treated on GD 7-16 with 50 mg/kg BP-3 showed "severe neuronal apoptosis accompanied by impaired ESR1/ESR2 expression, enhanced GPER1 expression, global DNA hypomethylation and altered methylation statuses of apoptosis-related and ERs genes" (Wnuk et al. 2018b).
- Mouse: Neocortical cells cultured from isolated brain tissue obtained from embryos of pregnant mice treated on GD 7-16, "using environmentally relevant doses" (e.g., 50 mg/kg) showed:
    - Impaired autophagy in terms of BECLIN-1, MAP1LC3B, autophagosomes, and autophagy-related factors;
    - Disrupted levels of retinoid X receptor (RXR) and peroxisome proliferator-activated receptor gamma (PPARγ);
    - Altered epigenetic status (i.e., attenuated HDAC and sirtuin activities);
    - Inhibited post-translational modifications in terms of global sumoylation;
    - Dysregulated expression of neurogenesis- and neurotransmitter-related genes, as well as miRNAs involved in pathologies of the nervous system (Wnuk et al. 2019).
- Mouse: Ovariectormized females were treated for 4 days, after which the mammary tissue was examined histologically. Acute exposure to BP-3 increased the formation of ERα-dependent R-loops (RNA:DNA triplex structures) and the DNA damage marker γ-H2AX (Majhi et al. 2020).

- Bioluminescence-based yeast (BLYES, BLYAS, and BLYR): BP-3 caused cytotoxicity and was estrogenic and anti-androgenic, estrogen assay showed non-monotonic concentration-response curve (Balázs et al. 2016).
- Zebrafish: In embryos treated 2 hours post fertilization, BP-3 prolonged hatching time and reduced hatching rate of embryos and some hatched larvae developed yolk sac edema and curved spines; BP-3 upregulated gene expression of CYP1A, CYP1B, CYP3A65, ERα, ERβ1, GPER, VTG1, BRCA2, CYP19A, DMRT1, GSTA, GSTM and GSTP, and downregulated gene expression of 11β-HSD, and RNA-seq data showed that BP-3 affects steroid hormone biosynthesis (estradiol-17 β) (Meng et al. 2020).

References cited in "BP-3"

Aker AM, Watkins DJ, Johns LE, Ferguson KK, Soldin OP, Anzalota Del Toro LV, et al. 2016. Phenols and parabens in relation to reproductive and thyroid hormones in pregnant women. Environ Res 151:30-37.

Aker AM, Johns L, McElrath TF, Cantonwine DE, Mukherjee B, Meeker JD. 2018. Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: A repeated measures study. Environ Int 113:341-349.

Aker AM, Ferguson KK, Rosario ZY, Mukherjee B, Alshawabkeh AN, Cordero JF, et al. 2019. The associations between prenatal exposure to triclocarban, phenols and parabens with gestational age and birth weight in northern Puerto Rico. Environ Res 169:41-51.

Bae J, Kim S, Kannan K, Buck Louis GM. 2016. Couples' urinary concentrations of benzophenone-type ultraviolet filters and the secondary sex ratio. Sci Total Environ 543:28-36.

Balázs A, Krifaton C, Orosz I, Szoboszlay S, Kovács R, Csenki Z, et al. 2016. Hormonal activity, cytotoxicity and developmental toxicity of UV filters. Ecotoxicol Environ Saf 131:45-53.

Berger K, Gunier RB, Chevrier J, Calafat AM, Ye X, Eskenazi B, et al. 2018. Associations of maternal exposure to triclosan, parabens, and other phenols with prenatal maternal and neonatal thyroid hormone levels. Environ Res 165:379-386.

Binder AM, Corvalan C, Calafat AM, Ye X, Mericq V, Pereira A, et al. 2018. Childhood and adolescent phenol and phthalate exposure and the age of menarche in Latina girls. Environ Health 17:32.

Biomonitoring California. 2020. Available: https://biomonitoring.ca.gov/results/chemical/all?field_chemical_name_target_id_selective%5B%5D=129. [accessed 28 August 2020].

Blüthgen N, Zucchi S, Fent K. 2012. Effects of the UV filter benzophenone-3 (oxybenzone) at low concentrations in zebrafish (*Danio rerio*). Toxicol Appl Pharmacol 263:184-194.

Broniowska Ż, Pomierny B, Smaga I, Filip M, Budziszewska B. 2016. The effect of UV-filters on the viability of neuroblastoma (SH-SY5Y) cell line. Neurotoxicology 54:44-52.

Buckley JP, Herring AH, Wolff MS, Calafat AM, Engel SM. 2016. Prenatal exposure to environmental phenols and childhood fat mass in the Mount Sinai Children's Environmental Health Study. Environ Int 91:350-356.

Buttke DE, Sircar K, Martin C. 2012. Exposures to endocrine-disrupting chemicals and age of menarche in adolescent girls in NHANES (2003-2008). Environ Health Perspect 120:1613-1618.

Campos D, Silva ARR, Loureiro S, Grabicová K, Staňová AV, Soares A, et al. 2019. Two-generational effects of benzophenone-3 on the aquatic midge *Chironomus riparius*. Sci Total Environ 669:983-990.

CDC (Center for Disease Control and Prevention) 2019. Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2019, Volume One. Available: https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2019-508.pdf [accessed 23 September 2020].

Coronado M, De Haro H, Deng X, Rempel MA, Lavado R, Schlenk D. 2008. Estrogenic activity and reproductive effects of the UV-filter oxybenzone (2-hydroxy-4-methoxyphenyl-methanone) in fish. Aquat Toxicol 90:182-187.

Guo J, Wu C, Zhang J, Li W, Lv S, Lu D, et al. 2020. Maternal and childhood urinary phenol concentrations, neonatal thyroid function, and behavioral problems at 10 years of age: The SMBCS study. Sci Total Environ 743:140678.

Harley KG, Berger KP, Kogut K, Parra K, Lustig RH, Greenspan LC, et al. 2019. Association of phthalates, parabens and phenols found in personal care products with pubertal timing in girls and boys. Hum Reprod 34:109-117.

Hines EP, Mendola P, von Ehrenstein OS, Ye X, Calafat AM, Fenton SE. 2015. Concentrations of environmental phenols and parabens in milk, urine and serum of lactating North Carolina women. Reprod Toxicol 54:120-128.

Huo W, Cai P, Chen M, Li H, Tang J, Xu C, et al. 2016. The relationship between prenatal exposure to BP-3 and Hirschsprung's disease. Chemosphere 144:1091-1097.

Joensen UN, Jørgensen N, Thyssen JP, Szecsi PB, Stender S, Petersen JH, et al. 2018. Urinary excretion of phenols, parabens and benzophenones in young men: Associations to reproductive hormones and semen quality are modified by mutations in the filaggrin gene. Environ Int 121:365-374.

Kim S, Jung D, Kho Y, Choi K. 2014. Effects of benzophenone-3 exposure on endocrine disruption and reproduction of Japanese medaka (*Oryzias latipes*)--a two generation exposure study. Aquat Toxicol 155:244-252.

Kinnberg KL, Petersen GI, Albrektsen M, Minghlani M, Awad SM, Holbech BF, et al. 2015. Endocrine-disrupting effect of the ultraviolet filter benzophenone-3 in zebrafish, *Danio rerio.* Environ Toxicol Chem 34:2833-2840.

Krause M, Frederiksen H, Sundberg K, Jørgensen FS, Jensen LN, Nørgaard P, et al. 2018. Maternal exposure to UV filters: Associations with maternal thyroid hormones, IGF-I/IGFBP3 and birth outcomes. Endocr Connect 7:334-346.

Krzyżanowska W, Pomierny B, Starek-Świechowicz B, Broniowska Ż, Strach B, Budziszewska B. 2018. The effects of benzophenone-3 on apoptosis and the expression of sex hormone receptors in the frontal cortex and hippocampus of rats. Toxicol Lett 296:63-72.

LaPlante CD, Bansal R, Dunphy KA, Jerry DJ, Vandenberg LN. 2018. Oxybenzone alters mammary gland morphology in mice exposed during pregnancy and lactation. J Endocr Soc 2:903-921.

Lee J, Kim S, Park YJ, Moon HB, Choi K. 2018. Thyroid hormone-disrupting potentials of major benzophenones in two cell lines (GH3 and FRTL-5) and embryo-larval zebrafish. Environ Sci Technol 52:8858-8865.

Majhi PD, Sharma A, Roberts AL, Daniele E, Majewski AR, Chuong LM, et al. 2020. Effects of benzophenone-3 and propylparaben on estrogen receptor-dependent r-loops and DNA damage in breast epithelial cells and mice. Environ Health Perspect 128:17002.

Matouskova K, Jerry DJ, Vandenberg LN. 2019. Exposure to low doses of oxybenzone during perinatal development alters mammary gland morphology in male and female mice. Reprod Toxicol.

Matta MK, Florian J, Zusterzeel R, Pilli NR, Patel V, Volpe DA, et al. 2020. Effect of sunscreen application on plasma concentration of sunscreen active ingredients: A randomized clinical trial. Jama 323:256-267.

Meng Q, Yeung K, Kwok ML, Chung CT, Hu XL, Chan KM. 2020. Toxic effects and transcriptome analyses of zebrafish (*Danio rerio*) larvae exposed to benzophenones. Environ Pollut 265:114857.

Messerlian C, Mustieles V, Minguez-Alarcon L, Ford JB, Calafat AM, Souter I, et al. 2018. Preconception and prenatal urinary concentrations of phenols and birth size of singleton infants born to mothers and fathers from the Environment And Reproductive Health (EARTH) Study. Environ Int 114:60-68.

Philippat C, Mortamais M, Chevrier C, Petit C, Calafat AM, Ye X, et al. 2012. Exposure to phthalates and phenols during pregnancy and offspring size at birth. Environ Health Perspect 120:464-470.

Philippat C, Wolff MS, Calafat AM, Ye X, Bausell R, Meadows M, et al. 2013. Prenatal exposure to environmental phenols: Concentrations in amniotic fluid and variability in urinary concentrations during pregnancy. Environ Health Perspect 121:1225-1231.

Philippat C, Heude B, Botton J, Alfaidy N, Calafat AM, Slama R. 2019. Prenatal exposure to select phthalates and phenols and associations with fetal and placental weight among male births in the EDEN cohort (France). Environ Health Perspect 127:17002.

Santamaria CG, Meyer N, Schumacher A, Zenclussen ML, Teglia CM, Culzoni MJ, et al. 2020. Dermal exposure to the UV filter benzophenone-3 during early pregnancy affects fetal growth and sex ratio of the progeny in mice. Arch Toxicol 94:2847-2859.

Santamaría CG, Abud JE, Porporato MM, Meyer N, Zenclussen AC, Kass L, et al. 2019. The UV filter benzophenone 3, alters early follicular assembly in rat whole ovary cultures. Toxicol Lett 303:48-54.

Schlumpf M, Cotton B, Conscience M, Haller V, Steinmann B, Lichtensteiger W. 2001. *In vitro* and *in vivo* estrogenicity of UV screens. Environ Health Perspect 109:239-244.

Scinicariello F, Buser MC. 2016. Serum testosterone concentrations and urinary bisphenol a, benzophenone-3, triclosan, and paraben levels in male and female children and adolescents: NHANES 2011-2012. Environ Health Perspect 124:1898-1904.

Tang R, Chen MJ, Ding GD, Chen XJ, Han XM, Zhou K, et al. 2013. Associations of prenatal exposure to phenols with birth outcomes. Environ Pollut 178:115-120.

Tao J, Bai C, Chen Y, Zhou H, Liu Y, Shi Q, et al. 2020. Environmental relevant concentrations of benzophenone-3 induced developmental neurotoxicity in zebrafish. Sci Total Environ 721:137686.

Wnuk A, Rzemieniec J, Lasoń W, Krzeptowski W, Kajta M. 2018a. Apoptosis induced by the UV filter benzophenone-3 in mouse neuronal cells is mediated via attenuation of Erα/Pparγ and stimulation of Erβ/Gpr30 signaling. Mol Neurobiol 55:2362-2383.

Wnuk A, Rzemieniec J, Litwa E, Lasoń W, Kajta M. 2018b. Prenatal exposure to benzophenone-3 (bp-3) induces apoptosis, disrupts estrogen receptor expression and alters the epigenetic status of mouse neurons. J Steroid Biochem Mol Biol 182:106-118.

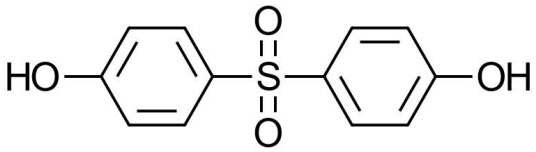
Wnuk A, Rzemieniec J, Staroń J, Litwa E, Lasoń W, Bojarski A, et al. 2019. Prenatal exposure to benzophenone-3 impairs autophagy, disrupts RXRS/PPARγ signaling, and alters epigenetic and post-translational statuses in brain neurons. Mol Neurobiol 56:4820-4837.

Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, et al. 2008. Prenatal phenol and phthalate exposures and birth outcomes. Environ Health Perspect 116:1092-1097.

Wolff MS, Teitelbaum SL, McGovern K, Pinney SM, Windham GC, Galvez M, et al. 2015. Environmental phenols and pubertal development in girls. Environ Int 84:174-180.

Bisphenol S (BPS)

(4,4'-Sulfonyldiphenol, CAS No. 80-09-1)



BPS is an analog of bisphenol A (BPA), and has become increasingly commonly used as a building block for polycarbonates, a reactant in polymer reactions, and a corrosion inhibitor in fast-drying epoxy glues. BPS is also used as a developer in thermal paper such as that used for cash register receipts. Human biomonitoring studies indicate that exposure to BPS is widespread and likely increasing. For example:

- A 2012 study reported that 81% of urine samples from men and women from the general population in the US and seven Asian countries contained BPS (Liao et al. 2012).
- BPS was detected in 89.4% of randomly selected urine samples from the 2013-2014 National Health and Nutrition Examination Survey (Lehmler et al. 2018).
- In Puerto Rico in 2010-2016, pregnant women's urine concentrations of BPS showed an increasing temporal trend while BPA concentrations decreased (Ashrap et al. 2018).

BPS passed the human and animal data screens, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies published from 2018 to August 2020 that were identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Numerous human studies reporting developmental and reproductive toxicity (DART)-related effects associated with BPS were identified in the recent literature. DART findings reported in epidemiologic studies published between 2018 and August 2020 are summarized here. The findings are organized by groups of outcomes.

Indicators of fetal growth

- Lower birth weight (prospective cohort studies) (Hu et al. 2019) (Goodrich et al. 2019).

- Lower birth weight of female infants (prospective cohort study) (Ferguson et al. 2018).
- Birth weight and head circumference not associated with preconception or prenatal exposure of mothers or fathers seeking fertility evaluation (prospective cohort study) (Mustieles et al. 2018).
- No associations with birth size (cohort study) (J Liang et al. 2020).
- No associations with ultrasound parameters of fetal growth in models adjusted for BPA and bisphenol F (BPF) (cross-sectional study) (Zhou et al. 2020).
- Lower ponderal index (prospective cohort study) (Hu et al. 2019).
- Shorter birth length (prospective cohort study) (Hu et al. 2019).
- No associations with birth weight or length (cross-sectional study) (Wan et al. 2018).

Gestation duration

- Shorter gestation length in women with negative life event stress scores (prospective cohort study) (Aker et al. 2020).
- Preterm birth (nested case-control study) (Aung et al. 2019).
- Preterm birth associated with maternal preconception exposure (prospective cohort study) (Mustieles et al. 2020).
- Increased gestational age and odds of late term birth in girls (cross-sectional study) (Wan et al. 2018).
- No significant association with gestational age (longitudinal cohort study) (Huang et al. 2019).

Other DART effects

- Lower psychomotor development at 2 years (prospective cohort study) (Jiang et al. 2020).
- Lower corticotropin releasing hormone in pregnant women (cross-sectional study with repeated measures) (Aker et al. 2019).
- Higher plasma fasting glucose in pregnant women at 24-28 weeks gestation, particularly for women carrying female fetuses (prospective cohort study) (Zhang et al. 2019).
- Decrease in maternal plasma free thyroxine and marginal increase in thyroid stimulating hormone (cross-sectional study with repeated measures) (Aker et al. 2018).
- No associations with serum levels of maternal thyroid hormones in early pregnancy (cross-sectional study) (Derakhshan et al. 2019).
- No consistent association with urinary markers of oxidative stress in pregnant women (cross-sectional study with repeated measures) (Ferguson et al. 2019).

- In women with history of recurrent unexplained spontaneous abortion, marginal association with increased serum interleukin-10, suggesting association with oxidative stress and immune imbalance (cross-sectional study) (F Liang et al. 2020).
- Lower semen volume among men attending a fertility center, and lower sperm concentration, count, and motility among those with body mass index ≥ 25 kg/m^2 (cross-sectional study) (Ghayda et al. 2019).

Animal studies

Findings reported in whole animal studies examining possible DART effects of exposure to BPS published between 2018 and August 2020 are summarized here.

Effects on placenta

- Mouse: A number of effects on the placenta-brain axis were observed, such as a decrease in the area occupied by spongiotrophoblast relative to trophoblast giant cells, reduced placental serotonin (5-HT) concentrations, reduced 5-HT giant cell immunoreactivity, increased concentrations of dopamine and 5-hydroxyindoleacetic acid (5-HIAA; main metabolite of serotonin), increased giant cell dopamine immunoreactivity. These effects on the placenta-brain axis were almost identical to those reported for BPA (Mao et al. 2020).
- Sheep: An effect on placental endocrine function, specifically, dysregulation of the fusogenic trophoblast signaling pathway, was observed (Gingrich et al. 2018).

Developmental effects on hormones

Developmental effects are associated with *in utero* exposures unless otherwise specified.

- Rat: Altered hormone concentrations and antioxidant enzymes (Ullah et al. 2019b).
- Rat: Sex-differentiated effects on hormones after exposure at higher dose (50 µg/kg/day) during pregnancy and lactation (da Silva et al. 2019).
- Mouse: F3 males (F1 exposed *in utero*): Dysregulated serum levels of estradiol-17β and testosterone, as well as expression of steroidogenic enzymes in F3 adult testis (Shi et al. 2019c).
- Mouse: F3 females (F1 exposed *in utero*): Serum estradiol-17β elevated at six months; dysregulated expression of steroidogenic enzymes observed in ovary at three or six months (Shi et al. 2019b).

- Zebrafish: Significant increases in T3 and/or T4, transcriptional changes of genes related to thyroid development, thyroid hormone transport, and metabolism in larvae; delayed hatching (Lee et al. 2019).
- Zebrafish: Decreased thyroxine (T4) and increased 3,5,3'-triiodothyronine (T3) in eggs (F1) (Wei et al. 2018).

Developmental effects on reproductive tissues or function

Developmental effects are associated with *in utero* exposures unless otherwise specified

- Rat: Changes in male reproductive tissues (Ullah et al. 2019b).
- Mouse: Accelerated mammary gland development and adverse mammary gland morphology, adenocarcinomas (Tucker et al. 2018).
- Mouse: Modest changes in the mammary gland at puberty following low doses (2, 200, or 2000 µg/kg/day) of BPS administered perinatally (Kolla and Vandenberg 2019).
- Mouse: Age and dose-specific effects on mammary gland development with perinatal exposure (Kolla et al. 2018).
- Mouse: Early puberty onset, abnormal estrous cyclicity, mating difficulties, fertility, disruption of early folliculogenesis (Shi et al. 2019a).
- Mouse: F3 females (F1 exposed *in utero*): Earlier puberty, abnormal estrous cyclicity, mating difficulties starting at six months, reduced pregnancy rates, parturition issues, nursing issues at six months that worsened at nine months (Shi et al. 2019b).
- Mouse: F3 males (F1 exposed *in utero*): Decreased sperm counts and/or motility and disrupted germ cell development (Shi et al. 2019c).

Other developmental effects

Developmental effects are associated with *in utero* exposures unless otherwise specified

- Rat: Sex-differentiated effects on behavior after exposure at higher dose (50 µg/kg/day) during pregnancy and lactation (da Silva et al. 2019).
- Rat: Increased preference for fat-enriched diet, which may increase risk of obesity (da Silva et al. 2019).
- Mouse: Increased body weight and weights of liver and epididymal white adipose tissue (epiWAT), serum alanine aminotransferase activity, and liver triglyceride and cholesterol levels. Increased expression of genes involved in inflammatory pathways in liver and epiWAT. Changes in levels of metabolites associated with lipid and glucose metabolism in liver and epiWAT. Relative expression of genes involved in lipid and glucose metabolism were significantly changed in liver and epiWAT in males (Meng et al. 2019).

- Zebrafish: Downregulated expression of six neurodevelopment genes (*alpha1-tubulin*, *elavl3*, *gap43*, *mbp*, *syn2a* and *gfap*) and increased oxidative stress; these may be mechanisms by which BPS affects locomotor behavior and alters retinal structure in zebrafish (Gu et al. 2019).
- Zebrafish: Reduced yolk lipid consumption (Wang et al. 2019).

Effects on hormones, postnatal exposure

- Rat: Reduced testosterone production (Ullah et al. 2018a).
- Rat: Decreased plasma testosterone, luteinizing hormone and follicle stimulating hormone concentrations; increased estradiol levels (Ullah et al. 2018b).
- Rat: Highest doses (50 mg/kg) resulted in increased plasma concentrations of testosterone and estradiol, while plasma progesterone, luteinizing hormone, and follicle stimulating hormone concentrations were reduced (Ahsan et al. 2018).
- Mouse: BPS administered during perinatal period did not appear to sensitize the female to an estrogenic challenge administered during the peripubertal period (Kolla and Vandenberg 2019).

Effects on reproductive tissues and function, postnatal exposure

- Rat: Changes to gonadosomatic index and relative reproductive organ weights, increased oxidative stress in testis (Ullah et al. 2018b).
- Rat: Adverse effects on testes and spermatogenesis. Reduced antioxidant enzyme activities and protein content, increased reactive oxygen species in male reproductive tissues (Ullah et al. 2018a).
- Rat: No difference in ovarian weight or ovulation (Demacopulo and Kreimann 2019).
- Rat: Reduced gonadosomatic index and absolute and relative uterus weight. Highest dose (50 mg/kg) resulted in delayed puberty onset and altered estrous cyclicity (Ahsan et al. 2018).
- Rat: Structural impairments in testes and altered sexual differentiation of a dimorphic population of dopaminergic neurons in the anteroventral periventricular nucleus region of the hypothalamus in males (John et al. 2019).
- Daphnids: Inhibition of reproduction and growth (Liu et al. 2019).

Effects on germ cells, postnatal exposure

- Rat: DNA damage in sperm, while motility was not affected (Ullah et al. 2019a).
- Rat: Decreased sperm motility and production (Ullah et al. 2018b).
- Rat: Increased number of cystic follicles in the ovaries and increased number of atretic follicles (Ahsan et al. 2018).
- Mouse: Effects on oocyte quality “even at concentrations…orders of magnitude below those measured in humans” (Prokesova et al. 2019).

- Mouse: Decreased number and increased mean volume of antral follicles. *In vivo* fertilization rate decreased after treatment with 10 ng/g/day and increased after treatment with 100 ng/g/day (Nevoral et al. 2018).

Other effects

- Rat: Increased proliferating cell nuclear antigen expression, which correlates with accumulation of ezrin, in the endometrium (Demacopulo and Kreimann 2019).

Mechanistic, in vitro, and other relevant data

BPS is a structural analog to BPA, thus its physiological effects may be similar (Rochester and Bolden 2015). BPA is a known endocrine disruptor and is listed under Proposition 65 as causing reproductive toxicity (female reproductive endpoint)[8].

- Human: BPS increased production of intracellular reactive oxygen species, decreased antioxidant capacity, and increased damage to biomacromolecules, the main targets of oxidative stress, in KGN cells (ovarian granulosa-like tumor cell line) (Huang et al. 2020).
- Human: BPS showed agonistic activity for human estrogen receptors α and β, and no activity (agonistic or antagonistic) for the following: androgen receptor, glucocorticoid receptor, pregnane X receptor, constitutive androstane receptor (Kojima et al. 2019).
- Human: BPS has estrogen receptor α agonistic activity, and no estrogen receptor β agonistic activity (Li et al. 2018).
- Cattle: Cumulus-oocyte complexes that were matured *in vitro* had spindle abnormalities and chromosome misalignment after exposure to BPS (Campen et al. 2018a).
- Cattle: Granulosa cell estradiol production was stimulated when cells were exposed to 100 μM BPS under basal conditions (Campen et al. 2018b).
- Pig: BPS inhibited estradiol production, cell proliferation, and nonenzymatic scavenging activity, and stimulated cell viability, superoxide and nitric oxide production in cultured granulosa cells (Berni et al. 2019).
- Rat: In an *in vitro* BPS exposure study in cultured testicular tissues, antioxidant enzyme activities and oxidative stress markers were induced, whereas testosterone production was reduced (Ullah et al. 2018a).
- Mouse: In the mouse GC-2 spermatocyte cell line BPS induced apoptosis and caused cellular damage, influenced GC-2 cell steroid receptor and steroidogenesis-related gene expression, and increased global DNA methylation.

[8] https://oehha.ca.gov/media/downloads/proposition-65//p65list010320.pdf

BPS also increased cell viability after 24-hour exposures, but this effect diminished after longer exposure (Sidorkiewicz et al. 2018).

- Mouse: Cell-matrix and cell-cell adhesion, and signal transduction pathways were altered in embryonic stem cells that were treated with BPS during differentiation into neuroectoderm/neural progenitor cells (Yin et al. 2019).
- Zebrafish: In a global transcriptome sequencing study, BPS treatment of embryos altered expression levels of 246 genes. Functional enrichment analysis indicated that metabolism was the main pathway for disruption (Qiu et al. 2019).

References cited in "BPS"

Ahsan N, Ullah H, Ullah W, Jahan S. 2018. Comparative effects of bisphenol S and bisphenol A on the development of female reproductive system in rats; a neonatal exposure study. Chemosphere 197:336-343.

Aker A, McConnell RER, Loch-Caruso R, Park SK, Mukherjee B, Rosario ZY, et al. 2020. Interactions between chemicals and non-chemical stressors: The modifying effect of life events on the association between triclocarban, phenols and parabens with gestational length in a Puerto Rican cohort. Sci Total Environ 708:134719.

Aker AM, Johns L, McElrath TF, Cantonwine DE, Mukherjee B, Meeker JD. 2018. Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: A repeated measures study. Environ Int 113:341-349.

Aker AM, Ferguson KK, Rosario ZY, Mukherjee B, Alshawabkeh AN, Calafat AM, et al. 2019. A repeated measures study of phenol, paraben and triclocarban urinary biomarkers and circulating maternal hormones during gestation in the Puerto Rico PROTECT cohort. Environ Health 18:28.

Ashrap P, Watkins DJ, Calafat AM, Ye X, Rosario Z, Brown P, et al. 2018. Elevated concentrations of urinary triclocarban, phenol and paraben among pregnant women in Northern Puerto Rico: Predictors and trends. Environ Int 121:990-1002.

Aung MT, Ferguson KK, Cantonwine DE, McElrath TF, Meeker JD. 2019. Preterm birth in relation to the bisphenol A replacement, bisphenol S, and other phenols and parabens. Environ Res 169:131-138.

Berni M, Gigante P, Bussolati S, Grasselli F, Grolli S, Ramoni R, et al. 2019. Bisphenol S, a Bisphenol A alternative, impairs swine ovarian and adipose cell functions. Domest Anim Endocrinol 66:48-56.

Campen KA, Kucharczyk KM, Bogin B, Ehrlich JM, Combelles CMH. 2018a. Spindle abnormalities and chromosome misalignment in bovine oocytes after exposure to low doses of bisphenol A or bisphenol S. Hum Reprod 33:895-904.

Campen KA, Lavallee M, Combelles C. 2018b. The impact of bisphenol S (BPS) on bovine granulosa and theca cells. Reprod Domest Anim 53:450-457.

da Silva BS, Pietrobon CB, Bertasso IM, Lopes BP, Carvalho JC, Peixoto-Silva N, et al. 2019. Short and long-term effects of bisphenol S (BPS) exposure during pregnancy and lactation on plasma lipids, hormones, and behavior in rats. Environ Pollut 250:312-322.

Demacopulo B, Kreimann EL. 2019. Bisphenol S increases EZRIN expression and the detrimental effects induced by dehydroepiandrosterone in rat endometrium. Mol Cell Endocrinol 483:64-73.

Derakhshan A, Shu H, Peeters RP, Kortenkamp A, Lindh CH, Demeneix B, et al. 2019. Association of urinary bisphenols and triclosan with thyroid function during early pregnancy. Environ Int 133:105123.

Ferguson KK, Meeker JD, Cantonwine DE, Mukherjee B, Pace GG, Weller D, et al. 2018. Environmental phenol associations with ultrasound and delivery measures of fetal growth. Environ Int 112:243-250.

Ferguson KK, Lan Z, Yu Y, Mukherjee B, McElrath TF, Meeker JD. 2019. Urinary concentrations of phenols in association with biomarkers of oxidative stress in pregnancy: Assessment of effects independent of phthalates. Environ Int 131:104903.

Ghayda RA, Williams PL, Chavarro JE, Ford JB, Souter I, Calafat AM, et al. 2019. Urinary bisphenol S concentrations: Potential predictors of and associations with semen quality parameters among men attending a fertility center. Environ Int 131:105050.

Gingrich J, Pu Y, Roberts J, Karthikraj R, Kannan K, Ehrhardt R, et al. 2018. Gestational bisphenol s impairs placental endocrine function and the fusogenic trophoblast signaling pathway. Arch Toxicol 92:1861-1876.

Goodrich JM, Ingle ME, Domino SE, Treadwell MC, Dolinoy DC, Burant C, et al. 2019. First trimester maternal exposures to endocrine disrupting chemicals and metals and fetal size in the Michigan Mother-Infant Pairs study. J Dev Orig Health Dis 10:447-458.

Gu J, Zhang J, Chen Y, Wang H, Guo M, Wang L, et al. 2019. Neurobehavioral effects of bisphenol S exposure in early life stages of zebrafish larvae (*Danio rerio*). Chemosphere 217:629-635.

Hu J, Zhao H, Braun JM, Zheng T, Zhang B, Xia W, et al. 2019. Associations of trimester-specific exposure to bisphenols with size at birth: A Chinese prenatal cohort study. Environ Health Perspect 127:107001.

Huang M, Liu S, Fu L, Jiang X, Yang M. 2020. Bisphenol A and its analogues bisphenol S, bisphenol F and bisphenol AF induce oxidative stress and biomacromolecular damage in human granulosa KGN cells. Chemosphere 253:126707.

Huang S, Li J, Xu S, Zhao H, Li Y, Zhou Y, et al. 2019. Bisphenol A and bisphenol S exposures during pregnancy and gestational age - a longitudinal study in China. Chemosphere 237:124426.

Jiang Y, Li J, Xu S, Zhou Y, Zhao H, Li Y, et al. 2020. Prenatal exposure to bisphenol A and its alternatives and child neurodevelopment at 2 years. J Hazard Mater 388:121774.

John N, Rehman H, Razak S, David M, Ullah W, Afsar T, et al. 2019. Comparative study of environmental pollutants bisphenol A and bisphenol S on sexual differentiation of anteroventral periventricular nucleus and spermatogenesis. Reprod Biol Endocrinol 17:53.

Kojima H, Takeuchi S, Sanoh S, Okuda K, Kitamura S, Uramaru N, et al. 2019. Profiling of bisphenol a and eight its analogues on transcriptional activity via human nuclear receptors. Toxicology 413:48-55.

Kolla S, Morcos M, Martin B, Vandenberg LN. 2018. Low dose bisphenol S or ethinyl estradiol exposures during the perinatal period alter female mouse mammary gland development. Reprod Toxicol 78:50-59.

Kolla SDD, Vandenberg LN. 2019. Data describing effects of perinatal exposure to bisphenol S on a peripubertal estrogen challenge in intact female CD-1 mice. Data Brief 25:103862.

Lee S, Kim C, Shin H, Kho Y, Choi K. 2019. Comparison of thyroid hormone disruption potentials by bisphenols A, S, F, and Z in embryo-larval zebrafish. Chemosphere 221:115-123.

Lehmler HJ, Liu B, Gadogbe M, Bao W. 2018. Exposure to Bisphenol A, Bisphenol F, and Bisphenol S in U.S. Adults and Children: The National Health and Nutrition Examination Survey 2013-2014. ACS Omega 3:6523-6532.

Li Y, Perera L, Coons LA, Burns KA, Tyler Ramsey J, Pelch KE, et al. 2018. Differential *in vitro* biological action, coregulator interactions, and molecular dynamic analysis of Bisphenol A (BPA), BPAF, and BPS ligand-ERα complexes. Environ Health Perspect 126:017012.

Liang F, Huo X, Wang W, Li Y, Zhang J, Feng Y, et al. 2020. Association of bisphenol A or bisphenol S exposure with oxidative stress and immune disturbance among unexplained recurrent spontaneous abortion women. Chemosphere 257:127035.

Liang J, Liu S, Liu T, Yang C, Wu Y, Jennifer Tan HJ, et al. 2020. Association of prenatal exposure to bisphenols and birth size in Zhuang ethnic newborns. Chemosphere 252:126422.

Liao C, Liu F, Alomirah H, Loi VD, Mohd MA, Moon H-B, et al. 2012. Bisphenol S in urine from the United States and seven Asian countries: Occurrence and human exposures. Environ Sci Technol 46:6860-6866.

Liu Y, Yan Z, Zhang L, Deng Z, Yuan J, Zhang S, et al. 2019. Food up-take and reproduction performance of daphnia magna under the exposure of bisphenols. Ecotoxicol Environ Saf 170:47-54.

Mao J, Jain A, Denslow ND, Nouri MZ, Chen S, Wang T, et al. 2020. Bisphenol A and bisphenol S disruptions of the mouse placenta and potential effects on the placenta-brain axis. Proc Natl Acad Sci U S A 117:4642-4652.

Meng Z, Wang D, Liu W, Li R, Yan S, Jia M, et al. 2019. Perinatal exposure to bisphenol S (BPS) promotes obesity development by interfering with lipid and glucose metabolism in male mouse offspring. Environ Res 173:189-198.

Mustieles V, Williams PL, Fernandez MF, Mínguez-Alarcón L, Ford JB, Calafat AM, et al. 2018. Maternal and paternal preconception exposure to bisphenols and size at birth. Hum Reprod 33:1528-1537.

Mustieles V, Zhang Y, Yland J, Braun JM, Williams PL, Wylie BJ, et al. 2020. Maternal and paternal preconception exposure to phenols and preterm birth. Environ Int 137:105523.

Nevoral J, Kolinko Y, Moravec J, Žalmanová T, Hošková K, Prokešová Š, et al. 2018. Long-term exposure to very low doses of bisphenol S affects female reproduction. Reproduction 156:47-57.

Prokesova S, Ghaibour K, Liska F, Klein P, Fenclova T, Stiavnicka M, et al. 2019. Acute low-dose bisphenol S exposure affects mouse oocyte quality. Reprod Toxicol 93:19-27.

Qiu W, Liu S, Yang F, Dong P, Yang M, Wong M, et al. 2019. Metabolism disruption analysis of zebrafish larvae in response to BPA and BPA analogs based on rna-seq technique. Ecotoxicol Environ Saf 174:181-188.

Rochester JR, Bolden AL. 2015. Bisphenol S and F: A systematic review and comparison of the hormonal activity of Bisphenol A substitutes. Environ Health Perspect 123:643-650.

Shi M, Sekulovski N, MacLean JA, Whorton A, Hayashi K. 2019a. Prenatal exposure to Bisphenol A analogues on female reproductive functions in mice. Toxicol Sci 168:561-571.

Shi M, Whorton AE, Sekulovski N, MacLean JA, Hayashi K. 2019b. Prenatal exposure to Bisphenol A, E, and S induces transgenerational effects on male reproductive functions in mice. Toxicol Sci 172:303-315.

Shi M, Whorton AE, Sekulovski N, MacLean JA, Hayashi K. 2019c. Prenatal exposure to Bisphenol A, E, and S induces transgenerational effects on female reproductive functions in mice. Toxicol Sci 170:320-329.

Sidorkiewicz I, Czerniecki J, Jarząbek K, Zbucka-Krętowska M, Wołczyński S. 2018. Cellular, transcriptomic and methylome effects of individual and combined exposure to BPA, BPF, BPS on mouse spermatocyte GC-2 cell line. Toxicol Appl Pharmacol 359:1-11.

Tucker DK, Hayes Bouknight S, Brar SS, Kissling GE, Fenton SE. 2018. Evaluation of prenatal exposure to bisphenol analogues on development and long-term health of the mammary gland in female mice. Environ Health Perspect 126:087003.

Ullah A, Pirzada M, Jahan S, Ullah H, Shaheen G, Rehman H, et al. 2018a. Bisphenol A and its analogs bisphenol B, bisphenol F, and bisphenol S: Comparative *in vitro* and *in vivo* studies on the sperms and testicular tissues of rats. Chemosphere 209:508-516.

Ullah A, Pirzada M, Jahan S, Ullah H, Turi N, Ullah W, et al. 2018b. Impact of low-dose chronic exposure to bisphenol A and its analogue bisphenol B, bisphenol F and bisphenol S on hypothalamo-pituitary-testicular activities in adult rats: A focus on the possible hormonal mode of action. Food Chem Toxicol 121:24-36.

Ullah A, Pirzada M, Jahan S, Ullah H, Khan MJ. 2019a. Bisphenol A analogues bisphenol B, bisphenol F, and bisphenol S induce oxidative stress, disrupt daily sperm production, and damage DNA in rat spermatozoa: A comparative *in vitro* and *in vivo* study. Toxicol Ind Health 35:294-303.

Ullah A, Pirzada M, Jahan S, Ullah H, Razak S, Rauf N, et al. 2019b. Prenatal BPA and its analogs BPB, BPF, and BPS exposure and reproductive axis function in the male offspring of Sprague Dawley rats. Hum Exp Toxicol 38:1344-1365.

Wan Y, Huo W, Xu S, Zheng T, Zhang B, Li Y, et al. 2018. Relationship between maternal exposure to bisphenol S and pregnancy duration. Environ Pollut 238:717-724.

Wang W, Zhang X, Qin J, Wei P, Jia Y, Wang J, et al. 2019. Long-term bisphenol S exposure induces fat accumulation in liver of adult male zebrafish (*Danio rerio*) and slows yolk lipid consumption in F1 offspring. Chemosphere 221:500-510.

Wei P, Zhao F, Zhang X, Liu W, Jiang G, Wang H, et al. 2018. Transgenerational thyroid endocrine disruption induced by bisphenol S affects the early development of zebrafish offspring. Environ Pollut 243:800-808.

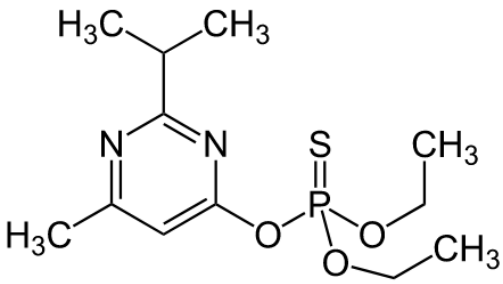
Yin N, Liang X, Liang S, Liang S, Yang R, Hu B, et al. 2019. Embryonic stem cell- and transcriptomics-based *in vitro* analyses reveal that bisphenols A, F and S have similar and very complex potential developmental toxicities. Ecotoxicol Environ Saf 176:330-338.

Zhang W, Xia W, Liu W, Li X, Hu J, Zhang B, et al. 2019. Exposure to bisphenol A substitutes and gestational diabetes mellitus: A prospective cohort study in China. Front Endocrinol (Lausanne) 10:262.

Zhou B, Yang P, Deng YL, Zeng Q, Lu WQ, Mei SR. 2020. Prenatal exposure to bisphenol A and its analogues (bisphenol F and S) and ultrasound parameters of fetal growth. Chemosphere 246:125805.

Diazinon

(CAS No. 333-41-5)



Diazinon is an organophosphate insecticide. Sales of diazinon for residential use have been banned by the US Environmental Protection Agency since January 1, 2005; however, there is still widespread agricultural use in California and elsewhere in the US. Diazinon is registered in California to control foliage and soil insects, and pests in a range of fruits (e.g., pears, peaches, watermelon), nuts (e.g., almonds) and vegetables (e.g., onions, lettuce, tomatoes). According to the California Department of Pesticide Regulation (DPR), 72,621 pounds of diazinon were used in California in 2017.

Diazinon passed the human and animal data screens, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of some relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

A limited number of studies reporting developmental and reproductive toxicity (DART)-related effects associated with diazinon were identified in the recent literature. DART findings reported in these studies are summarized here. The findings are organized by groups of outcomes.

Birth weight

- Lower birth weight (birth cohort study) (Jaacks et al. 2019).
- Cord plasma levels of diazinon were not associated with birth weight, length, or head circumference, while levels of diazinon and chlorpyrifos combined were associated with decreased birth weight and length (prospective cohort study) (Whyatt et al. 2004).
- Early pregnancy detection of urinary 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPy), a biomarker of diazinon exposure, was associated with increased risk of

low birth weight, but not stunting at one or two years of age (birth cohort study) (Jaacks et al. 2019).

Association with autism spectrum disorders

- Application of diazinon within 2000 meters of mothers' residences during pregnancy was associated with increased risk of autism spectrum disorder (ASD) and ASD with intellectual disability (case-control study) (von Ehrenstein et al. 2019).
- In a single-pesticide model, agricultural diazinon use within one kilometer of maternal residence during pregnancy was associated with a lower verbal comprehension intelligence quotient (IQ) score at 7 years of age. Diazinon use was also non-significantly associated with lower scores on other IQ scales (e.g., full scale IQ, perceptual reasoning) (prospective cohort study) (Gunier et al. 2017).

Female reproductive effects

- Levels in follicular fluid correlated with lower number of oocytes retrieved in women of couples undergoing *in vitro* fertility intervention for male factor infertility; significant association of levels in follicular fluid with lower implantation rate (cross sectional observation study) (Al-Hussaini et al. 2018).
- Women with urinary IMPy in the fourth quartile, compared to women in the first quartile, were at increased risk of endometriosis, suggesting that exposure to diazinon may be associated with endometriosis (prospective cohort study) (Li et al. 2020).
- Female spouses of pesticide applicators who used diazinon were at increased risk of ovarian cancer (prospective cohort study) (Lerro et al. 2015).

Male reproductive effects

- Urinary IMPy was associated with highly elevated risk of having low sperm concentration, percentage sperm with normal morphology, and percentage motile sperm (case-control study) (Swan et al. 2003).
- Among male pesticide applicators, diazinon exposure in the top tertiles was associated with non-significantly elevated risk of aggressive prostate cancer (prospective cohort study) (Jones et al. 2015).

Animal studies

Findings reported in whole animal studies examining possible DART effects of exposure to diazinon are summarized here. .

Maternal and developmental effects

- Rat: In a two generation guideline study with dietary exposure, lower gestational and lactational body weight gains; decreased live litter size for high dose (500 ppm) F2a litters, and decreased pup survival and pup body weights during lactation for the F1a mid (100 ppm) and high dose (500 ppm) groups and the F2a high dose group were observed (DPR 1999; US EPA 1997)
- Rat: In a one generation dietary study conducted according to FIFRA (Federal Insecticide Fungicide & Rodenticide Act) guidelines, observations included decreased gestational and lactational body weights and/or body weight gains in dams in the high dose group; decreased F1a and F1b live litter sizes; increased incidences of stillbirths in low dose F1b and high dose F1a and F1b litters; and decreased pup survival and pup body weights during lactation in mid and high dose F1a and F1b litters (DPR 1999; US EPA 1997)
- Rat: In a study conducted according to FIFRA guidelines, maternal exposure on gestational days (GD) 6-15 resulted in slight decreases in maternal weight gain and food consumption and structural changes seen in offspring at the high dose (DPR 1999)
- Rat: Maternal exposure on GD 6-15 resulted in fetal toxicity and increased number of litters with skeletal and visceral anomalies in the high dose group (7.6 mg/kg-day); cholinergic symptoms, including diarrhea, tremors, weakness, salivation, and decreased activity were observed in high dose dams; milder cholinergic symptoms in dams occurred in the 3.8 mg/kg-day dose group (Elmazoudy et al. 2011).
- Mouse: Maternal exposure throughout gestation resulted in significant delays in sexual maturity (descent of testes and vaginal opening) in offspring (Spyker and Avery 1977).
- Rabbit: In a standard guideline teratology study, no adverse effects were seen in offspring of dams exposed during gestation to doses of up to 100 mg/kg-day (DPR 1999).

Neurodevelopmental effects

- Mouse: Maternal exposure throughout gestation impaired offspring endurance and coordination on rod cling and inclined plane tests of neuromuscular function in offspring of the low and high dose groups, delayed the appearance of the

contact placing reflex in offspring of the low dose group, and resulted in neuropathologic changes in the forebrain of high dose group offspring (Spyker and Avery 1977).

- Rat: Maternal exposure prior to conception through gestation affected offspring by impairing novel-object recognition (test of cognitive function) significantly decreasing preference for the novel vs. familiar object; increasing percent time spent in the open arms of the elevated plus maze (index of risk-taking behavior), and increasing hyperactivity early in the Figure-8 apparatus test session during adolescence, but not adulthood (Hawkey et al. 2020).
- Rat: Pups were exposed to diazinon on postnatal days (PND) 1-4 and examined with cognitive battery tests. Findings in adolescence were significant hyperactivity in initial but not later trials in the T-maze, and no effects on locomotor activity in the Figure-8 test. Findings in adulthood were reduced pre-pulse inhibition (an index of sensorimotor gating) (only in males), impaired spatial learning in the radial-arm maze in the low dose group, and increased sensitivity to memory-impairing effects of the anticholinergic drug scopolamine in the low dose group (Timofeeva et al. 2008).
- Rat: Subcutaneous administration on PND 1-4 impaired neuritic outgrowth in the forebrain and brainstem and decreased choline acetyltransferase activity (a cholinergic neuronal marker) on PND 5; no effect on hemicholinium-3 binding to the presynaptic choline transporter (index of cholinergic neuronal activity) and no down-regulation of the m_2-muscarinic acetylcholine receptor was observed (Slotkin et al. 2006).
- Rat: Animals were exposed on PND 1-4, and levels of serotonin (5HT) receptors and 5HT transporters were assessed in the cerebral cortical region and brainstem at 30, 60 and 100 days of age. A lasting deficit in $5HT_{1A}$ receptors was observed in both brain regions in males, with greater effects seen at lower doses, and a significant increase in 5HT transporters was observed in both brain regions in females, with greater effects seen at lower doses (Slotkin et al. 2008).
- Rat: Maternal exposure (to doses that did not inhibit acetylcholinesterase [AChE]) prior to conception through PND 14 affected biochemical parameters in the brain of offspring (assessed from adolescence through adulthood). Specifically, deficits in presynaptic acetylcholine (ACh) activity and decreases in nicotinic acetylcholine receptors (AChRs) and serotonin receptors were observed to a greater extent in cerebrocortical regions and the hippocampus compared to the striatum, midbrain or brainstem; females were more sensitive than males (Slotkin et al. 2019).
- Rat: Exposure (to doses that did not inhibit AChE) on PND 1-4 altered emotional behavior in young adults (PND 52), with decreased time spent in the open arms of the elevated plus maze (males only), shorter latencies to begin eating novel

food (males only), and reduced preference for chocolate milk in the anhedonia test (both sexes) (Roegge et al. 2008).

- Zebrafish: Exposure to embryos for 24 h had no effect on embryonic spontaneous movement or heartbeat, or larval responses to touch or a light-dark transition (Velki et al. 2017a).
- Zebrafish: Exposure to embryos for varying lengths of time using different exposure and recovery scenarios resulted in alterations in AChE, caboxylesterase (CES), ethoxyresorufin-O-deethylase (EROD), glutathione-S-transferase (GST), catalase (CAT) and glutathione peroxidase (GPx), and in gene expression levels of acetylcholinesterase (*ache*), carboxylesterase (*ces2*), cytochrome P450 (*cyp1a*), glutathione-S-transferase (*gstp1*), catalase (*cat*), glutathione peroxidase (*gpx1a*) and glutathione reductase (*gsr*) (Velki et al. 2017b).

Male and female reproductive effects

- Mouse: Maternal exposure throughout gestation resulted in significant delays in sexual maturity (descent of testes decent and vaginal opening) in offspring [also mentioned above under Maternal and Developmental Effects] (Spyker and Avery 1977).
- Rat: In a one-generation dietary study conducted according to FIFRA guidelines, fecundity (number of live deliveries per number of cohoused male and female pairs) was decreased at the 1000 ppm treatment level for the F1a mating trial, and at the 10 ppm, 100 ppm and 1000 ppm treatment levels for the F1b mating trial; and absolute and/or relative ovary weights were decreased in the 100 ppm and 1000 ppm dams (DPR 1999).
- Rat: Administration of a single intraperitoneal dose of 20mg/kg to males significantly decreased sperm motility, progressive motility, and beat cross frequency (BCF) and increased the amplitude of lateral head displacement (ALH). Administration of 40 mg/L in drinking water for 90 days to males significantly increased sperm velocity average path, curvilinear velocity, and ALH, and decreased progressive motility and BCF (Toman et al. 2016).
- Rat: A single exposure to adult females decreased the number of proliferating ovarian follicles based on proliferating cell nuclear antigen staining, in secondary and Graffian ovarian follicles (Sargazi et al. 2019).
- Rat: Maternal exposure on GD 6-15 decreased net gravid uterine weight decreased in the 7.6 mg/kg-d dose group (Elmazoudy et al. 2011).

Mechanistic, in vitro, and other relevant data

- Rat: Primary cortical astrocyte cultures were prepared from GD 21 fetuses, cultured for 12 days and then exposed to diazinon or its oxon for 24 h, after which in some experiments freshly isolated GD 21 fetal hippocampal neurons were introduced in a "sandwich" co-culture system. Exposure to diazinon or its oxon increased production of reactive oxygen species (ROS) and decreased fibronectin levels in astrocyte cultures at concentrations devoid of any cytotoxicity. In the co-culture system, the ability of exposed astrocytes (exposed to either diazinon or its oxon) to foster neurite outgrowth in hippocampal neurons was impaired (Pizzurro et al. 2014a).
- Rat: Primary hippocampal neuronal cells were prepared from GD 21 fetuses, cultured in astrocyte-conditioned medium for 24 h, and then exposed to diazinon or its oxon for 24 h. Exposure to diaznon or its oxon increased production of reactive oxygen species in cultured neurons, and decreased neurite outgrowth (measured as a decrease in longest neurite length) at concentrations devoid of any cytotoxicity. Exposure to the oxon also decreased minor neurite length (Pizzurro et al. 2014b).
- Rat: Exposure of male pups on PND 1-4 (at either a dose that did not inhibit cholinesterase or a dose that resulted in <20% inhibition) altered expression of genes encoding fibroblastic growth factors (*Fgf)* and fibroblastic growth factor receptors (*Fgfr*) in the brainstem and forebrain on PND 5 by microarray analysis. The fibroblast growth factor superfamily of neurotrophic factors plays a critical role in neuronal cell development, brain assembly and recovery from neuronal injury. Specifically, there was decreased expression of *Fgf20* in the forebrain and of *Fgf2* and *Fgf22* in the brain stem, and increased expression of *Fgfr4* in the brain stem (Slotkin et al. 2007b).
- Rat: Neonatal rats were exposed on PND 1-4 at doses that did not inhibit AChE, and gene expression profiles in the brainstem and forebrain were examined on PND 5 using microarray technology. Exposure resulted in alterations in gene expression in pathways involved in general neural cell development, cell signaling, cytotoxicity, and neurotransmitter systems (Slotkin and Seidler 2007).
- Rat: In undifferentiated and differentiating neuronotypic adrenal pheochromocytoma (PC12) cells, diazinon inhibited DNA synthesis. In studies with differentiating cells, diazinon increased lipid peroxidation and increased the tyrosine hydroxylase to choline acetyltransferase activity ratio (Slotkin et al. 2007a).
- Rat: In gonadotropin-primed immature females, diazinon decreased ovarian steroidogenic acute regulatory protein (*Star*) mRNA levels and the diameter of corpus lutea (Siavashpour et al. 2018).

- Rat: In differentiating PC12 cells, diazinon reduced expression in many of the 21 Parkinson's Disease (PD)-related genes assessed. Analysis of microarray data generated in the study of Slotkin and Seidler (2007) described above (i.e., gene expression profiles from the brainstem and forebrain of rats exposed on PND 1-4) found significant changes in nine of the 21 PD-related genes assessed (Slotkin and Seidler 2011).

References cited in "Diazinon"

Al-Hussaini TK, Abdelaleem AA, Elnashar I, Shabaan OM, Mostafa R, El-Baz MAH, et al. 2018. The effect of follicullar fluid pesticides and polychlorinated biphenyls concentrations on intracytoplasmic sperm injection (ICSI) embryological and clinical outcome. Eur J Obstet Gynecol Reprod Biol 220:39-43.

DPR (Department of Pesticide Regulation Medical Toxicology Branch). 1999. Diazinon Summary Of Toxicological Data.

Elmazoudy RH, Attia AA, Abdelgawad HS. 2011. Evaluation of developmental toxicity induced by anticholinesterase insecticide, diazinon in female rats. Birth Defects Res B Dev Reprod Toxicol 92:534-542.

Gunier RB, Bradman A, Harley KG, Kogut K, Eskenazi B. 2017. Prenatal residential proximity to agricultural pesticide use and IQ in 7-year-old children. Environ Health Perspect 125:057002.

Hawkey A, Pippen E, White H, Kim J, Greengrove E, Kenou B, et al. 2020. Gestational and perinatal exposure to diazinon causes long-lasting neurobehavioral consequences in the rat. Toxicology 429:152327.

Jaacks LM, Diao N, Calafat AM, Ospina M, Mazumdar M, Ibne Hasan MOS, et al. 2019. Association of prenatal pesticide exposures with adverse pregnancy outcomes and stunting in rural Bangladesh. Environ Int 133:105243.

Jones RR, Barone-Adesi F, Koutros S, Lerro CC, Blair A, Lubin J, et al. 2015. Incidence of solid tumours among pesticide applicators exposed to the organophosphate insecticide diazinon in the agricultural health study: An updated analysis. Occup Environ Med 72:496-503.

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. Occup Environ Med 72:736-744.

Li AJ, Chen Z, Lin TC, Buck Louis GM, Kannan K. 2020. Association of urinary metabolites of organophosphate and pyrethroid insecticides, and phenoxy herbicides with endometriosis. Environ Int 136:105456.

Pizzurro DM, Dao K, Costa LG. 2014a. Diazinon and diazoxon impair the ability of astrocytes to foster neurite outgrowth in primary hippocampal neurons. Toxicol Appl Pharmacol 274:372-382.

Pizzurro DM, Dao K, Costa LG. 2014b. Astrocytes protect against diazinon- and diazoxon-induced inhibition of neurite outgrowth by regulating neuronal glutathione. Toxicology 318:59-68.

Roegge CS, Timofeeva OA, Seidler FJ, Slotkin TA, Levin ED. 2008. Developmental diazinon neurotoxicity in rats: Later effects on emotional response. Brain Res Bull 75:166-172.

Sargazi Z, Reza Nikravesh M, Jalali M, Reza Sadeghnia H, Rahimi Anbarkeh F. 2019. The protective effect of vitamin e on rats' ovarian follicles following an administration of diazinon: An experimental study. Int J Reprod Biomed (Yazd) 17:79-88.

Siavashpour A, Ghasemi Y, Khalvati B, Jeivad F, Azarpira N, Niknahad H. 2018. Diazinon interrupts ovarian steroidogenic acute regulatory (*Star*) gene transcription in gonadotropin-stimulated rat model. Iran J Pharm Res 17:535-542.

Slotkin TA, Levin ED, Seidler FJ. 2006. Comparative developmental neurotoxicity of organophosphate insecticides: Effects on brain development are separable from systemic toxicity. Environ Health Perspect 114:746-751.

Slotkin TA, MacKillop EA, Ryde IT, Tate CA, Seidler FJ. 2007a. Screening for developmental neurotoxicity using PC12 cells: Comparisons of organophosphates with a carbamate, an organochlorine, and divalent nickel. Environ Health Perspect 115:93-101.

Slotkin TA, Seidler FJ. 2007. Comparative developmental neurotoxicity of organophosphates *in vivo*: Transcriptional responses of pathways for brain cell development, cell signaling, cytotoxicity and neurotransmitter systems. Brain Res Bull 72:232-274.

Slotkin TA, Seidler FJ, Fumagalli F. 2007b. Exposure to organophosphates reduces the expression of neurotrophic factors in neonatal rat brain regions: Similarities and differences in the effects of chlorpyrifos and diazinon on the fibroblast growth factor superfamily. Environ Health Perspect 115:909-916.

Slotkin TA, Bodwell BE, Levin ED, Seidler FJ. 2008. Neonatal exposure to low doses of diazinon: Long-term effects on neural cell development and acetylcholine systems. Environ Health Perspect 116:340-348.

Slotkin TA, Seidler FJ. 2011. Developmental exposure to organophosphates triggers transcriptional changes in genes associated with parkinson's disease *in vitro* and *in vivo*. Brain Res Bull 86:340-347.

Slotkin TA, Skavicus S, Ko A, Levin ED, Seidler FJ. 2019. Perinatal diazinon exposure compromises the development of acetylcholine and serotonin systems. Toxicology 424:152240.

Spyker JM, Avery DL. 1977. Neurobehavioral effects of prenatal exposure to the organophosphate diazinon in mice. J Toxicol Environ Health 3:989-1002.

Swan SH, Kruse RL, Liu F, Barr DB, Drobnis EZ, Redmon JB, et al. 2003. Semen quality in relation to biomarkers of pesticide exposure. Environ Health Perspect 111:1478-1484.

Timofeeva OA, Roegge CS, Seidler FJ, Slotkin TA, Levin ED. 2008. Persistent cognitive alterations in rats after early postnatal exposure to low doses of the organophosphate pesticide, diazinon. Neurotoxicol Teratol 30:38-45.

Toman R, Hluchy S, Cabaj M, Massanyi P, Roychoudhury S, Tunegova M. 2016. Effect of separate and combined exposure of selenium and diazinon on rat sperm motility by computer assisted semen analysis. J Trace Elem Med Biol 38:144-149.

US EPA (United States Environmental Protection Agency). 1997. Rfd/Review Report of Diazinon {o,o,-d.lethyl 0-[6-methyl-2-(1-methylethyl)-4-pyritnidinyl] phosphorothioate}.

Velki M, Di Paolo C, Nelles J, Seiler TB, Hollert H. 2017a. Diuron and diazinon alter the behavior of zebrafish embryos and larvae in the absence of acute toxicity. Chemosphere 180:65-76.

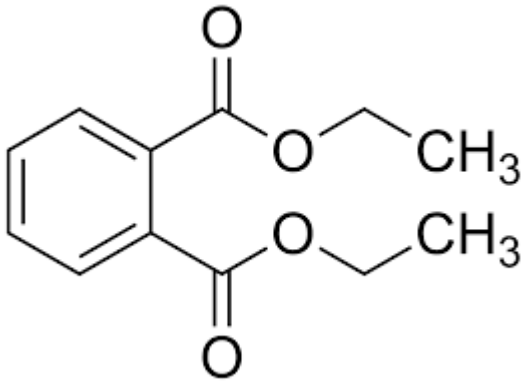
Velki M, Meyer-Alert H, Seiler TB, Hollert H. 2017b. Enzymatic activity and gene expression changes in zebrafish embryos and larvae exposed to pesticides diazinon and diuron. Aquat Toxicol 193:187-200.

von Ehrenstein OS, Ling C, Cui X, Cockburn M, Park AS, Yu F, et al. 2019. Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: Population based case-control study. Bmj 364:l962.

Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R, et al. 2004. Prenatal insecticide exposures and birth weight and length among an urban minority cohort. Environ Health Perspect 112:1125-1132.

Diethyl phthalate (DEP)

(Diethyl benzene-1,2-dicarboxylate, CAS No. 84-66-2)



Diethyl phthalate (DEP) is a plasticizer used in resins and elastomers, a solvent used in the manufacture of some plastics, mosquito repellents and personal care and consumer products, particularly those containing fragrances, and an excipient used in pharmaceuticals. DEP is also a component of tobacco smoke (Zhao et al. 2017).

Human biomonitoring studies indicate that exposure to DEP is widespread. For example, Table 3 below summarizes data on urinary concentrations of the DEP metabolite monoethyl phthalate (MEP) (geometric mean and 95% confidence interval [CI]) measured in studies conducted by the Biomonitoring California Program between 2005 and 2013 (Biomonitoring California 2020).

DEP passed the human and animal data screens, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies that were identified during the preliminary toxicological evaluation.

Table 3. Urinary concentration (ng/ml) of MEP, a metabolite and biomarker of exposure to DEP, in studies in California residents. Data from Biomonitoring California (https://biomonitoring.ca.gov/) (Biomonitoring California 2020).

Project	Sample Year	Geometric mean (ng/ml)	95% Lower CI	95% Upper CI	N	Detection Frequency
Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS)	2005 to 2006	85	63.1	115	49	100%
Markers of Autism Risk in Babies– Learning Early Signs (MARBLES)-1	2007 to 2008	65.1	26	163	13	100%
Markers of Autism Risk in Babies– Learning Early Signs (MARBLES)-2	2007 to 2008	62.4	32.2	121	15	100%
Firefighter Occupational Exposures (FOX) Project	2010 to 2011	52.9	38.1	73.4	101	79.20%
Maternal and Infant Environmental Exposure Project (MIEEP)	2010 to 2011	95.5	69.3	132	89	Not reported
Biomonitoring Exposures Study (BEST) - 1.Pilot	2011 to 2012	57.1	45.2	72.1	109	97.20%
Biomonitoring Exposures Study (BEST) - 2.Expanded	2013	52.4	43.9	62.6	218	100%

Human epidemiologic studies

Numerous human studies reporting DEP-related development and reproductive toxicity (DART) effects were identified in the recent literature. A number of DART findings reported in epidemiologic studies are summarized here, with an emphasis on those published within the last two years. The findings are organized by groups of outcomes.

Maternal and Developmental effects

- Urinary MEP was marginally associated with delayed implantation (prospective cohort study) (Chin et al. 2019).
- Increased risk of preterm birth associated with phthalate exposure during third trimester was due mainly to DEP (nested case-control study) (Broe et al. 2019).
- Averaged first and third trimester urinary MEP was associated with increased risk of gestational diabetes (prospective cohort study) (Shaffer et al. 2019).
- Among subfertile couples, maternal preconception urinary MEP was associated with lower birth weight:placental weight ratio and prenatal urinary MEP was associated with lower placental weight (prospective cohort study) (Mustieles et al. 2019).
- First trimester urinary MEP was associated with shorter cord blood telomere length (a marker of biological aging) in female infants (prospective cohort study) (Song et al. 2019).
- Prenatal urinary MEP was associated with a greater ano-clitoral distance (masculinizing effect) in newborn girls (prospective cohort study) (Arbuckle 2018).

Neurodevelopmental effects

- Prenatal urinary MEP was associated with lower gross motor function in girls at 11 years of age (prospective cohort study) (Balalian et al. 2019).

Reproductive system effects

- Urinary concentration of MEP in women was associated with decreased fecundity (prospective cohort study) (Thomsen et al. 2017).
- Urinary MEP was associated with DNA damage in sperm among men at an infertility clinic (cross-sectional study) (Hauser et al. 2007).

Animal studies

Findings reported in animal studies examining possible DART effects of exposure to DEP are summarized here.

Maternal and developmental effects

- Rat: Maternal exposure on gestation day (GD) 8 through postnatal day (PND) 30 increased the mitotic index (based on Ki-67 antigen expression) and decreased lactase and sucrase activities in the small intestine of offspring on PND 30 (Setti Ahmed et al. 2018).
- Rat: Exposure on GD 6-15 had no effect on parameters of embryo/fetal development (indices of prenatal viability, such as resorption incidence, or live litter size), except an increased incidence of supernumerary ribs (a variation) at the high dose level (5% of the feed); maternal body weight gain and feed consumption were also decreased at the high dose (Field et al. 1993).
- Rat: In a two-generation reproductive toxicity study, exposure had no effect on food consumption, although decreases in body weight gains were observed in F1 and F2 pups before weaning. Vaginal opening was slightly delayed in F1 females at 15000 ppm, and F0 males exhibited an increase in liver content of CYP3A2, a cytochrome P450 isozyme, at 15000 ppm, and a decrease in serum testosterone at 3000 and 15000 ppm. Exposure did not result in changes in reproductive performance or other reproductive parameters, or gross or histopathological findings at dose levels up to 15000 ppm in the diet (Fujii et al. 2005).
- Rat: Maternal exposure on GD12-20 to doses ranging up to 1000 mg/kg-day had no effect on birth rate, sex ratio, or number of pups per dam. Exposure decreased birth weight of male pups, decreased anogenital distance at the top two doses, decreased testis volume, testicular testosterone (at the top dose), Leydig cell size, Leydig cell protein levels of INSL3 and CYP11A1, and gene expression of several steroidogenic-related genes in the testis (with no effect on *star*) (Hu et al. 2018).

Mechanistic, in vitro, and other relevant data

- Rat: In a two-generation chronic toxicity study of effects on the adrenal and thyroid glands, the parental generation was exposed via diet over a 100-day premating period and through mating, gestation and lactation for a total of 150 days, and the F1 progeny, at body weights of 75-100 g, were similarly exposed for 150 days to half the dietary dose given to the previous generation. Exposure altered the adrenal gland cortex in the zona fasciculata region (involved in the production of glucocorticoids), with vacuolations and degeneration observed in parental and F1 males, but not females. In the thyroid gland, exposure resulted in shrinkage of follicles, loss of thyroglobulin, and fibrosis of the interfollicular epithelium, in parental and F1 animals of both sexes (Pereira et al. 2007).

- Rat: Exposure of mesencephalic neural stem cell cultures decreased cell migration and cell proliferation; apoptosis was not observed (Ishido and Suzuki 2014).
- Mouse: Exposure of mouse embryonic stem cells decreased viability in a dose-dependent manner, stimulated intracellular ROS production, and upregulated gene expression of the neural ectoderm markers *Pax6*, *Nestin*, *Sox1* and *Sox3* at non-cytotoxic concentrations (Yin et al. 2018).
- Zebrafish: Exposure of embryos from 4-hour post-fertilization (hpf) to 96 hpf inhibited acetylcholinesterase activity and upregulated expression of the following neuron-related genes: growth associated protein 43 (*gap43*), embryonic lethal abnormal vision-like 3 (*elavl3*), glial fibrillary acidic protein (*gfap*), myelin basic protein (*mbp*), α1-tubulin, and neurogenin1 (*ngn1*) (Xu et al. 2013a).
- Zebrafish: Exposure of embryos from 4 hpf to 96 hpf enhanced the production of reactive oxygen species (ROS) and lipid peroxidation (LPO) in a concentration-dependent manner, increased the activity of antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)) in a concentration-dependent manner, and increased expression of the following innate immune-related genes: interferon γ (*ifnγ*), interleukin-1β (*iliβ*), Myxovirus resistance (*mx*), tumor necrosis factor α (*tnfα*), CC-chemokine, CXCL-clc, lysozyme (*lyz*) and complement factor C3B (*c3*) (Xu et al. 2013b).
- Frog: Exposure of *Xenopus laevis* embryos in the 96h frog embryo teratogenesis assay-Xenopus (FETAX) assay resulted in malformations (edema, abnormal gut coiling and notochord malformations) at 50 and 100 ppm (effective concentration [EC]50 = 51.2 ppm); the mean minimum concentration to inhibit growth was 41.7 ppm, and the teratogenic index was 1.25, indicating a low teratogenic risk to developing embryos (Gardner et al. 2016).
- *Caenorhabditis elegans*: Exposure from larval stage 1 to the young adult stage reduced fecundity at a concentration of 1 micromolar (µM), increased lipid content at a concentration of 10 µM, upregulated expression of genes associated with lipid metabolism, including *fasn-1*, *pod-2*, *fat-5*, *acs-6* and *sbp-1*, and vitellogenin, altered genes associated with stress response (upregulated: *ced-1 wah-1*, *daf-21* and *gst-4*; downregulated: *ctl-1*, *cdf-2*, *hsp-16.1*, *hsp-16.48*, and *sip-1*), reduced the average lifespan from 14 to 12 days, and altered expression of genes associated with lifespan (Pradhan et al. 2018).

References cited in "DEP"

Arbuckle TE. 2018. Prenatal exposure to phthalates and phenols and infant endocrine-sensitive outcomes: The mirec study. Environ Int 20:572-583.

Balalian AA, Whyatt RM, Liu X, Insel BJ, Rauh VA, Herbstman J, et al. 2019. Prenatal and childhood exposure to phthalates and motor skills at age 11 years. Environmental research 171:416-427.

Biomonitoring California. 2020. Available: https://biomonitoring.ca.gov/results/chemical/2183?field_chemical_name_target_id_selective%5B%5D=158&field_chemical_name_target_id_selective%5B%5D=161&field_chemical_name_target_id_selective%5B%5D=162&field_chemical_name_target_id_selective%5B%5D=1345 [accessed 28 August 2020].

Broe A, Pottegård A, Hallas J, Ahern TP, Lamont RF, Damkier P. 2019. Phthalate exposure from drugs during pregnancy and possible risk of preterm birth and small for gestational age. European Journal of Obstetrics and Gynecology and Reproductive Biology 240:293-299.

Chin HB, Jukic AM, Wilcox AJ, Weinberg CR, Ferguson KK, Calafat AM, et al. 2019. Association of urinary concentrations of phthalate metabolites and bisphenol A with early pregnancy endpoints. Environmental research 168:254-260.

Field EA, Price CJ, Sleet RB, George JD, Marr MC, Myers CB, et al. 1993. Developmental toxicity evaluation of diethyl and dimethyl phthalate in rats. Teratology 48:33-44.

Fujii S, Yabe K, Furukawa M, Hirata M, Kiguchi M, Ikka T. 2005. A two-generation reproductive toxicity study of diethyl phthalate (DEP) in rats. J Toxicol Sci 30 Spec No.:97-116.

Gardner ST, Wood AT, Lester R, Onkst PE, Burnham N, Perygin DH, et al. 2016. Assessing differences in toxicity and teratogenicity of three phthalates, diethyl phthalate, di-n-propyl phthalate, and di-n-butyl phthalate, using X*enopus laevis* embryos. J Toxicol Environ Health A 79:71-82.

Hauser R, Meeker JD, Singh NP, Silva MJ, Ryan L, Duty S, et al. 2007. DNA damage in human sperm is related to urinary levels of phthalate monoester and oxidative metabolites. Hum Reprod 22:688-695.

Hu G, Li J, Shan Y, Li X, Zhu Q, Li H, et al. 2018. *In utero* combined di-(2-ethylhexyl) phthalate and diethyl phthalate exposure cumulatively impairs rat fetal leydig cell development. Toxicology 395:23-33.

Ishido M, Suzuki J. 2014. Classification of phthalates based on an *in vitro* neurosphere assay using rat mesencephalic neural stem cells. J Toxicol Sci 39:25-32.

Mustieles V, Mínguez-Alarcón L, Christou G, Ford JB, Dimitriadis I, Hauser R, et al. 2019. Placental weight in relation to maternal and paternal preconception and prenatal urinary phthalate metabolite concentrations among subfertile couples. Environmental research 169:272-279.

Pereira C, Mapuskar K, Vaman Rao C. 2007. A two-generation chronic mixture toxicity study of clophen a60 and diethyl phthalate on histology of adrenal cortex and thyroid of rats. Acta Histochem 109:29-36.

Pradhan A, Olsson PE, Jass J. 2018. Di(2-ethylhexyl) phthalate and diethyl phthalate disrupt lipid metabolism, reduce fecundity and shortens lifespan of *Caenorhabditis elegans*. Chemosphere 190:375-382.

Setti Ahmed K, Kharoubi O, Aoues AEK, Bouchekara M, Khaladi B, Taleb M. 2018. Effect of gestational and lactational exposure to dehp, dinp, and dep on intestinal morphology, disaccharidases, and alkaline phosphatase in rats during postnatal development. Am J Perinatol 35:1251-1259.

Shaffer RM, Ferguson KK, Sheppard L, James-Todd T, Butts S, Chandrasekaran S, et al. 2019. Maternal urinary phthalate metabolites in relation to gestational diabetes and glucose intolerance during pregnancy. Environment international 123:588-596.

Song L, Liu B, Wu M, Zhang L, Wang L, Zhang B, et al. 2019. Prenatal exposure to phthalates and newborn telomere length: A birth cohort study in Wuhan, China. Environmental health perspectives 127:87007-87007.

Thomsen AM, Riis AH, Olsen J, Jönsson BA, Lindh CH, Hjollund NH, et al. 2017. Female exposure to phthalates and time to pregnancy: A first pregnancy planner study. Hum Reprod 32:232-238.

Xu H, Shao X, Zhang Z, Zou Y, Chen Y, Han S, et al. 2013a. Effects of di-n-butyl phthalate and diethyl phthalate on acetylcholinesterase activity and neurotoxicity related gene expression in embryonic zebrafish. Bull Environ Contam Toxicol 91:635-639.

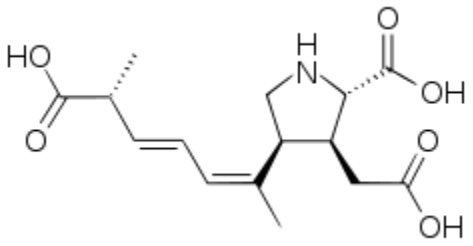
Xu H, Shao X, Zhang Z, Zou Y, Wu X, Yang L. 2013b. Oxidative stress and immune related gene expression following exposure to di-n-butyl phthalate and diethyl phthalate in zebrafish embryos. Ecotoxicol Environ Saf 93:39-44.

Yin N, Liang S, Liang S, Hu B, Yang R, Zhou Q, et al. 2018. DEP and DBP induce cytotoxicity in mouse embryonic stem cells and abnormally enhance neural ectoderm development. Environ Pollut 236:21-32.

Zhao R, Wu Y, Zhao F, Lv Y, Huang D, Wei J, et al. 2017. The risk of missed abortion associated with the levels of tobacco, heavy metals and phthalate in hair of pregnant woman: A case control study in chinese women. Medicine (Baltimore) 96:e9388.

Domoic acid

(*CAS No. 14277-97-5*)



Domoic acid is a neurotoxin produced by marine planktonic diatoms of the genus *Pseudo-nitzschia.* Edible species of fish and shellfish become contaminated by consuming domoic acid-producing plankton. Affected species include Dungeness crab, Rock crab, lobsters, razor clams, anchovies, sardines, and more. People can be exposed by eating contaminated fish and shellfish. In pregnant women, domoic acid readily crosses the placenta and enters the fetal brain (Grant et al. 2010), and can be transferred to offspring in breast milk (Maucher and Ramsdell 2005).

Since 2015, fishing/harvesting seasons in various California coastal areas have been delayed or canceled due to excessively high domoic acid levels. The US Food and Drug Administration (FDA) has set action levels at >30 ppm domoic acid in Dungeness crab viscera, and ≥ 20 ppm for all fish, shellfish and crab meat (US FDA 2020); (Wekell et al. 2004). An acute reference dose of 0.075 mg domoic acid per kg body weight per day has been proposed (Marien 1996; Costa et al. 2010), but does not address the possibility of chronic exposures to lower dose levels (Costa et al. 2010; Ferriss et al. 2017; Grattan et al. 2018; Petroff et al. 2019).

Domoic acid passed the animal data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

No human epidemiologic studies reporting developmental and reproductive toxicity (DART)-related effects associated with domoic acid were identified.

Animal studies

Findings reported in whole animal studies examining possible DART effects of exposure to domoic acid are summarized here. The primary concern for DART effects of domoic acid is developmental neurotoxicity (DNT). The molecular pathway by which domoic acid exerts neurotoxicity is well understood, and is consistent with reported DNT outcomes.

Neurodevelopmental effects, prenatal exposure only

- Monkey (macaque): Impaired recognition memory assessed at 1-2 months postnatal age following prenatal exposure to doses below 0.075 mg/kg-day, the proposed human Tolerable Daily Intake level (Grant et al. 2019).
- Mouse: Histological evidence of progressive neuronal damage in the hippocampus with increasing postnatal age and functional evidence of excitotoxicity, including increased neuronal calcium influx via kainite receptor activation into cortical and hippocampal slices prepared on postnatal day (PND) 30 (Dakshinamurti et al. 1993).
- Mouse: Persistent anomalies in social interactions and related resting state functional connectivity in the anterior cingulate that may be relevant to autism spectrum disorder (Mills et al. 2016; Zuloaga et al. 2016), and behavioral changes resembling diagnostic features of schizophrenia (Mills, 2016).
- Mouse: Altered motor coordination and activity, and gender-specific persistent neurobehavioral effects at doses not inducing maternal toxicity (Shiotani et al. 2017).
- Mouse: Alterations in parvalbumin-positive subtype GABAergic neurons in the dentate gyrus and lateral amygdala (Zuloaga, 2016).
- Rat: Memory impairments and increased susceptibility to the amnesic effects of scopolamine following prenatal exposure to doses below those causing overt clinical toxicity (Levin et al. 2005).
- Zebrafish: Tonic-clonic convulsions, loss of touch-response reflexes, and stereotypic fin movements by hatched embryos following microinjection of fertilized eggs with domoic acid. The fin movements, in particular, were considered potentially related to observations in rodents of stereotypic scratching following domoic acid exposure (Tiedeken et al. 2005).
- Zebrafish: Reduced threshold to chemically-induced seizures in hatched embryos following microinjection of fertilized eggs with domoic acid (Tiedeken and Ramsdell 2007).

Neurodevelopmental effects, perinatal/neonatal exposure

- Rat: Perinatal exposure of pups resulted in permanent changes in neuronal excitability of the adult animal (Gill and Kumara 2019). Both generalized seizure and focal after-discharge thresholds were lowered, and "massive fiber sprouting" was significantly increased.
- Rat: Neonatally-exposed adults spent significantly less time in "paradoxical sleep" then unexposed controls (Gill et al. 2009).
- Rat: Chronic exposure of neonatal rats to low, non-convulsive doses of domoic acid produced lasting changes in the response to the rewarding properties of nicotine (Burt et al. 2008b).
- Rat: Exposure to a single, non-lethal dose on PND 1 resulted in significant hypoactivity in a figure-8 maze when tested during adolescence (Levin et al. 2006).
- Rat: Exposure to a single dose on PND 7 resulted in motor abnormalities, spinal cord lesions, and death (Wang et al. 2000).
- Rat: The neurotoxic effects of a single domoic acid exposure on PND 0, 5, 14, or 22 were compared, and the effects of domoic acid exposure on PND 8 or 14 were compared with those of kainic acid. Domoic acid was the more potent neurotoxin, particularly when exposure occurred at earlier ages (Doucette et al. 2000).
- Rat: Characteristic neural cytotoxic effects of domoic acid occurred at exposures 40 times lower by body weight in neonates than in adult rats (Xi et al. 1997).
- Neurodevelopmental Effects, Early Postnatal Exposure Only
- Rat: Long-term changes in α(2)-adrenoceptor binding in brain tissues of young males (Thomsen et al. 2016).
- Rat: Altered spontaneous behavior in adults (Jandová et al. 2014).
- Rat: Results of maze tasks given to adults indicated a modified behavioral stress/anxiety response and male-specific deficits in cognitive flexibility. 80% of male and 20% of females exhibited seizure behaviors. Biochemical level effects included changes (some of which were male-specific) in adrenergic receptors, expression of mineralocorticoid and glucocorticoid receptors, and a significant decrease in glucocorticoid/mineralocorticoid ratio (Gill et al. 2012).
- Rat: Domoic acid-treated male pups demonstrated early alterations in glutamate signaling, resulting in social withdrawal as adults; this experimental system may provide a model for schizophrenia (Ryan et al. 2011).
- Rat: Exposure on PND 8-14 increased the incidence of behavioral seizures in adults (Perry et al. 2009).
- Rat: Low dose (20 microgram per kilogram) exposures on PND 8-14 resulted in lasting changes to learning and memory (Adams et al. 2009).

- Rat: Alterations in behaviors dependent upon functional integrity of the midbrain dopamine system, namely novelty-related behaviors and in nicotine-induced reinforcement, were observed in juvenile, adolescent, and adult rats tested following domoic acid exposure on PND 8 -14 (Burt et al. 2008a; Burt et al. 2008b).
- Rat: Exposure on PND 8-14 significantly increased mossy fiber sprouting in the hippocampus of mature rat brains (Bernard et al. 2007).
- Rat: Low dose (20 microgram per kilogram) exposures on PND 8-14 altered odor conditioning in a manner considered to involve NMDA glutamate receptors as well as kainate receptors (Tasker et al. 2005).
- Rat: Low dose (5 or 20 microgram per kilogram) exposures on PND 8-14 produced behavioral changes (Doucette et al. 2003), as well as permanent and reproducible behavioral-seizure syndrome when animals were tested as adults (Doucette et al. 2004). Treated rats also showed significant increases in hippocampal mossy fiber staining and reductions in hippocampal cell count, as well as molecular level changes (Doucette et al. 2004).

Neurodevelopmental effects in wild marine mammals following pre and/or postnatal exposure

- Sea lion: Studies of stranded California sea lions unable to survive in the wild suggested an association between neurological disease and potential neonatal exposure to domoic acid (Simeone et al. 2019). Adult sea lions died acutely or sometime after exposure, often expressing persistent seizures with characteristic necropsy findings (Silvagni et al. 2005).
- Sea lion: Pregnant female California sea lions are regularly exposed to domoic acid in their diet (Ramsdell 2010; Ramsdell and Zabka 2008). Domoic acid was detected in 79% of amniotic fluid samples from 24 animals (Lefebvre et al. 2018). The distribution of domoic acid in fetal fluid samples suggested recirculation through swallowing and hence continuous exposure to the developing brain.
- Sea lion: Co-exposure to domoic acid and legacy DDTs may contribute to observation of an epilepsy syndrome observed among young sea lions that may have been exposed *in utero* (Ramsdell et al. 2010). Such an effect is supported by studies using a zebrafish model of susceptibility to domoic acid-induced seizures after DDT/DDE exposure during neurodevelopment, which found enhanced seizure behaviors at levels of DDTs (e.g., p,p'-DDE) similar to body burden levels found in fetal California sea lions (Tiedeken and Ramsdell 2009).
- Sea lion: Examination of 67 aborted or prematurely live-born pups provided evidence of domoic acid contributing to reproductive failure on California sea lion rookeries (Goldstein et al. 2009).

Non-DNT DART outcomes, prenatal exposure

- Monkey (macaque): Exposure prior to and during pregnancy to low doses of domoic acid showed no evidence of reproductive toxicity or physical developmental toxicity. While DNT outcomes were not evaluated, maternal animals evidenced a dose-dependent increase in intention tremors (Burbacher et al. 2019).
- Zebrafish: Exposure of embryos increased mortality and cardiac defects, and altered expression of some cardiac development-correlated genes at concentrations between one and 1000 ng/L culture media (Hong et al. 2015).

Mechanistic, in vitro, and other relevant data

Domoic acid is a high-affinity structural analog of kainic acid, and hence a direct agonist of glutamic kainite receptors (Larm et al. 1997). In sufficient concentrations, agonists of glutamic kainite receptors can overstimulate neurons and result in neuronal cell death through a process known as excitotoxicity. Loss of these neurons can lead to functional deficits in learning and memory (see for example the proposed adverse outcome pathway for ionotrophic glutamate receptors in adult brain at https://aopwiki.org/aops/48). While this proposed pathway has not been specifically validated for developing brains, current evidence tends to support its applicability.

- Mouse: Exposure of cultured dopaminergic neurons prepared from embryonic mesencephalaresulted in cytotoxicity, as measured by increased release of lactate dehydrogenase into the culture medium, increased apoptotic cell death, decreased expression of neuronal nuclear antigen, and decreased numbers of dopaminergic neurons. These effects were attributed to activation of α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid/kainic acid (AMPA/KA) receptors on dopaminergic neurons (Radad et al. 2018).
- Rat: Chronic exposure of cultured primary cortical neurons to a low concentration of domoic acid altered spontaneous electrical activity, measured using microelectrode arrays, leading to possible neuronal malfunction (Hogberg et al. 2011a). These effects were considered to be primarily mediated by the AMPA/KA receptor (Hogberg and Bal-Price 2011b).

References cited in "Domoic acid"

Adams AL, Doucette TA, James R, Ryan CL. 2009. Persistent changes in learning and memory in rats following neonatal treatment with domoic acid. Physiol Behav 96:505-512.

Bernard PB, Macdonald DS, Gill DA, Ryan CL, Tasker RA. 2007. Hippocampal mossy fiber sprouting and elevated TRKB receptor expression following systemic administration of low dose domoic acid during neonatal development. Hippocampus 17:1121-1133.

Burbacher TM, Grant KS, Petroff R, Shum S, Crouthamel B, Stanley C, et al. 2019. Effects of oral domoic acid exposure on maternal reproduction and infant birth characteristics in a preclinical nonhuman primate model. Neurotoxicol Teratol 72:10-21.

Burt MA, Ryan CL, Doucette TA. 2008a. Altered responses to novelty and drug reinforcement in adult rats treated neonatally with domoic acid. Physiol Behav 93:327-336.

Burt MA, Ryan CL, Doucette TA. 2008b. Low dose domoic acid in neonatal rats abolishes nicotine induced conditioned place preference during late adolescence. Amino Acids 35:247-249.

Costa LG, Giordano G, Faustman EM. 2010. Domoic acid as a developmental neurotoxin. Neurotoxicology 31:409-423.

Dakshinamurti K, Sharma SK, Sundaram M, Watanabe T. 1993. Hippocampal changes in developing postnatal mice following intrauterine exposure to domoic acid. J Neurosci 13:4486-4495.

Doucette TA, Strain SM, Allen GV, Ryan CL, Tasker RA. 2000. Comparative behavioural toxicity of domoic acid and kainic acid in neonatal rats. Neurotoxicol Teratol 22:863-869.

Doucette TA, Bernard PB, Yuill PC, Tasker RA, Ryan CL. 2003. Low doses of non-nmda glutamate receptor agonists alter neurobehavioural development in the rat. Neurotoxicol Teratol 25:473-479.

Doucette TA, Bernard PB, Husum H, Perry MA, Ryan CL, Tasker RA. 2004. Low doses of domoic acid during postnatal development produce permanent changes in rat behaviour and hippocampal morphology. Neurotox Res 6:555-563.

Ferriss BE, Marcinek DJ, Ayres D, Borchert J, Lefebvre KA. 2017. Acute and chronic dietary exposure to domoic acid and recreational harvesters: A survey of shellfish consumption behavior. Environment International 101:70-70,.

Gill DA, Bastlund JF, Anderson NJ, Tasker RA. 2009. Reductions in paradoxical sleep time in adult rats treated neonatally with low dose domoic acid. Behav Brain Res 205:564-567.

Gill DA, Perry MA, McGuire EP, Pérez-Gómez A, Tasker RA. 2012. Low-dose neonatal domoic acid causes persistent changes in behavioural and molecular indicators of stress response in rats. Behav Brain Res 230:409-417.

Gill S, Kumara VMR. 2019. Detecting neurodevelopmental toxicity of domoic acid and ochratoxin a using rat fetal neural stem cells. Mar Drugs 17.

Goldstein T, Zabka TS, Delong RL, Wheeler EA, Ylitalo G, Bargu S, et al. 2009. The role of domoic acid in abortion and premature parturition of California sea lions (*Zalophus californianus*) on San Miguel Island, California. J Wildl Dis 45:91-108.

Grant KS, Burbacher TM, Faustman EM, Gratttan L. 2010. Domoic acid: Neurobehavioral consequences of exposure to a prevalent marine biotoxin. Neurotoxicol Teratol 32:132-141.

Grant KS, Crouthamel B, Kenney C, McKain N, Petroff R, Shum S, et al. 2019. Preclinical modeling of exposure to a global marine bio-contaminant: Effects of *in utero* domoic acid exposure on neonatal behavior and infant memory. Neurotoxicol Teratol 73:1-8.

Grattan LM, Boushey CJ, Liang Y, Lefebvre KA, Castellon LJ, Roberts KA, et al. 2018. Repeated dietary exposure to low levels of domoic acid and problems with everyday memory: Research to public health outreach. Toxins (Basel) 10:1-10.

Hogberg HT, Sobanski T, Novellino A, Whelan M, Weiss DG, Bal-Price AK. 2011a. Application of micro-electrode arrays (MEAS) as an emerging technology for developmental neurotoxicity: Evaluation of domoic acid-induced effects in primary cultures of rat cortical neurons. Neurotoxicology 32:158-168.

Hogberg HT, Bal-Price AK. 2011b. Domoic acid-induced neurotoxicity is mainly mediated by the ampa/ka receptor: Comparison between immature and mature primary cultures of neurons and glial cells from rat cerebellum. J Toxicol 2011:543512.

Hong Z, Zhang Y, Zuo Z, Zhu R, Gao Y. 2015. Influences of domoic acid exposure on cardiac development and the expression of cardiovascular relative genes in zebrafish (*Danio rerio*) embryos. J Biochem Mol Toxicol 29:254-260.

Jandová K, Kozler P, Langmeier M, Marešová D, Pokorný J, Riljak V. 2014. Influence of low-dose neonatal domoic acid on the spontaneous behavior of rats in early adulthood. Physiol Res 63 Suppl 4:S521-528.

Larm JA, Beart PM, Cheung NS. 1997. Neurotoxin domoic acid produces cytotoxicity via kainate- and ampa-sensitive receptors in cultured cortical neurones. Neurochem Int 31:677-682.

Lefebvre KA, Hendrix A, Halaska B, Duignan P, Shum S, Isoherranen N, et al. 2018. Domoic acid in California sea lion fetal fluids indicates continuous exposure to a neuroteratogen poses risks to mammals. Harmful Algae 79:53-57.

Levin ED, Pizarro K, Pang WG, Harrison J, Ramsdell JS. 2005. Persisting behavioral consequences of prenatal domoic acid exposure in rats. Neurotoxicol Teratol 27:719-725.

Levin ED, Pang WG, Harrison J, Williams P, Petro A, Ramsdell JS. 2006. Persistent neurobehavioral effects of early postnatal domoic acid exposure in rats. Neurotoxicol Teratol 28:673-680.

Marien K. 1996. Establishing tolerable dungeness crab (Cancer magister) and razor clam (Siliqua patula) domoic acid contaminant levels. Environ Health Perspect 104:1230-1236.

Maucher JM, Ramsdell JS. 2005. Domoic acid transfer to milk: Evaluation of a potential route of neonatal exposure. Environ Health Perspect 113:461-464.

Mills BD, Pearce HL, Khan O, Jarrett BR, Fair DA, Lahvis GP. 2016. Prenatal domoic acid exposure disrupts mouse pro-social behavior and functional connectivity MRI. Behav Brain Res 308:14-23.

Perry MA, Ryan CL, Tasker RA. 2009. Effects of low dose neonatal domoic acid administration on behavioural and physiological response to mild stress in adult rats. Physiol Behav 98:53-59.

Petroff R, Richards T, Crouthamel B, McKain N, Stanley C, Grant KS, et al. 2019. Chronic, low-level oral exposure to marine toxin, domoic acid, alters whole brain morphometry in nonhuman primates. Neurotoxicology 72:114-124.

Radad K, Al-Shraim M, Al-Emam A, Moldzio R, Rausch WD. 2018. Neurotoxic effects of domoic acid on dopaminergic neurons in primary mesencephalic cell culture. Folia Neuropathol 56:39-48.

Ramsdell JS, Zabka TS. 2008. In utero domoic acid toxicity: A fetal basis to adult disease in the California sea lion (*Zalophus californianus*). Mar Drugs 6:262-290.

Ramsdell JS. 2010. Neurological disease rises from ocean to bring model for human epilepsy to life. Toxins (Basel) 2:1646-1675.

Ryan CL, Robbins MA, Smith MT, Gallant IC, Adams-Marriott AL, Doucette TA. 2011. Altered social interaction in adult rats following neonatal treatment with domoic acid. Physiol Behav 102:291-295.

Shiotani M, Cole TB, Hong S, Park JJY, Griffith WC, Burbacher TM, et al. 2017. Neurobehavioral assessment of mice following repeated oral exposures to domoic acid during prenatal development. Neurotoxicol Teratol 64:8-19.

Silvagni PA, Lowenstine LJ, Spraker T, Lipscomb TP, Gulland FM. 2005. Pathology of domoic acid toxicity in California sea lions (*Zalophus californianus*). Vet Pathol 42:184-191.

Simeone C, Fauquier D, Skidmore J, Cook P, Colegrove K, Gulland F, et al. 2019. Clinical signs and mortality of non-released stranded California sea lions housed in display facilities: The suspected role of prior exposure to algal toxins. Vet Rec 185:304.

Tasker RA, Perry MA, Doucette TA, Ryan CL. 2005. Nmda receptor involvement in the effects of low dose domoic acid in neonatal rats. Amino Acids 28:193-196.

Thomsen MB, Lillethorup TP, Jakobsen S, Nielsen EH, Simonsen M, Wegener G, et al. 2016. Neonatal domoic acid alters *in vivo* binding of [(11)c]yohimbine to α(2)-adrenoceptors in adult rat brain. Psychopharmacology (Berl) 233:3779-3785.

Tiedeken JA, Ramsdell JS, Ramsdell AF. 2005. Developmental toxicity of domoic acid in zebrafish (*Danio rerio*). Neurotoxicol Teratol 27:711-717.

Tiedeken JA, Ramsdell JS. 2007. Embryonic exposure to domoic acid increases the susceptibility of zebrafish larvae to the chemical convulsant pentylenetetrazole. Environ Health Perspect 115:1547-1552.

Tiedeken JA, Ramsdell JS. 2009. Ddt exposure of zebrafish embryos enhances seizure susceptibility: Relationship to fetal p,p'-dde burden and domoic acid exposure of California sea lions. Environ Health Perspect 117:68-73.

US FDA (Food and Drug Administration). 2020. Appendix 5: FDA and EPA Safety Levels and Regulations and Guidance. Available: https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=&cad=rja&uact=8&ved=2ahUKEwipi7jf_6zrAhWFtp4KHdQOCTMQFjAAegQIBRAB&url=https%3A%2F%2Fwww.fda.gov%2Fmedia%2F80400%2Fdownload&usg=AOvVaw3gZChY_lOemec-gay1fSuD [accessed 17 September 2020].

Wang GJ, Schmued LC, Andrews AM, Scallet AC, Slikker W, Jr., Binienda Z. 2000. Systemic administration of domoic acid-induced spinal cord lesions in neonatal rats. J Spinal Cord Med 23:31-39.

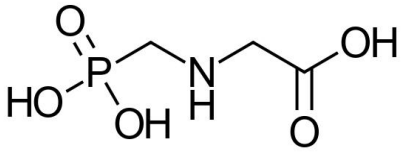
Wekell JC, Hurst J, Lefebvre KA. 2004. The origin of the regulatory limits for PSP and ASP toxins in shellfish. J of Shellfish Res 23:927-930.

Xi D, Peng YG, Ramsdell JS. 1997. Domoic acid is a potent neurotoxin to neonatal rats. Nat Toxins 5:74-79.

Zuloaga DG, Lahvis GP, Mills B, Pearce HL, Turner J, Raber J. 2016. Fetal domoic acid exposure affects lateral amygdala neurons, diminishes social investigation and alters sensory-motor gating. Neurotoxicology 53:132-140.

Glyphosate and its salts

[N-(Phosphonomethyl)glycine]



Glyphosate is an organophosphorous non-selective herbicide. It is used as either the acid form, or as a salt (e.g., glyphosate monoammonium salt, glyphosate diammonium salt, glyphosate isopropylamine salt, glyphosate potassium salt). There are over 750 products containing glyphosate for sale in the US. Commercial products containing glyphosate may have concentrations ranging from 0.96 to 94% weight per weight, according to the information by the Agency for Toxic Substances and Disease Registry (ATSDR) (ATSDR 2020). A total of approximately 11.7 million pounds of glyphosate and its salts were used in 2017 in California (CDPR 2020).

The general population may be exposed to glyphosate via dermal contact, inhalation, or ingestion, through use of glyphosate products at home, living in proximity to agricultural areas where glyphosate is used, and consumption of residues present in foods (ATSDR 2020).

Glyphosate passed the human and animal data screens, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies published within the last five years and those included in the Toxicological Profile for Glyphosate by ATSDR (ATSDR 2020) that were identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Human studies reporting -developmental and reproductive toxicity (DART)-related effects associated with glyphosate were identified in the recent literature (published within the last five years) and in the Toxicological Profile for glyphosate (ATSDR 2020). DART findings reported in these epidemiologic studies are summarized here. The findings are organized by groups of outcomes.

Pregnancy outcomes

- Increased risks of miscarriage and preterm delivery when exposure to multiple pesticides including glyphosate was assessed as a combined group (retrospective cohort study) (Savitz et al. 1997).
- Marginally significantly increased risk of late spontaneous abortion associated with preconception exposure (retrospective cohort study) (Arbuckle et al. 2001).
- Shortened gestational length significantly correlated with higher maternal glyphosate urine levels. However, maternal glyphosate urine levels were not significantly correlated with birth weight percentile or head circumference (prospective cohort study) (Parvez et al. 2018).
- No effects on birth weight (retrospective cohort study) (Sathyanarayana et al. 2010).
- Paternal exposure was not associated with congenital malformations (case-control study) (García et al. 1998).
- Atrial septal defects were positively associated with higher levels of maternal exposure to glyphosate. (case-control study) (Rappazzo et al. 2019).

Neurodevelopmental effects

In the studies below, neurodevelopmental effects are associated with prenatal exposures.

- Increased risk of autism spectrum disorder (ASD) (with or without intellectual disability) and ASD with intellectual disability (case-control study) (von Ehrenstein et al. 2019).
- Increased risk of attention deficit disorder or attention-deficit/hyperactivity disorder (retrospective cohort study) (Garry et al. 2002).

Female reproductive effects

- No effect on time to pregnancy (retrospective cohort study) (Curtis et al. 1999).

Animal studies

Findings reported in whole animal studies examining possible DART effects of exposure to glyphosate published within the last five years or included in the 2020 ATSDR Toxicological Profile for glyphosate are summarized here.

Developmental effects on fetal growth and development

- Rat: Pregnant rats (F0) received a GBH in the diet at doses of 2 mg (GBH-LD: GBH-low dose group) or 200 mg (GBH-HD: GBH-high dose group) of

glyphosate/kg bw/day from gestational day (GD) 9 until weaning. F2 offspring from both dose groups showed delayed growth, associated with a higher incidence of small for gestational age fetuses. Structural congenital anomalies (conjoined fetuses and abnormally developed limbs) were detected in the F2 offspring from the GBH-HD group (Milesi et al. 2018).

- Rat: Depressed weight and increased incidence of unossified sternebrae were observed in fetuses from rat dams treated by gavage at 3,500 mg/kg/day during GDs 6–19 (US EPA 1992e, as cited by ATSDR 2020).
- Rat: Exposure of F0 dams perinatally to glyphosate or a GBH in drinking water at doses comparable to the US glyphosate ADI (1.75 mg/kg bw/day) resulted in significant and distinctive changes in overall bacterial composition in fecal samples from F1 pups (Mao et al. 2018).
- Rat: a transient exposure of gestating F0 generation female rats found negligible impacts of glyphosate on the directly exposed F0 generation, or F1 generation offspring pathology. In contrast, dramatic increases in pathologies in the F2 generation grand-offspring, and F3 transgenerational great-grand-offspring were observed. The transgenerational pathologies observed include prostate disease, obesity, kidney disease, ovarian disease, and parturition (birth) abnormalities. Epigenetic analysis of the F1, F2 and F3 generation sperm identified differential DNA methylation regions (DMRs) (Kubsad et al. 2019).

Neurodevelopmental effects

- Rat: Maternal exposure via drinking water affects cholinergic and glutamatergic neurotransmission in the hippocampus of immature and adult offspring (Cattani et al. 2017).
- Rat: Perinatal exposure to a glyphosate-based herbicide (GBH) modified the set point of the hypothalamic-pituitary-thyroid (HPT) axis in male offspring, with lower levels of TSH likely reflecting post-translational events (de Souza et al. 2017).
- Mouse: Perinatal exposure altered expression patterns of microRNA (miRNA) involved in the Wnt and Notch pathways (Ji et al. 2018).

Female reproductive effects

- Rat: Perinatal exposure to glyphosate, or a GBH affected maternal behavior and modulated neuroplasticity and gut microbiota in the dam (Dechartres et al. 2019).
- Rat: Female Wistar pups exposed to a GBH by subcutaneous (s.c.) injection from postnatal day (PND) 1 to PND 7 showed morphological changes in the uterus, characterized by increases in the incidence of luminal epithelial hyperplasia and increases in stromal and myometrial thickness. Altered expression of proteins involved in uterine organogenetic differentiation was also observed (Guerrero Schimpf et al. 2017).

- Rat: Female rats postnatally exposed to a GBH were bilaterally ovariectomized at weaning (PND21) and submitted to chronic estrogen stimulation until PND60 to evaluate uterine morphology, cell proliferation, expression of the estrogen receptors (ERs) (ESR1 and ESR2) and the expression of WNT7A and β-catenin (CTNNB1), which are E2-modulated targets that have been implicated in altered uterine E2 responsiveness and neoplasia. Early postnatal exposure to a GBH enhanced the sensitivity of the rat uterus to estradiol, and induced histomorphological and molecular changes associated with uterine hyperplasia (Guerrero Schimpf et al. 2018). This was a follow up study to the study report by Guerrero Schimpf (2017).
- Rat: Subchronic exposure to a GBH impaired ovary development, including folliculogenesis, decreased estrogen secretion, and promoted oxidative stress (Hamdaoui et al. 2018).
- Rat: GBH administered from postnatal day (PND) 1 to PND 7 altered expression of genes involved in embryo implantation process, including Wnt5a, β-catenin, Wnt7a and β-catenin, Dkk1 and sFRP4 (Ingaramo et al. 2017).
- Rat: Glyphosate and a GBH caused alterations in anogenital distance (AGD), age at first estrus, and serum levels of testosterone in the offspring, following perinatal treatment. Hormonal status imbalances were more pronounced in GBH-treated rats after prolonged exposure (Manservisi et al. 2019).
- Mouse: Gestational treatment with glyphosate or a GBH in drinking water caused decreases in body weight gain and ovary and liver weight in glyphosate-treated pregnant animals. Additionally, histopathological alterations in the ovary including increased atretic follicles, interstitial fibrosis and decreased mature follicles were observed in dams treated with glyphosate. The serum concentrations of both progesterone and estrogen were markedly altered after glyphosate exposure, and there were also changes in the expression of GnRH, LHR, FSHR, 3β-HSD and Cyp19a1 genes at the hypothalamic-pituitary-ovarian axis. The sex ratio of female:male fetuses was increased following gestational treatment with glyphosate or GBH; no effects were observed on fetal weights or anogenital distance at birth (Ren et al. 2018).
- Sheep: Treatment of ewe lambs (young females) with GBH by s.c. injection from PND 1 to 14 did not affect ovarian or uterine weight. However, on PND 45, the ovary of GBH-exposed lambs showed altered follicular dynamics, increased proliferation of granulosa and theca cells, and decreased mRNA expression of FSHR and GDF9, whereas the uterus showed decreased cell proliferation but no alteration in histomorphology or gene expression (Alarcón et al. 2019).

Male reproductive effects

- Rat: ATSDR (2020) stated “[an] increased percentage of morphologically abnormal sperm was reported among rats receiving a glyphosate formulation from the drinking water for 8 days at 640 mg/kg/day.”

- Rat: ATSDR (2020) discussed one study that reported an increased incidence of prostatitis among male rats receiving glyphosate (technical) in the diet for up to two years at estimated doses of approximately 361 and 1,214 mg/kg/day.
- Rat: Postnatal exposure to GBH by s.c. injection resulted in greater development of the mammary gland with increased stromal collagen organization and terminal end buds (TEBs) in male rats (Altamirano et al. 2018).
- Rat: Glyphosate administration at 375 mg/kg-day in diet to 28 Wistar male rats decreased sperm motility, sperm plasma membrane integrity, glutathione, and protein levels of superoxide dismutase in the testicular tissue of rats (Avdatek et al. 2018).
- Rat: Perinatal exposure to a GBH at 3.5 or 350 mg /kg bw/day altered mammary gland development and methylation status of estrogen receptor alpha in male offspring at PND 60 (Gomez et al. 2019).
- Rat: Treatment with glyphosate for two weeks at 2.5 or 25 mg/kg-day had no effect on testicular histopathology or testosterone synthesis. An equivalent dose of a GBH induced minor effects on steroidogenic gene expression (Johansson et al. 2018).
- Rat: Exposure to GBH in drinking water for 12 weeks caused significant alterations in the level of all the reproductive hormones, reductions in sperm count, percentage motility, increase in abnormal sperm cells, and severe degenerative testicular architectural lesions in males (Owagboriaye et al. 2017).
- Mouse: GBH impaired spermatogenesis, decreased sperm motility and concentration, and increased the sperm deformity rate (Jiang et al. 2018).
- Mouse: Perinatal exposure in drinking water to glyphosate affected testis morphology, decreased serum testosterone, and decreased the number of spermatozoa; no effects seen with a GBH (Pham et al. 2019).

Mechanistic, in vitro, and other relevant data

- Rat: Pregnant rats (F0) were orally treated with a GBH at a dose of 350 mg glyphosate/kg bw/day through food from GD 9 until weaning. F1 females were bred and uterine samples were collected on GD5 (preimplantation period) for evaluation on epigenetic changes in estrogen receptor alpha (ERα). Expression of total ERα mRNA in uterine tissues was upregulated. A decrease in DNA methylation was observed in one of the three sites evaluated in the O promoter. Moreover, histone H4 acetylation and histone H3 lysine 9 trimethylation (H3K9me3) were enriched in the O promoter in uterine tissues from GBH-exposed rats, whereas H3K27me3 was decreased (Lorenz et al. 2019).
- Pig: Incubation of fresh commercial semen with different concentrations of glyphosate or a GBH showed decreased sperm motility, viability, mitochondrial activity and acrosome integrity (Nerozzi et al. 2020).

- Pig: Glyphosate inhibited cell growth, 17-β estradiol and non-enzymatic scavenging activity and increased progesterone and nitric oxide secretion in cultured granulosa cells. It also decreased cell viability and inhibited adipogenic differentiation in cultured adipose stromal cells (Gigante et al. 2018).
- Chicken: Decreased hatchability rate of eggs treated *in ovo* with a GBH (Fathi et al. 2019).
- Mouse: Oocytes treated with glyphosate *in vitro* had reduced rates of germinal vesicle breakdown and first polar body extrusion (Zhang et al. 2019).

References cited in "Glyphosate and its salts"

Alarcón R, Ingaramo PI, Rivera OE, Dioguardi GH, Repetti MR, Demonte LD, et al. 2019. Neonatal exposure to a glyphosate-based herbicide alters the histofunctional differentiation of the ovaries and uterus in lambs. Mol Cell Endocrinol 482:45-56.

Altamirano GA, Delconte MB, Gomez AL, Ingaramo PI, Bosquiazzo VL, Luque EH, et al. 2018. Postnatal exposure to a glyphosate-based herbicide modifies mammary gland growth and development in wistar male rats. Food Chem Toxicol 118:111-118.

Arbuckle TE, Lin Z, Mery LS. 2001. An exploratory analysis of the effect of pesticide exposure on the risk of spontaneous abortion in an ontario farm population. Environ Health Perspect 109:851-857.

ATSDR (Agency for Toxic Substances and Disease Registry). 2020. Toxicological Profile for Glyphosate. Available: https://www.atsdr.cdc.gov/toxprofiles/TP.asp?id=1488&tid=293 [accessed 3 September 2020].

Avdatek F, Birdane YO, Türkmen R, Demirel HH. 2018. Ameliorative effect of resveratrol on testicular oxidative stress, spermatological parameters and DNA damage in glyphosate-based herbicide-exposed rats. Andrologia 50:e13036.

Cattani D, Cesconetto PA, Tavares MK, Parisotto EB, De Oliveira PA, Rieg CEH, et al. 2017. Developmental exposure to glyphosate-based herbicide and depressive-like behavior in adult offspring: Implication of glutamate excitotoxicity and oxidative stress. Toxicology 387:67-80.

CDPR (California Department of Pesticide Regulation). 2020. 2017 Annual Statewide Pesticide Use Report Indexed by Chemical. Available: https://www.cdpr.ca.gov/docs/pur/pur17rep/statewide_ai_2017.htm [accessed 3 September 2020.

Curtis KM, Savitz DA, Weinberg CR, Arbuckle TE. 1999. The effect of pesticide exposure on time to pregnancy. Epidemiology 10:112-117.

de Souza JS, Kizys MM, da Conceição RR, Glebocki G, Romano RM, Ortiga-Carvalho TM, et al. 2017. Perinatal exposure to glyphosate-based herbicide alters the thyrotrophic axis and causes thyroid hormone homeostasis imbalance in male rats. Toxicology 377:25-37.

Dechartres J, Pawluski JL, Gueguen MM, Jablaoui A, Maguin E, Rhimi M, et al. 2019. Glyphosate and glyphosate-based herbicide exposure during the peripartum period affects maternal brain plasticity, maternal behaviour and microbiome. J Neuroendocrinol 31:e12731.

Fathi MA, Abdelghani E, Shen D, Ren X, Dai P, Li Z, et al. 2019. Effect of in ovo glyphosate injection on embryonic development, serum biochemistry, antioxidant status and histopathological changes in newly hatched chicks. J Anim Physiol Anim Nutr (Berl) 103:1776-1784.

García AM, Benavides FG, Fletcher T, Orts E. 1998. Paternal exposure to pesticides and congenital malformations. Scand J Work Environ Health 24:473-480.

Garry VF, Harkins ME, Erickson LL, Long-Simpson LK, Holland SE, Burroughs BL. 2002. Birth defects, season of conception, and sex of children born to pesticide applicators living in the red river valley of minnesota, USA. Environ Health Perspect 110 Suppl 3:441-449.

Gigante P, Berni M, Bussolati S, Grasselli F, Grolli S, Ramoni R, et al. 2018. Glyphosate affects swine ovarian and adipose stromal cell functions. Anim Reprod Sci 195:185-196.

Gomez AL, Altamirano GA, Leturia J, Bosquiazzo VL, Muñoz-de-Toro M, Kass L. 2019. Male mammary gland development and methylation status of estrogen receptor alpha in wistar rats are modified by the developmental exposure to a glyphosate-based herbicide. Mol Cell Endocrinol 481:14-25.

Guerrero Schimpf M, Milesi MM, Ingaramo PI, Luque EH, Varayoud J. 2017. Neonatal exposure to a glyphosate based herbicide alters the development of the rat uterus. Toxicology 376:2-14.

Guerrero Schimpf M, Milesi MM, Luque EH, Varayoud J. 2018. Glyphosate-based herbicide enhances the uterine sensitivity to estradiol in rats. J Endocrinol.

Hamdaoui L, Naifar M, Rahmouni F, Harrabi B, Ayadi F, Sahnoun Z, et al. 2018. Subchronic exposure to kalach 360 sl-induced endocrine disruption and ovary damage in female rats. Arch Physiol Biochem 124:27-34.

Ingaramo PI, Varayoud J, Milesi MM, Guerrero Schimpf M, Alarcón R, Muñoz-de-Toro M, et al. 2017. Neonatal exposure to a glyphosate-based herbicide alters uterine decidualization in rats. Reprod Toxicol 73:87-95.

Ji H, Xu L, Wang Z, Fan X, Wu L. 2018. Differential microrna expression in the prefrontal cortex of mouse offspring induced by glyphosate exposure during pregnancy and lactation. Exp Ther Med 15:2457-2467.

Jiang X, Zhang N, Yin L, Zhang WL, Han F, Liu WB, et al. 2018. A commercial roundup® formulation induced male germ cell apoptosis by promoting the expression of xaf1 in adult mice. Toxicol Lett 296:163-172.

Johansson HKL, Schwartz CL, Nielsen LN, Boberg J, Vinggaard AM, Bahl MI, et al. 2018. Exposure to a glyphosate-based herbicide formulation, but not glyphosate alone, has only minor effects on adult rat testis. Reprod Toxicol 82:25-31.

Kubsad D, Nilsson EE, King SE, Sadler-Riggleman I, Beck D, Skinner MK. 2019. Assessment of glyphosate induced epigenetic transgenerational inheritance of pathologies and sperm epimutations: Generational toxicology. Sci Rep 9:6372.

Lorenz V, Milesi MM, Schimpf MG, Luque EH, Varayoud J. 2019. Epigenetic disruption of estrogen receptor alpha is induced by a glyphosate-based herbicide in the preimplantation uterus of rats. Mol Cell Endocrinol 480:133-141.

Manservisi F, Lesseur C, Panzacchi S, Mandrioli D, Falcioni L, Bua L, et al. 2019. The ramazzini institute 13-week pilot study glyphosate-based herbicides administered at human-equivalent dose to sprague dawley rats: Effects on development and endocrine system. Environ Health 18:15.

Mao Q, Manservisi F, Panzacchi S, Mandrioli D, Menghetti I, Vornoli A, et al. 2018. The ramazzini institute 13-week pilot study on glyphosate and roundup administered at human-equivalent dose to sprague dawley rats: Effects on the microbiome. Environ Health 17:50.

Milesi MM, Lorenz V, Pacini G, Repetti MR, Demonte LD, Varayoud J, et al. 2018. Perinatal exposure to a glyphosate-based herbicide impairs female reproductive outcomes and induces second-generation adverse effects in wistar rats. Arch Toxicol 92:2629-2643.

Nerozzi C, Recuero S, Galeati G, Bucci D, Spinaci M, Yeste M. 2020. Effects of roundup and its main component, glyphosate, upon mammalian sperm function and survival. Sci Rep 10:11026.

Owagboriaye FO, Dedeke GA, Ademolu KO, Olujimi OO, Ashidi JS, Adeyinka AA. 2017. Reproductive toxicity of roundup herbicide exposure in male albino rat. Exp Toxicol Pathol 69:461-468.

Parvez S, Gerona RR, Proctor C, Friesen M, Ashby JL, Reiter JL, et al. 2018. Glyphosate exposure in pregnancy and shortened gestational length: A prospective indiana birth cohort study. Environ Health 17:23.

Pham TH, Derian L, Kervarrec C, Kernanec PY, Jégou B, Smagulova F, et al. 2019. Perinatal exposure to glyphosate and a glyphosate-based herbicide affect spermatogenesis in mice. Toxicol Sci 169:260-271.

Rappazzo KM, Warren JL, Davalos AD, Meyer RE, Sanders AP, Brownstein NC, et al. 2019. Maternal residential exposure to specific agricultural pesticide active ingredients and birth defects in a 2003-2005 north carolina birth cohort. Birth Defects Res 111:312-323.

Ren X, Li R, Liu J, Huang K, Wu S, Li Y, et al. 2018. Effects of glyphosate on the ovarian function of pregnant mice, the secretion of hormones and the sex ratio of their fetuses. Environ Pollut 243:833-841.

Sathyanarayana S, Basso O, Karr CJ, Lozano P, Alavanja M, Sandler DP, et al. 2010. Maternal pesticide use and birth weight in the agricultural health study. J Agromedicine 15:127-136.

Savitz DA, Arbuckle T, Kaczor D, Curtis KM. 1997. Male pesticide exposure and pregnancy outcome. Am J Epidemiol 146:1025-1036.

von Ehrenstein OS, Ling C, Cui X, Cockburn M, Park AS, Yu F, et al. 2019. Prenatal and infant exposure to ambient pesticides and autism spectrum disorder in children: Population based case-control study. BMJ 364:l962.

Zhang JW, Xu DQ, Feng XZ. 2019. The toxic effects and possible mechanisms of glyphosate on mouse oocytes. Chemosphere 237:124435.

Manganese

(CAS #: 7439-96-5)

Manganese (Mn) is a naturally occurring element. It is an essential nutrient involved in the synthesis and activation of many enzymes and in the regulation of glucose and lipid metabolism in humans. It is also toxic at higher levels. Manganese is used primarily in steel production. It is also used in a number of other products including: dry-cell batteries; animal feed; fertilizer; livestock nutritional supplement; paint, glazes and varnishes; ceramics; cosmetics; and pesticides (ATSDR 2012).

The most common source of exposure to Mn is the diet. Reviews of typical Western and vegetarian diets showed typical Mn intakes of 0.7 to 10.9 mg/day (WHO, 2004). However, Mn in drinking water or air has been shown in some circumstances to result in significant exposure. Occupational exposures are most likely to occur via inhalation[9].

Manganese has been shown to cross the blood-brain barrier and a limited amount of manganese is also able to cross the placenta during pregnancy, enabling it to reach the developing fetus.

Manganese passed the human and animal data screens, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Numerous human studies reporting developmental and reproductive toxicity (DART)-related effects associated with manganese were identified in the recent literature. A number of DART findings reported in epidemiologic studies are summarized here, with an emphasis on those published within the last five years. The findings are organized by groups of outcomes.

Birth weight

- Lower birth weight, inverted U-shaped dose-response curve, only in male infants, associated with maternal blood Mn levels during the 3rd trimester (prospective cohort study) (Yamamoto et al. 2019).

[9] https://biomonitoring.ca.gov/sites/default/files/downloads/102110Manganese.pdf

- Lower birth weight, associated with increases in the ratio of cord to maternal 3rd trimester blood Mn levels (prospective cohort study) (Ashley-Martin et al. 2018).
- Lower birth weight, inverted U-shaped dose-response curve, associated with maternal urinary Mn levels in the 3rd trimester (prospective cohort study) (Hu et al. 2018).
- Lower birth weight, associated with higher maternal erythrocyte Mn levels in the 2nd trimester (prospective cohort study) (Tsai et al. 2015).
- Low birth weight, inverted U-shaped dose-response curve, associated with maternal urine Mn levels (case-control study) (Xia et al. 2016).
- No association of birth weight with Mn in drinking water (prospective cohort study) (Rahman et al. 2015).
- Higher birth weight Z-score, only in female infants, associated with higher Mn levels in teeth (retrospective cohort study) (Cassidy-Bushrow et al. 2019).

Birth length

- Reduced birth length, inverted U-shaped dose-response curve, associated with urinary Mn levels in the 3rd trimester (prospective cohort study) (Hu et al. 2018).
- Reduced birth length, associated with higher Mn in drinking water (prospective cohort study) (Rahman et al. 2015).
- No association with maternal blood Mn levels (prospective cohort study) (Yamamoto et al. 2019).

Ponderal index

- Reduced ponderal index, inverted U-shaped dose-response curve, associated with urinary Mn levels in the 2nd trimester (prospective cohort study) (Hu et al. 2018).

Head circumference

- Slight reduction in head circumference with low maternal blood Mn (prospective cohort study) (Yamamoto et al. 2019).
- Reduced head circumference with low Mn cord blood (prospective cohort study) (Eguchi et al. 2019).
- Reduced head circumference, with higher maternal erythrocyte Mn levels in the 2nd trimester (prospective cohort study) (Tsai et al. 2015).

Chest circumference

- Reduced chest circumference, associated with higher maternal erythrocyte Mn levels in the 2nd trimester (prospective cohort study) (Tsai et al. 2015).
- Increased chest circumference, associated with higher maternal hair Mn concentration (prospective cohort study) (Mora et al. 2015b).

Small for gestational age

- Increased risk of small for gestational age, U-shaped dose-response curve, only in males, associated with maternal blood Mn levels during the 3rd trimester (prospective cohort study) (Yamamoto et al. 2019).

Preterm birth

- Increased risk of preterm birth in a dose-response manner, modified by single nucleotide polymorphisms, with higher maternal Mn levels in 1st trimester (nested case-control study) (Hao et al. 2020).

Birth defects - neural tube defects

- Increased risk in a dose-response manner, with higher placental Mn levels (case-control study) (Liu et al. 2013).
- Increased risk, with higher 2nd trimester maternal blood Mn levels (case-control study) (Özel et al. 2019).
- Increased risk in a dose-response manner, with higher placental Mn (case-control study) (Yin et al. 2020).
- No increased risk of neural tube defects associated with maternal hair Mn levels (case-control study) (Yan et al. 2017).

Neurodevelopmental effects

- Lower cognitive score (Bayley Scales of Infant and Toddler Development), with a mediating effect through birth length, in 2-3 year-olds, associated with higher umbilical cord blood Mn levels (prospective cohort study) (Lee et al. 2018).
- Lower cognitive, language and motor scores (Bayley Scales of Infant Development) associated with higher 3rd trimester maternal blood Mn levels. Maternal depressive symptoms were negatively associated with neurodevelopment scores. Association of higher Mn levels (maternal as well as cord blood), and lower 24-month language scores was stronger among women with depressive symptoms, evaluated during the third trimester. Inverted U-

shaped dose-response curves associated with cord blood Mn and neurodevelopment scores (prospective cohort study) (Muñoz-Rocha et al. 2018).

- Lower mental development index (MDI) and psychomotor development index (PDI) scores in 2-year-old children living near the Tar Creek Superfund Site, associated with higher maternal and cord blood Mn at delivery (prospective cohort study) (Claus Henn et al. 2017).
- Decreased visuospatial learning and memory, in 10-14 year-old girls only, U-shaped dose-response curve, associated with prenatal Mn in deciduous teeth. No significant associations for postnatal Mn exposure (prospective cohort study) (Bauer et al. 2017).
- Decreased cognitive score (Bayley Scale of Infant and Toddler Development) in 20-40 month-old children associated with cord blood Mn levels in a nonlinear manner, using Bayesian kernel machine regression to study the joint effect of co-exposure to arsenic, Mn, and lead. Arsenic was seen to be a potentiator of Mn toxicity (prospective cohort study) (Valeri et al. 2017).
- Lower mental and psychomotor development scores at 6 months of age, inverted U shape dose-response curve, associated with maternal blood Mn at term (prospective cohort study) (Chung et al. 2015).
- Poorer behavioral outcomes, including internalizing, externalizing, and hyperactivity problems, in 7 and 10.5 year-olds associated with higher Mn in deciduous teeth and in boys only, better motor function, memory, and/or cognitive abilities. Higher Mn was also associated with poorer visuospatial memory and cognitive scores in children with higher prenatal lead levels (prospective cohort study) (Mora et al. 2015a).
- Lower PDI scores in 2-year-olds associated with higher maternal urine Mn before delivery. Higher MDI scores in girls only with increasing Mn until levels reached the 30th percentiles, with no effect after that threshold using Bayesian kernel machine regression (prospective cohort study) (Li et al. 2020).
- Decrement in perceptual-performance skills in 4-5 year-olds associated with increased placental Mn in a dose-response manner. Better memory span and quantitative skills associated with higher placental Mn (prospective cohort study) (Freire et al. 2018).
- Lower overall cognitive and language quotients using the Comprehensive Developmental Inventory for Infants and Toddlers in 2 year-olds, associated with increased cord blood Mn (prospective cohort study) (Lin et al. 2013).
- Lower Neonatal Behavioral Neurological Assessments scores in 3-day-old infants: cord blood Mn greater than or equal to 5.0 µg/L had adverse effects on behavior, active tone and general reactions of clusters, in a non-linear pattern (prospective cohort study) (Yu et al. 2014).

Female reproductive effects

- Higher prolactin and luteinizing hormone levels in 7-12 year-olds (possible trigger for early onset of puberty) associated with higher Mn measured in children's toenails, hair and blood, in a non-linear manner (cross-sectional study) (Dos Santos et al. 2019).
- Lower free triiodothyronine (FT3) in pregnant women at approximately 25 weeks gestation associated with higher maternal blood Mn (cross-sectional study) (Guo et al. 2018).
- Higher serum total testosterone levels in 12-19-year-old female adolescents, National Health and Nutrition Examination Survey (NHANES) 2011-2012, associated with higher blood Mn (cross-sectional study) (Yao et al. 2019).
- Increased gestational blood pressure throughout pregnancy, associated with higher maternal blood Mn (prospective cohort study) (Vigeh et al. 2016).

Male reproductive effects

- Lower sperm concentration associated with higher seminal Mn in men recruited from a hospital (cross-sectional study) (Liu et al. 2020).
- In Mn-exposed workers compared to controls: higher gonadotropin-releasing hormone and luteinizing hormone levels; lower testosterone levels; lower sperm progressive motility and total motility, associated with higher urinary Mn (occupational study) (Yang et al. 2019).
- Increasing percentage of Annexin V+/PI- spermatozoa (indicating apoptosis) associated with increasing urine Mn levels, with a dose-dependent trend; two urine samples collected a few hours apart from male partners of couples attending an infertility clinic (cross-sectional study) (Wang et al. 2016).
- Increased risk of low sperm motility and low sperm concentration; U-shaped dose–response curve associated with urinary Mn levels for sperm motility, sperm concentration and morphology (cross-sectional study) (Wirth et al. 2007).

Animal studies

Relevant whole animal studies examining possible DART effects of exposure to manganese were identified. Findings reported in a number of these studies are summarized here.

Developmental effects

- Mouse: Maternal subcutaneous exposure at doses of 0, 2, 4, 8 and 16 mg/kg per day from gestation day (GD) 6-15 reduced maternal body weight and food

consumption, and increased maternal mortality (10% and 32%, respectively) at the two highest doses. At the three highest doses, reduced fetal (GD 18) body weights, increased number of late resorptions, and delayed ossification of sternabrae were observed (Sánchez et al. 1993).

- Mouse: Exposure of 21-day old males and females via gavage (0, 0.013, 0.13 and 1.3 mg/kg-day) for 60 days prior to mating, with continued dosing of dams throughout pregnancy and lactation, resulted in dose-dependent Mn bioaccumulation in pup brain, as well as decreased non-protein thiol levels and glutathione S-transferase and acetylcholinesterase activities, and increased oxidized lipids and proteins in the brains of offspring (Okada et al. 2016).
- Rat: Maternal exposure via drinking water during gestation through weaning resulted in higher body weights in offspring for the first week of life, learning and memory deficits in female offspring, and hypoactivity and increased anxiety in male offspring (Betharia and Maher 2012).
- Rat: Maternal intravenous exposure at doses of 0, 5, 20, or 40 µmol/kg on GD 6-17 significantly reduced mean fetal weights at the mid and high dose, increased post-implantation losses at the high dose, increased the number of dead fetuses (one at the low dose and five at the high dose), increased the number of litters (and fetuses) with abnormal flexure in a dose-dependent manner, increased skeletal malformations in a dose-dependent manner, and increased the occurrence of reduced ossifications in a dose-dependent manner (Treinen et al. 1995).

Female reproductive effects

- Rat: Exposure to doses of 0, 2.5, 5 or 10 mg/kg-day during postnatal days (PND) 21–32 resulted in earlier puberty onset age and advanced ovary and uterus development at the high dose, and in the preoptic area-anterior hypothalamus, decreased levels of gamma-aminobutyric acid receptor ($GABA_AR$) at all doses and increased levels of nitric oxide at the mid and high dose (Yang et al. 2020).
- Rat: Exposure of females to 10 mg/kg-day from PND 12-29 increased serum levels of luteinizing hormone, follicle stimulating hormone and estradiol and increased Mn levels in the medial basal hypothalamus and preoptic area. In a second experiment, continuation of exposure from PND 12 to the date of vaginal opening resulted in earlier age at vaginal opening (Pine et al. 2005).
- Rat: Exposure of females from PND 22-35 to 0, 1.0, 3.3, or 10 mg/kg-day decreased ovarian weights at the low and mid dose, decreased uterine and oviduct weights at the mid dose, decreased the thickness of the uterine myometrium at the low dose and increased the thickness at the mid and high

dose, increased serum luteinizing hormone at the low dose and decreased serum follicle stimulating hormone at the low and high dose (Kim et al. 2012).

Male reproductive effects

- Mouse: Daily exposure of 22-day old males to $MnCl_2$ (0, 15, 30 or 60 mg/kg) for 45 days resulted in the following: reduced androgen-dependent organ weights; altered levels of fecal androgenic metabolites, sperm parameters (e.g., decreased progressive motility, vitality, sperm concentration, and daily sperm production), and antioxidant enzyme activities in the testis (decreased catalase and glutathione reductase, increased superoxide dismutase and glutathione S-transferase); decreased testis non-protein thiol content; increased lipid peroxidation in the testis; and reduced acetylcholinesterase activity in the hypothalamus (Souza et al. 2020).
- Mouse: Exposure of males and females via gavage (0, 0.013, 0.13 and 1.3 mg/kg-day) for 60 days prior to mating, with continued dosing of dams throughout pregnancy and lactation, and direct exposure of some male offspring (F1 males) post-weaning for 60 days resulted in the following: decreased sperm concentration in F0 and F1 males (both with or without direct exposure post-weaning); altered F1 sperm parameters (decreased progressive motility with or without direct exposure post-weaning, decreased vitality without post-weaning exposure); and decreased antioxidant enzyme activity in the testis (catalase, glutathione S-transferase) and seminal vesicles (superoxide dismutase) in F1 males without post-weaning exposure (Souza et al. 2019).
- Mouse: Exposure of adult males for 14 days via intraperitoneal injection (0, 12.5, 25, and 50 mg/kg/day) reduced sperm count at the mid and high dose, slightly increased the number of sperm shape deformities at the high dose, increased serum levels of gonadotropin-releasing hormone, follicle stimulating hormone, luteinizing hormone, and prostaglandin E_2, decreased serum testosterone levels, and increased gene and protein expression in the hypothalamus of the EP1 and EP2 receptors (Wu et al. 2020).
- Rat: Exposure of adult males to an oral dose of 15 mg/kg-day for 45 days, decreased sperm count and progressive motility, and increased sperm morphological defects without affecting sperm viability. Histopathological observations in the testis include decreased germinal epithelium and vacuolization, and in the epididymis include tubule vacuolization with very few sperm cells. The following biochemical measurements were observed in testis, epididymis, and brain: decreased superoxide dismutase, catalase and glutathione-S-transferase activities; increased myeloperoxidase activity; decreased levels of reduced glutathione; and increased levels of hydrogen

peroxide, nitric oxide, lipid peroxidation, tumor necrosis factor alpha, and caspase-3. In addition, acid phosphatase, alkaline phosphatase and lactate dehydrogenase activities were decreased in the testis, and serum levels of luteinizing hormone, follicle stimulating hormone and testosterone were decreased (Adedara et al. 2017).

- Rat: Exposure of adult males for 30 days to supraphysiologic levels (5 mg/kg or 15 mg/kg, intraperitoneal) decreased body weight at the high dose, reduced relative epididymis and ventral prostate weights, reduced the percentage of normal seminiferous tubules and increased the percentage of abnormal seminiferous tubules in a dose dependent manner, decreased the height of seminiferous epithelium at the high dose, increased vacuoles at both doses and degeneration of the seminiferous epithelium at the high dose, decreased sperm number and accelerated sperm transit time in the cauda epididymis, decreased sperm motility (progressive movement) at both doses, and increased sperm head abnormalities at both doses (Gomes Silva et al. 2018).
- Rat: Exposure of adult males to an oral dose of 15 mg/kg-day for 14 days, decreased epididymal sperm count and progressive motility, and increased sperm abnormalities without affecting sperm viability. The following biochemical measurements were observed in testis and epididymis: decreased superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase activities; increased myeloperoxidase activity; decreased levels of reduced glutathione; and increased levels of reactive oxygen and nitrogen species, nitric oxide, and lipid peroxidation. In addition, acid phosphatase, alkaline phosphatase, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase activities were decreased in the testis, and serum levels of luteinizing hormone, follicle stimulating hormone and testosterone were decreased (Owumi et al. 2020).
- Rat: Exposure of adult males to an oral dose of 50 mg/kg-day for 30 days increased sperm abnormalities; decreased gonadosomatic index, sperm motility, and sperm count; reduced serum levels of testosterone and luteinizing hormone; elevated testes levels of malondialdehyde, nitric oxide, and 8-OH-2′-deoxyguanosine; decreased testes levels of superoxide dismutase, glutathione, and catalase; and decreased the diameter of seminiferous tubules (ST), height of germinal epithelium, number of spermatogonia/ST, spermatocytes/ST, spermatids/ ST, and Leydig cells/intertubular area (Mohammed et al. 2018).

Mechanistic, in vitro and other relevant data

- Human: In healthy term singleton pregnancies, higher Mn in infant toenails was associated with increased methylation of the placental glucocorticoid receptor

(NR3C1) gene; this receptor is a key regulator of hypothalamic–pituitary–adrenal axis activity. When stratified by sex, the effect was strengthened in females, although the interaction was not significant and the effect in males was no longer significant (Appleton et al. 2017).

- Rat: Exposure of immature females to 10 mg/kg $MnCl_2$ from PND 12 through 22 or 29 increased serum estradiol levels and gonadotropin-releasing hormone gene expression in the preoptic area and rostral hypothalamus on PND 29, along with upregulation of other genes associated with puberty (Srivastava et al. 2013).
- Zebrafish: Exposure of fertilized embryos to $MnCl_2$ (0, 10, 25, 50 µmol/L) for 5 days resulted in decreased hatching rate at 48 h, increased mortality and malformations (primarily pericardial edema) at 96 and 120 h, and reduced larval swim distance and average velocity (assessed on day 5) in the mid and high dose groups. Central nervous system expression of the *nrxn2aa* and *nrxn2ab* genes, which encode for synaptic adhesion proteins, was altered (*nrxn2ab* was upregulated at 24 h and downregulated at 48 h; *nrxn2aa* was downregulated at 72 h) (Tu et al. 2017).

References cited in "Manganese"

Adedara IA, Subair TI, Ego VC, Oyediran O, Farombi EO. 2017. Chemoprotective role of quercetin in manganese-induced toxicity along the brain-pituitary-testicular axis in rats. Chem Biol Interact 263:88-98.

Appleton AA, Jackson BP, Karagas M, Marsit CJ. 2017. Prenatal exposure to neurotoxic metals is associated with increased placental glucocorticoid receptor DNA methylation. Epigenetics 12:607-615.

Ashley-Martin J, Dodds L, Arbuckle TE, Ettinger AS, Shapiro GD, Fisher M, et al. 2018. Maternal and cord blood manganese (Mn) levels and birth weight: The MIREC birth cohort study. Int J Hyg Environ Health 221:876-882.

ATSDR (Agency for Toxic Substances and Disease Registry). 2012. Toxicological Profile for Manganese. Atlanta (GA). Available: https://www.atsdr.cdc.gov/toxprofiles/tp151.pdf [accessed 22 September 2020].

Bauer JA, Claus Henn B, Austin C, Zoni S, Fedrighi C, Cagna G, et al. 2017. Manganese in teeth and neurobehavior: Sex-specific windows of susceptibility. Environ Int 108:299-308.

Betharia S, Maher TJ. 2012. Neurobehavioral effects of lead and manganese individually and in combination in developmentally exposed rats. Neurotoxicology 33:1117-1127.

Cassidy-Bushrow AE, Wu KH, Sitarik AR, Park SK, Bielak LF, Austin C, et al. 2019. *In utero* metal exposures measured in deciduous teeth and birth outcomes in a racially-diverse urban cohort. Environ Res 171:444-451.

Chung SE, Cheong HK, Ha EH, Kim BN, Ha M, Kim Y, et al. 2015. Maternal blood manganese and early neurodevelopment: The Mothers and Children's Environmental Health (MOCEH) study. Environ Health Perspect 123:717-722.

Claus Henn B, Bellinger DC, Hopkins MR, Coull BA, Ettinger AS, Jim R, et al. 2017. Maternal and cord blood manganese concentrations and early childhood neurodevelopment among residents near a mining-impacted superfund site. Environ Health Perspect 125:067020.

dos Santos NR, Rodrigues JLG, Bandeira MJ, Anjos A, Araújo CFS, Adan LFF, et al. 2019. Manganese exposure and association with hormone imbalance in children living near a ferro-manganese alloy plant. Environ Res 172:166-174.

Eguchi A, Yanase K, Yamamoto M, Sakurai K, Watanabe M, Todaka E, et al. 2019. The relationship of maternal PCB, toxic, and essential trace element exposure levels with birth weight and head circumference in Chiba, Japan. Environ Sci Pollut Res Int 26:15677-15684.

Freire C, Amaya E, Gil F, Fernández MF, Murcia M, Llop S, et al. 2018. Prenatal co-exposure to neurotoxic metals and neurodevelopment in preschool children: The Environment and Childhood (INMA) project. Sci Total Environ 621:340-351.

Gomes Silva AP, da Silva Araujo Santiago M, Maranho LA, de Oliveira RP, Constantino DHJ, Pereira CDS, et al. 2018. Could male reproductive system be the main target of subchronic exposure to manganese in adult animals? Toxicology 409:1-12.

Guo J, Lv N, Tang J, Zhang X, Peng L, Du X, et al. 2018. Associations of blood metal exposure with thyroid hormones in Chinese pregnant women: A cross-sectional study. Environ Int 121:1185-1192.

Hao Y, Yan L, Pang Y, Yan H, Zhang L, Liu J, et al. 2020. Maternal serum level of manganese, single nucleotide polymorphisms, and risk of spontaneous preterm birth: A nested case-control study in China. Environ Pollut 262:114187.

Hu J, Wu C, Zheng T, Zhang B, Xia W, Peng Y, et al. 2018. Critical windows for associations between manganese exposure during pregnancy and size at birth: A longitudinal cohort study in Wuhan, China. Environ Health Perspect 126:127006.

Kim SI, Jang YS, Han SH, Choi MJ, Go EH, Cheon YP, et al. 2012. Effect of manganese exposure on the reproductive organs in immature female rats. Dev Reprod 16:295-300.

Lee JJ, Valeri L, Kapur K, Ibne Hasan MOS, Quamruzzaman Q, Wright RO, et al. 2018. Growth parameters at birth mediate the relationship between prenatal manganese

exposure and cognitive test scores among a cohort of 2- to 3-year-old Bangladeshi children. Int J Epidemiol 47:1169-1179.

Li C, Xia W, Jiang Y, Liu W, Zhang B, Xu S, et al. 2020. Low level prenatal exposure to a mixture of Sr, Se and Mn and neurocognitive development of 2-year-old children. Sci Total Environ 735:139403.

Lin CC, Chen YC, Su FC, Lin CM, Liao HF, Hwang YH, et al. 2013. *In utero* exposure to environmental lead and manganese and neurodevelopment at 2 years of age. Environ Res 123:52-57.

Liu J, Jin L, Zhang L, Li Z, Wang L, Ye R, et al. 2013. Placental concentrations of manganese and the risk of fetal neural tube defects. J Trace Elem Med Biol 27:322-325.

Liu P, Yuan G, Zhou Q, Liu Y, He X, Zhang H, et al. 2020. The association between metal exposure and semen quality in Chinese males: The mediating effect of androgens. Environ Pollut 264:113975.

Mohammed AT, Ebraheim LLM, Metwally MMM. 2018. Ebselen can protect male reproductive organs and male fertility from manganese toxicity: Structural and bioanalytical approach in a rat model. Biomed Pharmacother 102:739-748.

Mora AM, Arora M, Harley KG, Kogut K, Parra K, Hernández-Bonilla D, et al. 2015a. Prenatal and postnatal manganese teeth levels and neurodevelopment at 7, 9, and 10.5 years in the CHAMACOS cohort. Environ Int 84:39-54.

Mora AM, van Wendel de Joode B, Mergler D, Córdoba L, Cano C, Quesada R, et al. 2015b. Maternal blood and hair manganese concentrations, fetal growth, and length of gestation in the ISA cohort in Costa Rica. Environ Res 136:47-56.

Muñoz-Rocha TV, Tamayo YOM, Romero M, Pantic I, Schnaas L, Bellinger D, et al. 2018. Prenatal co-exposure to manganese and depression and 24-months neurodevelopment. Neurotoxicology 64:134-141.

Okada MA, Neto FF, Noso CH, Voigt CL, Campos SX, Alberto de Oliveira Ribeiro C. 2016. Brain effects of manganese exposure in mice pups during prenatal and breastfeeding periods. Neurochem Int 97:109-116.

Owumi SE, Danso OF, Nwozo SO. 2020. Gallic acid and omega-3 fatty acids mitigate epididymal and testicular toxicity in manganese-treated rats. Andrologia 52:e13630.

Özel Ş, Ozyer S, Aykut O, Çinar M, Yılmaz OH, Caglar A, et al. 2019. Maternal second trimester blood levels of selected heavy metals in pregnancies complicated with neural tube defects. J Matern Fetal Neonatal Med 32:2547-2553.

Pine M, Lee B, Dearth R, Hiney JK, Dees WL. 2005. Manganese acts centrally to stimulate luteinizing hormone secretion: A potential influence on female pubertal development. Toxicol Sci 85:880-885.

Rahman SM, Kippler M, Ahmed S, Palm B, El Arifeen S, Vahter M. 2015. Manganese exposure through drinking water during pregnancy and size at birth: A prospective cohort study. Reprod Toxicol 53:68-74.

Sánchez DJ, Domingo JL, Llobet JM, Keen CL. 1993. Maternal and developmental toxicity of manganese in the mouse. Toxicol Lett 69:45-52.

Souza TL, Batschauer AR, Brito PM, Oliveira Ribeiro CA, Martino-Andrade AJ, Ortolani-Machado CF. 2019. Multigenerational analysis of the functional status of male reproductive system in mice after exposure to realistic doses of manganese. Food Chem Toxicol 133:110763.

Souza TL, Batschauer AR, Brito PM, Leão-Buchir J, Spercoski KM, Neto FF, et al. 2020. Evaluation of Mn exposure in the male reproductive system and its relationship with reproductive dysfunction in mice. Toxicology 441:152504.

Srivastava VK, Hiney JK, Dees WL. 2013. Early life manganese exposure upregulates tumor-associated genes in the hypothalamus of female rats: Relationship to manganese-induced precocious puberty. Toxicol Sci 136:373-381.

Treinen KA, Gray TJ, Blazak WF. 1995. Developmental toxicity of mangafodipir trisodium and manganese chloride in Sprague-Dawley rats. Teratology 52:109-115.

Tsai MS, Liao KW, Chang CH, Chien LC, Mao IF, Tsai YA, et al. 2015. The critical fetal stage for maternal manganese exposure. Environ Res 137:215-221.

Tu H, Fan C, Chen X, Liu J, Wang B, Huang Z, et al. 2017. Effects of cadmium, manganese, and lead on locomotor activity and neurexin 2a expression in zebrafish. Environ Toxicol Chem 36:2147-2154.

Valeri L, Mazumdar MM, Bobb JF, Claus Henn B, Rodrigues E, Sharif OIA, et al. 2017. The joint effect of prenatal exposure to metal mixtures on neurodevelopmental outcomes at 20-40 months of age: Evidence from rural Bangladesh. Environ Health Perspect 125:067015.

Vigeh M, Nishioka E, Yokoyama K, Ohtani K, Matsukawa T. 2016. Increased prenatal blood manganese may induce gestational blood pressure. Hypertens Pregnancy 35:583-592.

Wang YX, Sun Y, Huang Z, Wang P, Feng W, Li J, et al. 2016. Associations of urinary metal levels with serum hormones, spermatozoa apoptosis and sperm DNA damage in a Chinese population. Environ Int 94:177-188.

Wirth JJ, Rossano MG, Daly DC, Paneth N, Puscheck E, Potter RC, et al. 2007. Ambient manganese exposure is negatively associated with human sperm motility and concentration. Epidemiology 18:270-273.

Wu F, Yang H, Liu Y, Yang X, Xu B, Liu W, et al. 2020. Manganese exposure caused reproductive toxicity of male mice involving activation of gnrh secretion in the hypothalamus by prostaglandin E2 receptors Ep1 and Ep2. Ecotoxicol Environ Saf 201:110712.

Xia W, Zhou Y, Zheng T, Zhang B, Bassig BA, Li Y, et al. 2016. Maternal urinary manganese and risk of low birth weight: A case-control study. BMC Public Health 16:142.

Yamamoto M, Sakurai K, Eguchi A, Yamazaki S, Nakayama SF, Isobe T, et al. 2019. Association between blood manganese level during pregnancy and birth size: The Japan environment and children's study (JECS). Environ Res 172:117-126.

Yan L, Wang B, Li Z, Liu Y, Huo W, Wang J, et al. 2017. Association of essential trace metals in maternal hair with the risk of neural tube defects in offspring. Birth Defects Res 109:234-243.

Yang H, Wang J, Yang X, Wu F, Qi Z, Xu B, et al. 2019. Occupational manganese exposure, reproductive hormones, and semen quality in male workers: A cross-sectional study. Toxicol Ind Health 35:53-62.

Yang X, Tan J, Xu X, Yang H, Wu F, Xu B, et al. 2020. Prepubertal overexposure to manganese induce precocious puberty through GABA(A) receptor/nitric oxide pathway in immature female rats. Ecotoxicol Environ Saf 188:109898.

Yao Q, Zhou G, Xu M, Dai J, Qian Z, Cai Z, et al. 2019. Blood metal levels and serum testosterone concentrations in male and female children and adolescents: NHANES 2011-2012. PLoS One 14:e0224892.

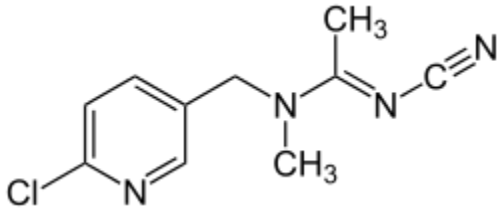
Yin S, Wang C, Wei J, Wang D, Jin L, Liu J, et al. 2020. Essential trace elements in placental tissue and risk for fetal neural tube defects. Environ Int 139:105688.

Yu XD, Zhang J, Yan CH, Shen XM. 2014. Prenatal exposure to manganese at environment relevant level and neonatal neurobehavioral development. Environ Res 133:232-238.

Neonicotinoid pesticides:

Acetamiprid

(CAS No. 135410-20-7)



Acetamiprid is a systemic neonicotinoid insecticide used to control sucking insects like aphids and the cherry fruit fly (US EPA 2020). According to the California Department of Pesticide Regulation (DPR), approximately 61,000 pounds of acetamiprid were used for nut crops (walnuts, almonds, pistachios, pecans) and cotton in California in 2017 (CDPR 2017).

Acetamiprid passed the animal data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of recent relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

One human study reporting developmental and reproductive toxicity (DART)-related effects of acetamiprid was identified.

- N-Desmethylacetamiprid (DMAP), a metabolite of acetamiprid, was measured in urine samples collected from very low birth weight infants at birth (n = 57, detection frequency 24.6%, median level 0.048 ppb) or on postnatal day (PND) 14 (n = 59, detection frequency 11.9%, median level 0.09 ppb). DMAP was detected at higher rates and levels in small for gestational-age infants compared to appropriate gestational-age infants (cross-sectional study) (Ichikawa et al. 2019).

Animal studies

Findings reported in whole animal studies examining possible DART effects of exposure to acetamiprid are summarized here.

Parental and developmental effects

- Rat: In a developmental toxicity study with gestational exposure, conducted according to FIFRA (Federal Insecticide Fungicide & Rodenticide Act) guidelines, an increase in a skeletal variation (shortening of the 13th rib) was observed at the high dose of 50 mg/kg-day (CDPR 2000) (US EPA 2013).
- Rat: In a standard reproduction study conducted according to FIFRA guidelines with exposure during premating, gestation and lactation, reduced food consumption and body weights were observed in parental animals of both sexes of both generations at the high dose (800 ppm). Decrements in pup weights (F1 and F2 pups) and reduced pup survival throughout the lactation period (in F2 pups) occurred at the high dose, as well as modest decrements in absolute brain weights in F1 adult males and females, reductions in litter size, viability, and weaning indices among F2 offspring, and significant delays in the age to attain vaginal opening and preputial separation in F2 offspring (CDPR 2000) (US EPA 2013).
- Rat: In a developmental neurotoxicity study, maternal effects observed at the high dose of 45 mg/kg-day consisted of decreased body weight and body weight gains, while offspring effects at the high dose included decreased body weights, body weight gains, and survival on postnatal days (PNDs) 0-1, with 3 dams experiencing total litter loss on PND 1 (12% of litters). No effects on developmental landmarks were noted (US EPA 2008) (US EPA 2013).
- Rabbit: In a developmental toxicity study conducted according to FIFRA guidelines, thoracic vertebral arches and fused ribs were observed in two fetuses (one from each litter) at the high dose of 30 mg/kg-day (CDPR 2000).

Neurodevelopmental effects

- Rat: In a developmental neurotoxicity study with maternal exposure from gestation day (GD) 6 to lactation day 21, a decrease in maximum auditory startle response was observed on PND 20 and PND 60 in high dose (45 mg/kg-day) F1 males, as well as a slight increase in the number of errors in the Biel maze just after weaning. There were no treatment-related macroscopic or microscopic findings noted for brain spinal cord or peripheral nerves or brain morphometry measurements in F1 animals, nor were any effects on motor activity noted at any age or dose level (US EPA 2008) (Sheets et al. 2016).
- Mouse: Exposure to acetamiprid from PND 12 to PND 26 by oral gavage impaired neurogenesis and altered microglial profiles (an increase in the number of amoeboid-type and activated M1-type microglia) in the developing hippocampal dendate gyrus (Nakayama et al. 2019).

- Mouse: Exposure on GD 6-13 induced neurodevelopmental toxicity and increased microglial activation (increased Iba1-immunoreactive and amoeboid-type microglia, increased M1/M2 microglial ratio) in the developing brain with hypoplasia of the cortical plate and decreased neurogenesis on GD 14 in the dorsal telencephalon and neocortex, and abnormal neuronal distribution in the neocortex (Kagawa and Nagao 2018).
- Mouse: Maternal exposure from GD 6 to lactation day 21 at 0, 1 or 10 mg/kg-day altered anxiety-related and socio-sexual behaviors in male offspring, with a reduction of anxiety level in the light-dark transition test at both doses and an increase in sexual and aggressive behaviors at the low dose. No behavioral effects were observed in female offspring, and no effects were observed in either sex on behavioral flexibility, numbers of vasopressin-immunoreactive cells in the paraventricular nucleus of the hypothalamus, or serum testosterone levels (Sano et al. 2016).

Male reproductive system effects

- Rat: Exposure of males for 90 days decreased sperm concentration in a dose dependent manner, non-significantly decreased plasma testosterone, increased plasma gonadotropin-releasing hormone, follicle-stimulating hormone, and luteinizing hormone at the low and mid doses, and in the testis increased lipid peroxidation and apoptosis at the mid and high doses and decreased glutathione and cell proliferation at all doses (Arıcan et al. 2020).
- Rat: Exposure of males 5 days/week for 9 weeks decreased body weight gain, absolute weights of the testes, epididymis, and seminal vesicles, number of spermatids, sperm count, sperm motility, and sperm viability, increased abnormal sperm, decreased plasma testosterone, and increased the plasma level of thiobarbituric acid-reactive substances (an indicator of lipid peroxidation) (Mosbah et al. 2018).
- Rat: Exposure of males for 35 days caused oxidative stress and mitochondrial damage in Leydig cells and inhibited the synthesis of testicular ATP and cAMP. Subsequent testosterone biosynthesis was disrupted by a decrease in the rate of conversion of cholesterol to testosterone and by preventing cholesterol from entering the mitochondria within the Leydig cells (Kong et al. 2017).
- Mouse: Exposure of 3-week-old males via drinking water to an acetamiprid-containing insecticide formulation (two levels) for 180 days decreased body weight, affected testicular histopathology (abnormal seminiferous tubules), and decreased expression of testosterone metabolism-associated genes (*Lhr*, *Star*, *Cyp11a1*, *Cyp17a1* and *Hsd17b1*), cell proliferation-associated marker genes

(*Ki67* and *Top2a*), and the neonicotinoid receptor *nAChRα7* gene (Terayama et al. 2018).

- Mouse: Exposure of adult males via intraperitoneal injection once, or twice (on successive days) had no effect on sperm morphology (Rasgele 2014).
- Guinea pig: Exposure of adult males to an acetamiprid-containing insecticide formulation for 90 days affected a measure of sexual behavior (increased time-to-mounting of females), decreased serum testosterone, decreased relative testes and accessory gland weights, decreased sperm count, motility, and the number of spermatozoa with intact plasma membranes, increased sperm head and tail abnormalities, affected testicular histopathology (decreased immature germinal cells in lumen, alterations in basal membrane of seminiferous tubules), increased testis catalase and superoxide dismutase activities and lipid peroxidation, and decreased testis glutathione levels (Guiekep et al. 2019).

Mechanistic, in vitro, and other relevant data

- Zebrafish: Exposure of embryos from 6 to 120 hours post-fertilization affected development, with impaired spontaneous movement identified as the most sensitive end point. Mortality and teratogenic effects were increased at concentrations greater than 263 mg/L, with bent spine being the main malformation (Ma et al. 2019)
- Pig: *In vitro* exposure of oocytes for 44 hours decreased the nuclear maturation rate, and increased the occurrence of dispersed, irregular chromosomes in matured (metaphase II stage) oocytes (Ishikawa et al. 2015).
- Mouse: Exposure of 2-cell stage embryos for 72 h decreased the number of embryos reaching the blastocyst stage (at concentrations of 100 µM) and the average number of cells per blastocyst (at concentrations of 10 µM and above) (Babeľová et al. 2017).
- Rat: Exposure for 14 days of neuron-enriched cultures from neonatal cerebellum resulted in a slight disturbance in Purkinje cell dendritic arborization, with no effects on neuron or glial cell morphology. Transcriptome microarray analysis identified differential expression in exposed versus control cultures in 48 genes, including nine genes essential for neurodevelopment, which were similarly altered by nicotine (Kimura-Kuroda et al. 2016).
- Mouse: Exposure of spermatozoa to 5 mM for 30 minutes had no effect on sperm motility or DNA fragmentation; however, use of exposed sperm prior to the *in vitro* fertilization process decreased numbers of 2-cell embryos and blastocysts. Exposure of a mixture of spermatozoa and oocytes (i.e., during the *in vitro* fertilization process) to 500 µM affected fertilization and embryonic development *in vitro*, decreasing the number of morulae and blastocysts; similar results were

observed following exposure of naturally fertilized zygotes to 500 µM, while similar exposure of 2-cell embryos was without effect (Gu et al. 2013).

References cited in "Acetamiprid"

Arıcan EY, Gökçeoğlu Kayalı D, Ulus Karaca B, Boran T, Öztürk N, Okyar A, et al. 2020. Reproductive effects of subchronic exposure to acetamiprid in male rats. Sci Rep 10:8985.

Babeľová J, Šefčíková Z, Čikoš Š, Špirková A, Kovaříková V, Koppel J, et al. 2017. Exposure to neonicotinoid insecticides induces embryotoxicity in mice and rabbits. Toxicology 392:71-80.

CDPR (California Department of Pesticide Regulation). 2000. Acetamiprid. Summary of Toxicology Data.

CDPR (California Pesticide Information Portal (CALPIP) Application). 2017. Pesticide Use Reporting (PUR) Data Update. Version 2019.04. Available at: Https://calpip.Cdpr.Ca.Gov/main.Cfm.

Gu YH, Li Y, Huang XF, Zheng JF, Yang J, Diao H, et al. 2013. Reproductive effects of two neonicotinoid insecticides on mouse sperm function and early embryonic development *in vitro*. PLoS One 8:e70112.

Guiekep AJN, Kenfack A, Ngoula F, Vemo BN, Nguemmeugne KS, Tedonkeng EP. 2019. Attenuating effects of mangifera indica leaves ethanolic extract against acetamiprid induced reproductive toxicity in male guinea pigs. Vet Res Forum 10:187-192.

Ichikawa G, Kuribayashi R, Ikenaka Y, Ichise T, Nakayama SMM, Ishizuka M, et al. 2019. LC-ESI/MS/MS analysis of neonicotinoids in urine of very low birth weight infants at birth. PLoS One 14:e0219208.

Ishikawa S, Hiraga K, Hiradate Y, Tanemura K. 2015. The effects analysis of two neonicotinoid insecticides on *in vitro* maturation of porcine oocytes using hanging drop monoculture method. J Vet Med Sci 77:725-728.

Kagawa N, Nagao T. 2018. Neurodevelopmental toxicity in the mouse neocortex following prenatal exposure to acetamiprid. J Appl Toxicol 38:1521-1528.

Kimura-Kuroda J, Nishito Y, Yanagisawa H, Kuroda Y, Komuta Y, Kawano H, et al. 2016. Neonicotinoid insecticides alter the gene expression profile of neuron-enriched cultures from neonatal rat cerebellum. Int J Environ Res Public Health 13.

Kong D, Zhang J, Hou X, Zhang S, Tan J, Chen Y, et al. 2017. Acetamiprid inhibits testosterone synthesis by affecting the mitochondrial function and cytoplasmic adenosine triphosphate production in rat leydig cells. Biol Reprod 96:254-265.

Ma X, Li H, Xiong J, Mehler WT, You J. 2019. Developmental toxicity of a neonicotinoid insecticide, acetamiprid to zebrafish embryos. J Agric Food Chem 67:2429-2436.

Mosbah R, Djerrou Z, Mantovani A. 2018. Protective effect of nigella sativa oil against acetamiprid induced reproductive toxicity in male rats. Drug Chem Toxicol 41:206-212.

Nakayama A, Yoshida M, Kagawa N, Nagao T. 2019. The neonicotinoids acetamiprid and imidacloprid impair neurogenesis and alter the microglial profile in the hippocampal dentate gyrus of mouse neonates. J Appl Toxicol 39:877-887.

Rasgele PG. 2014. Abnormal sperm morphology in mouse germ cells after short-term exposures to acetamiprid, propineb, and their mixture. Arh Hig Rada Toksikol 65:47-56.

Sano K, Isobe T, Yang J, Win-Shwe TT, Yoshikane M, Nakayama SF, et al. 2016. *In utero* and lactational exposure to acetamiprid induces abnormalities in socio-sexual and anxiety-related behaviors of male mice. Front Neurosci 10:228.

Sheets LP, Li AA, Minnema DJ, Collier RH, Creek MR, Peffer RC. 2016. A critical review of neonicotinoid insecticides for developmental neurotoxicity. Crit Rev Toxicol 46:153-190.

Terayama H, Qu N, Endo H, Ito M, Tsukamoto H, Umemoto K, et al. 2018. Effect of acetamiprid on the immature murine testes. Int J Environ Health Res 28:683-696.

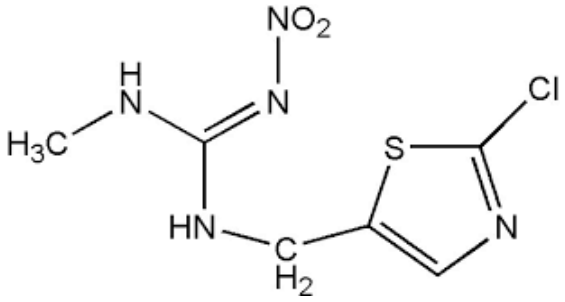
US EPA (US Environmental Protection Agency). 2008. Acetamiprid. Developmental Neurotoxicity Study, Data Evaluation Record (DER). MRID 46255619.

US EPA (US Environmental Protection Agency). 2013. Acetamiprid. Pesticide Tolerances. Rules and Regulations. Federal Register /Vol. 78, No. 118 /Wednesday, June 19, 2013 pp. 36671-36677.

US EPA (US Environmental Protection Agency). 2020. Acetamiprid. Proposed Interim Registration Review Decision. Docket Number EPA-HQ-OPP-2012-0329 www.regulations.gov.

Clothianidin

(CAS No. 210880-92-5)



Clothianidin is a systemic, neonicotinoid insecticide used to control sucking insects, some chewing insects including termites, and soil insects. Clothianidin is also a major metabolite and degradate of another neonicotinoid insecticide, thiamethoxam (US EPA 2020). Clothianidin is used on a variety of crops, including lettuce, broccoli, and wine grapes, and for structural pest control. According to the California Department of Pesticide Regulation (DPR), 25,949 pounds of clothianidin were used in California in 2017 (CDPR 2017).

Clothianidin passed the animal data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

No human studies reporting developmental and reproductive toxicity (DART)-related effects of clothianidin were identified.

Animal studies

Findings reported in whole animal studies examining possible DART effects of exposure to clothianidin are summarized here.

Maternal and developmental effects

- Rat: In a guideline developmental (teratology) study with exposure on gestation days (GD) 6-19, no adverse effects were observed up to a high dose of 125 mg/kg-day (CDPR 2003).
- Rat: In a two-generation dietary reproduction study conducted according to FIFRA guidelines, body weight decrements were observed in both parental generations at the high dose level of 2500 ppm (10 mg/kg-day M; 213 mg/kg-day F) and in F0 dams in the top two dose levels on lactation day 14. Decreased birth weights were observed in F1 pups, along with marked pup body weight decrements during and after the lactation period, decrements in weanling brain weight in both generations (F1 and F2 weanlings), as well as delays in developmental landmarks of preputial separation and vaginal patency in F1 pups (CDPR 2003).
- Mouse: In two separate studies, maternal exposure during gestation and lactation had no effect on litter size, litter weight, or sex ratio at birth, and increased the average body weight of male and female offspring during the early lactation period in a dose-related manner (Tanaka 2012a),(Tanaka 2012b).
- Rabbit: In a guideline developmental (teratology) study with exposure on GD 6-26, increased maternal mortality, abortions and premature deliveries were observed at the top two dose levels (75 and 100 mg/kg-day), and increased resorptions and decreased mean pup weight were observed at the top dose. Fetal effects included increased absence of the intermediate lung lobe and delays in bone ossification in the top two dose groups (CDPR 2003).

Neurodevelopmental effects

- Rat: In a guideline developmental neurotoxicity study with exposure in the diet from GD 0 through lactation day 22, findings included reduced body weights of dams and pups at the high dose (1,750 ppm), increased mortality of offspring between postnatal day (PND) 25 and 27 (2 males, 3 females) at the high dose, body weight decrements at PND 22 in female offspring, and a slight (transient) decrease in auditory startle response in the 500 ppm group on PND 22-23 (CDPR 2003).
- Rat: 90-day exposure of males, starting at 7 days of age to doses ranging from 2-24 mg/kg-day decreased performance at the high dose in the probe test measuring consolidation of memory, while there was no effect on spatial learning in the Morris water maze test or on the expression of related genes in the hippocampus (N-methyl D-aspartate 1 *[Grin1*], muscarinic receptor M1, synoptophysin [*Syp*] and growth-associated protein 43 [*Gap-43*) (Ozdemir et al. 2014).
- Rat: 30-day exposure of 8- to 9-week-old males to doses ranging from 2-24 mg/kg-day had no effect on spatial learning in the Morris water maze test,

consolidated memory in the probe test, or on the expression of related genes in the hippocampus (*Grin1*, muscarinic receptor M1, *Syp* and *Gap*-43) (Ozdemir et al. 2014).

- Mouse: Maternal exposure during gestation and lactation produced adverse effects in developmental neurobehavioral parameters in offspring. Specifically, in PND 7 female offspring, surface righting was accelerated in a dose-related manner, in 3-week old male offspring, the average speed in an exploratory behavior test was increased in a dose-related manner, and male offspring in the mid-dose group showed more activity in some measured variables for spontaneous behavior (Tanaka 2012a).
- Mouse: Exposure from 5 weeks of age in the F0 generation to 11 weeks of age in the F1 generation produced adverse effects in neurobehavioral parameters in F0 adult males (increased exploratory behavior), and in F1 offspring of both sexes. Specifically, in F1 males on PND 7 swimming head angle was accelerated in a dose-related manner, in F1 females on PND 7 negative geotaxis was accelerated in a dose-related manner, and in tests of exploratory behavior, the rearing of F1 females was increased in a dose-related manner (Tanaka 2012a).

Male reproductive effects

- Mouse: Maternal exposure from gestation day (GD) 1 to postnatal day (PND) 14 decreased testis weights and the number of germ cells per seminiferous tubule in male offspring on PND 14 (Yanai et al. 2017).
- Mouse: Exposure of adult males for four weeks resulted in a dose-dependent increase in vacuolated seminiferous tubule epithelia and decrease in staining for the antioxidant enzyme glutathione peroxidase 4 in spermatids (Hirano et al. 2015).
- Rat: 90-day exposure of 8- to 9-week-old males to doses ranging from 2-24 mg/kg-d (selected to fall below the reported no-observable-adverse-effect level for male rat reproductive system effects), resulted in decreased weights of epididymis, right cauda epididymis and seminal vesicles, and increased lipid peroxidation, cholesterol, and palmitic, linoleic and arachidonic acid levels in the testis. No effects were observed on serum testosterone, sperm parameters (e.g. concentration, motility and morphology), sperm DNA fragmentation, the apoptotic index in the seminiferous tubules, or α-tocopherol or glutathione in the testis (Bal et al. 2013).
- Rat: 90-day exposure of males, starting at 7 days of age, to doses ranging up to 32 mg/kg-d (the reported no-observable-adverse-effect level for male rat reproductive system effects) resulted in decreases in the absolute weights of right cauda epididymis and seminal vesicles and in body weight at the high dose; decreases in epididymal sperm concentration and increases in sperm head and tail abnormalities at the mid (8 mg/kg-d) and high dose, and increased apoptosis of germ cells, seminal DNA fragmentation, and cholesterol in the testes, and

decreased testicular glutathione and serum testosterone at the high dose (Bal et al. 2012).

Female reproductive effects

- Rat: In a chronic two-year (guideline) study with doses up to 3000 ppm in the diet (157 mg/kg-day M; 193 mg/kg-day F), a dose-related increase in the incidence of ovarian interstitial gland hyperplasia was observed (CDPR 2003).

Mechanistic, in vitro, and other relevant data

- Mouse: Exposure of 2-cell stage embryos for 72 h to 100 µM decreased the number of embryos reaching the blastocyst stage and the average number of cells per blastocyst (Babeľová et al. 2017).

References cited in "Clothianidin"

Babeľová J, Šefčíková Z, Čikoš Š, Špirková A, Kovaříková V, Koppel J, et al. 2017. Exposure to neonicotinoid insecticides induces embryotoxicity in mice and rabbits. Toxicology 392:71-80.

Bal R, Türk G, Yılmaz Ö, Etem E, Kuloğlu T, Baydaş G, et al. 2012. Effects of clothianidin exposure on sperm quality, testicular apoptosis and fatty acid composition in developing male rats. Cell Biol Toxicol 28:187-200.

Bal R, Türk G, Tuzcu M, Yılmaz Ö, Kuloğlu T, Baydaş G, et al. 2013. Effects of the neonicotinoid insecticide, clothianidin, on the reproductive organ system in adult male rats. Drug Chem Toxicol 36:421-429.

CDPR (California Department of Pesticide Regulation). 2003. Clothianidin., Summary of Toxicology Data.

CDPR (California Pesticide Information Portal (CALPIP) Application. 2017. Version 2019.04. Pesticide Use Reporting (PUR) Data Update. Available at: Https://calpip.Cdpr.Ca.Gov/main.Cfm..

Hirano T, Yanai S, Omotehara T, Hashimoto R, Umemura Y, Kubota N, et al. 2015. The combined effect of clothianidin and environmental stress on the behavioral and reproductive function in male mice. J Vet Med Sci 77:1207-1215.

Ozdemir HH, Kara M, Yumrutas O, Uckardes F, Eraslan E, Demir CF, et al. 2014. Determination of the effects on learning and memory performance and related gene expressions of clothianidin in rat models. Cogn Neurodyn 8:411-416.

Tanaka T. 2012a. Reproductive and neurobehavioral effects of clothianidin administered to mice in the diet. Birth Defects Res B Dev Reprod Toxicol 95:151-159.

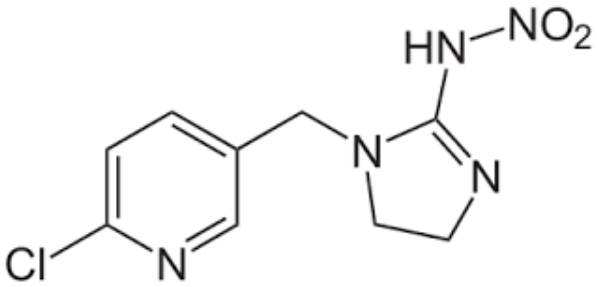
Tanaka T. 2012b. Effects of maternal clothianidin exposure on behavioral development in F_1 generation mice. Toxicol Ind Health 28:697-707.

US EPA (US Environmental Protection Agency). 2020. Clothianidin and Thiamethoxam Proposed Interim Registration Review Decision. Docket Numbers EPA-HQ-OPP-2011-0865 and EPA-HQ-OPP-2011-0581.

Yanai S, Hirano T, Omotehara T, Takada T, Yoneda N, Kubota N, et al. 2017. Prenatal and early postnatal noael-dose clothianidin exposure leads to a reduction of germ cells in juvenile male mice. J Vet Med Sci 79:1196-1203.

Imidacloprid

(CAS No.138261-41-3)



Imidacloprid is a neonicotinoid insecticide used to control sucking insects; some chewing insects including termites; soil insects; and fleas, flies and lice on livestock (topical use) and dogs and cats (oral and topical use)[10]. It is used on a variety of crops, mostly on vegetables (e.g., lettuce, broccoli), fruits (peaches, grapes, oranges, cantaloupe) and cotton. According to the California Department of Pesticide Regulation (DPR), in 2017 approximately 587,000 pounds of imidacloprid were applied to over 1.6 million acres in California (CDPR 2017).

Imidacloprid passed the animal data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Human epidemiologic studies reporting developmental and reproductive toxicity (DART)-related effects associated with imidacloprid were identified in the recent literature and are summarized here.

Birth defects

- In a hypothesis generating study of California infants or fetuses with congenital heart defects from the California Birth Defects Monitoring Program with exposure estimated categorically (none/any) from state pesticide use records between 1997–2006 and proximity of residence during pregnancy to use, an increase in adjusted odds ratio was observed for a group of four congenital heart defects, i.e., Tetralogy of Fallot (case-control study) (Carmichael et al. 2014).

[10] https://parasitipedia.net/index.php?option=com_content&view=article&id=2467&Itemid=2735

- In a hypothesis generating study of California infants or fetuses with birth defects from the California Birth Defects Monitoring Program with exposure estimated categorically (none/any) from state pesticide use records between 1997–2006 and proximity of residence during pregnancy to use, an increase in adjusted odds ratio was observed for anencephaly (marginally significant) (case control study) (Yang et al. 2014).
- In a hypothesis generating study of California infants or fetuses from the California Birth Defects Monitoring Program with exposure estimated categorically (none/any) from state pesticide use records between 1997–2006 and proximity of residence during pregnancy to use, no association was found for a birth defect (gastroschisis); when stratified by maternal age an increase in odds ratio was observed for women 20-24 years old (case control study) (Shaw et al. 2014).

Neurodevelopmental effects

- In a study of autism spectrum disorder, exposure was estimated categorically (never/ever; and prenatal never/ever/consistent/occasional) based on maternal-reported household usage of flea or tick control on pets (*in utero* through childhood). A nonsignificant increase in adjusted odds ratio was observed for exposure during pregnancy (compared to early life exposure, though results were considered imprecise) and an increase in adjusted odds ratio was observed for consistent/frequent users (marginally significant) (case control study) (Keil et al. 2014).

Animal studies

Findings reported in whole animal studies examining possible DART effects of exposure to imidacloprid are summarized here.

Maternal and developmental effects

- Rabbit: Increased resorptions, lower fetal body weight at the high dose in a study with gestational exposure (gestation day [GD] 6-18) conducted according to FIFRA (Federal Insecticide Fungicide & Rodenticide Act) guidelines (CDPR 2013).
- Rat: Decreased body weight gain and reduced food consumption of dams, and a high percentage of male fetuses and increased incidence of wavy ribs in the fetuses of the high dose (94.1 mg/kg/day) group in a study with gestational exposure (GD 6-15) conducted according to FIFRA guidelines (CDPR 2013) (Sheets et al. 2016).

- Rat: Decreased body weight and weight gain of dams and pups on postnatal day (PND) 21 in a developmental neurotoxicity (DNT) study conducted according to FIFRA guidelines with exposure from GD 0-PND 21 (CDPR 2013).
- Rat: Gestational exposure induced hyperglycemia, insulin resistance and dyslipidemia in Wistar rat dams and their offspring and the effects on offspring persisted until adult age (Ndonwi et al. 2020).
- Rat: Age-dependent adverse effects on the developing immune system after exposure during GD 6–PND 21, which was aggravated when exposure continued through PND 42, leading to a compromised immune system (Gawade et al. 2013).
- Rat: Adult females (parent generation 1 (P1) and P2) were exposed in diet (0, 100, 250, 700 ppm) before mating, through mating, gestation and lactation for 84 and 105 days, respectively. Exposure decreased food consumption and body weight gain in P1 at 700 ppm, and decreased body weight gain in P1-offspring until weaning, with decreased premating body weights in male P1-offspring (Suter et al. 1990, as cited in (CDPR 2013) (Mikolić and Karačonji 2018).
- Rat: Exposure of adult F0 females for 10 weeks or more and F1 parents for 8 weeks or more via the oral route at 20 mg/kg-day resulted in decreased ovarian weights and food consumption in F0 and F1 females, increased F1 birth weights, decreased F1 and F2 body weights at PND 21, and increased serum alanine aminotransferase (ALT) in F1 and F2 females (Vohra and Khera 2016).
- Mouse: Exposure over the early developmental period (GD 4-PND 21) reduced the number of offspring that were born and survived for more than one week (fecundity), decreased body weight in young adult male offspring, and decreased triglycerides levels in young adult offspring (Burke et al. 2018).

Neurodevelopmental effects

- Rat: Prenatal exposure (GD 0-PND 21) DNT study conducted according to FIFRA guidelines resulted in reduced motor activity levels and changes in dimensions of brain structures (reduction in the thickness of corpus callosum and decreased width of caudate putamen) on PND 11, as well as decreased body weight on PND 21 in F1 animals in the high dose group (750 ppm). Information on brain morphology in lower dose groups was not available (CDPR 2013) (Sheets et al. 2016).
- Rat: Newborn males were dosed daily via gavage from birth for 3 months to examine the effects of exposure on learning and memory during this critical period of neurodevelopment, and assessed using the Morris water maze and probe trial tests. Imidacloprid increased escape latency in the Morris water maze on test days 3-5, and decreased time spent swimming in the target quadrant in the probe trial test. No significant treatment-related differences in expression of genes synthesizing proteins known to be associated with learning in brain tissues

such as *Grin1*, *Syp Gap*43 or M1 were observed in the hippocampus (Kara et al. 2015).

- Rat: Gestational exposure (GD 9) to a single large, nonlethal dose of imidacloprid produced significant neurobehavioral deficits on PND 30, which corresponds to early adolescence in humans. Neurobehavioral deficits included sensorimotor impairments that were associated with increased acetylcholineesterase (AChE) activity in plasma and in the midbrain, cortex and brainstem. Exposure was also associated with increased ligand-biding densities for [^{3}H]AFDX 384, a ligand for m2mAChR, but not [^{3}H]cytosine, a ligand for alpha4beta2 type nAchR, in the cortex. Histopathological evaluation found no alterations in surviving neurons in various brain regions, while increased expression of glial fibrillary acidic protein (GFAP) was observed in glia in the motor cortex layer III, CA1, CA3, and the dentate gyrus (DG) subfield of the hippocampus of the offspring on PND 30 (Abou-Donia MB 2008).
- Mouse: Exposure from PND 12–26 reduced neurogenesis in the hippocampal DG and increased the number of amoeboid-type microglia and activated M1-type microglia in the DG (Nakayama et al. 2019).
- Mouse: Exposure over the early developmental period (GD 4-PND 21) induced long-lasting changes in behavior, including increased motor activity (assessed between PND 43-47), enhanced social dominance in the tube test (assessed between PND 54-64), reduced depressive-like behavior in the forced swim test (assessed between PND 56-64), and reduced social aggression in the intruder test (assessed between PND 66-72) (Burke et al. 2018).
- Mouse: Exposure of male pups via lactation on PND 1-28 significantly decreased the total thickness of pyramidal cell layers in the hippocampus, along with shrinkage and degeneration of pyramidal neurons in the CA1 and CA3 hippocampal regions on PNDs 29 and 63 (Bhaskar et al. 2017).

Endocrine effects

- Rat: Exposure to adult males decreased serum testosterone and luteinizing hormone (Tetsatsi et al. 2019).
- Mouse: Exposure of male pups via lactation on PND 1-28 decreased serum testosterone levels on PND 29, but not PND 63, and increased serum corticosterone levels at PND 29 and 63 (Bhaskar et al. 2017).
- Mouse: Exposure of adult males for 10 weeks decreased serum testosterone, testicular androgen receptor levels and aromatase activity, and inhibited expression of genes involved in the production of testosterone (Yuan et al. 2020).

Female reproductive effects

- Rat: Exposure of mature females to a pesticide formulation containing imidacloprid for 60 days caused ovarian damage, including decreased ovary

weight, interference with ovarian follicle development (variations in diameter of follicles at different stages of folliculogenesis), decreased number of follicles, increased follicular atresia, increased markers of lipid peroxidation and protein oxidation and decreased levels of reduced glutathione, vitamin E, catalase, superoxide dismutase, and glutathione peroxidase in the ovary, as well as decreases in serum estradiol levels (Mzid et al. 2018).

- Rat: Exposure of females for 90 days resulted in decreased ovarian weight and significant patho-morphological changes in follicles, including changes in antral and atretic follicles, increased markers of lipid peroxidation and decreased levels of reduced glutathione, catalase, superoxide dismutase, and glutathione peroxidase in the ovary, as well as decreased serum levels of luteinizing hormone and progesterone and increased levels of follicle stimulating hormone (Kapoor et al. 2011).
- Rat: Exposure of pregnant females from GD 7-21 resulted in decreased serum estradiol and progesterone levels, reduced ovarian weights and diameters, and reduced numbers and diameters of follicles and corpus luteum in 55-day-old female offspring. Female offspring also had lower rates of successful mating, lower numbers of fetuses, and decreased ovarian expression of the *Dax*1 gene (Nabiuni et al. 2015, as reported in (Mikolić and Karačonji 2018).
- Rat: Exposure of adult F0 females for 10 weeks or more and F1 females for 8 weeks or more decreased ovarian weight (reported above under Maternal and Developmental effects), and increased the number of atretic follicles (Vohra and Khera 2016).

Male reproductive effects

- Rat: Effects of imidacloprid on reproduction included testicular degeneration in the 2 year chronic guideline study (CDPR 2006).
- Rat: Exposure to adult males decreased sex organ weights, spermatozoa count, motility and viability, altered sperm morphology (increased head and tail abnormalities) and testis histology, increased testicular levels of lipid peroxidation, superoxide dismutase and catalase, and, as noted under Endocrine Effects, decreased serum testosterone and luteinizing hormone (Tetsatsi et al. 2019).
- Rat: Exposure of adult males for 28 days increased testicular markers of lipid peroxidation and protein oxidation, reduced testicular activity of several antioxidant enzymes (e.g., superoxide dismutase, catalase, glutathione peroxidase), and caused progressive congestion in blood vessels and mild edema of the interstitial spaces of the testes (Mahajan et al. 2018).
- Rat: Exposure of adult males for 28 days decreased total epididymal sperm count, sperm motility, and live sperm count, increased head and tail sperm abnormalities, and increased histopathologic alterations in the testis and epididymis (e.g., reduced spermatogenesis, increased interstitial edema).

Exposure also increased lipid peroxidation in the testis, and decreased antioxidant enzyme activities (e.g., superoxide dismutase, catalase, glutathione peroxidase) and 3β-hydroxysteroid dehydrogenase (HSD) and 17β-HSD enzymatic activities, and increased gamma-glutamyl transpeptidase, lactate dehydrogenase-x, and sorbitol dehydrogenase activities in the testis; testosterone levels were also decreased in testis and plasma (Lonare et al. 2016).

- Rat: 90-day exposure of males, starting at 7 days of age, resulted in suppression of testicular function with decreased testosterone levels, epididymis weight and epididymal sperm concentrations; increased apoptosis of germ cells, seminal DNA fragmentation, lipid peroxidation, and abnormal sperm; depletion of reduced glutathione; and increased levels of certain fatty acids (steric, oleic, linoleic, and arachidonic acids) (Bal et al. 2012a; Bal et al. 2012b).
- Rat: 90-day exposure of 8- to 9-week-old males to doses ranging up to 8 mg/kg-d (the reported no-observable-adverse-effect level for reproductive system effects) resulted in decreased serum testosterone, decreased absolute and relative weights of the epididymis, right cauda epididymis, and seminal vesicles, decreased sperm motility and epididymal sperm concentrations, increased apoptosis of germ cells, seminal DNA fragmentation, and abnormal sperm, depletion of reduced glutathione, and increased levels of certain fatty acids (steric, oleic, linoleic, and arachidonic acids) (Bal et al. 2012a; Bal et al. 2012b).
- Rat: Exposure of adult males for 60 days decreased sperm quality (e.g., decreased sperm viability and motile sperm velocity), produced a number of adverse histological changes in the testis (e.g., atrophied seminiferous tubules, arrested spermatogenesis), and decreased serum testosterone levels (Najafi et al. 2010).
- Rat: Exposure of adult males for 15 days resulted in decreases in luteinizing hormone, follicle stimulating hormone, testosterone, estradiol and prolactin in testis, decreases in sperm counts, motility and vitality, and increases in sperm abnormalities (Hafez et al. 2016).
- Rabbit: Exposure of male rabbits to 1/10th the LD50 for 10-20 consecutive days reduced the number of Leydig cells and widened the interstitial space in the testis (Memon et al. 2014).
- Mouse: Exposure for 14 or 28 days increased sperm head abnormalities, and 28-day exposure resulted in dominant lethal mutations at the spermatogonial stage (Bagri et al. 2015).
- Dog: In a chronic guideline study, testicular degeneration was observed (CDPR 2006).

Mechanistic, in vitro, and other relevant data

- Human: In an *in vitro* co-culture model of fetoplacental steroidogenesis, consisting of H295R human adrenocortical carcinoma cells with fetal characteristics and BeWo human choriocarcinoma cells with villous cytotrophoblast characteristics, imidacloprid induced CYP19 (aromatase) and CYP3A7 activities, and estradiol and estrone production, and decreased estriol production (Caron-Beaudoin et al. 2017).
- Rat: Exposure for 14 days of neuron-enriched cultures from neonatal cerebellum resulted in a slight disturbance in Purkinje cell dendritic arborization, with no effects on neuron or glial cell morphology. Transcriptome microarray analysis identified differential expression in exposed versus control cultures in 67 genes, including nine genes essential for neurodevelopment, which were similarly altered by nicotine (Kimura-Kuroda et al. 2012).
- Mouse: Exposure of spermatozoa to 5 mM for 30 minutes had no effect on sperm motility or DNA fragmentation; however, use of exposed sperm prior to the *in vitro* fertilization process decreased fertilization rate and numbers of 2-cell embryos, increased numbers of fragmented embryos, and decreased numbers of morulae and blastocysts. Exposure of a mixture of spermatozoa and oocytes (i.e., during the *in vitro* fertilization process) to 500 µM affected fertilization and embryonic development *in vitro*, decreasing the number of 2-cell embryos, morulae and blastocysts; exposure of naturally fertilized zygotes to 500 µM decreased the number of 4-cell embryos, morulae and blastocytes, while similar exposure of 2-cell embryos was without effect (Gu et al. 2013).
- Pig: *In vitro* exposure of oocytes for 44 hours decreased the nuclear maturation rate, and increased the occurrence of dispersed, irregular chromosomes in matured (metaphase II stage) oocytes (Ishikawa et al. 2015).
- Japanese medaka: Exposure of embryos post fertilization for 14 days to concentrations ranging from 0.2 to 2000 mg/L increased developmental anomalies at all doses tested (the percentage of total anomalies was 67% at 0.2 mg/L and >80% at concentrations of 2 mg/L and above); anomalies included lordosis/scoliosis, hemorrhage, jaw/skull deformity, edema of the yolk and bones, tail deformities and disorganization of the retinal pigment epithelium (Vignet et al. 2019).
- Zebrafish: Exposure of embryos post fertilization for 5 days to concentrations ranging from 0.2 to 2000 mg/L resulted in a marked thickening of muscle fibers in the highest dose group (Vignet et al. 2019).

References cited in "Imidacloprid"

Abou-Donia MB GL, Bullman S, Tu T, Khan WA, Dechkovskaia AM, Abdel-Rahman AA. 2008. Imidacloprid induces neurobehavioral deficits and increases expression of glial

fibrillary acidic protein in the motor cortex and hippocampus in offspring rats following *in utero* exposure. J Toxicol Environ Health (A) 71:119-130.

Bagri P, Kumar V, Sikka AK. 2015. An *in vivo* assay of the mutagenic potential of imidacloprid using sperm head abnormality test and dominant lethal test. Drug Chem Toxicol 38:342-348.

Bal R, Naziroğlu M, Türk G, Yilmaz Ö, Kuloğlu T, Etem E, et al. 2012a. Insecticide imidacloprid induces morphological and DNA damage through oxidative toxicity on the reproductive organs of developing male rats. Cell Biochem Funct 30:492-499.

Bal R, Türk G, Tuzcu M, Yilmaz O, Kuloglu T, Gundogdu R, et al. 2012b. Assessment of imidacloprid toxicity on reproductive organ system of adult male rats. J Environ Sci Health B 47:434-444.

Bhaskar R, Mishra AK, Mohanty B. 2017. Neonatal exposure to endocrine disrupting chemicals impairs learning behaviour by disrupting hippocampal organization in male Swiss albino mice. Basic Clin Pharmacol Toxicol 121:44-52.

Burke AP, Niibori Y, Terayama H, Ito M, Pidgeon C, Arsenault J, et al. 2018. Mammalian susceptibility to a neonicotinoid insecticide after fetal and early postnatal exposure. Sci Rep 8:16639.

Carmichael SL, Yang W, Roberts E, Kegley SE, Padula AM, English PB, et al. 2014. Residential agricultural pesticide exposures and risk of selected congenital heart defects among offspring in the San Joaquin Valley of California. Environ Res 135:133-138.

Caron-Beaudoin E, Viau R, Hudon-Thibeault AA, Vaillancourt C, Sanderson JT. 2017. The use of a unique co-culture model of fetoplacental steroidogenesis as a screening tool for endocrine disruptors: The effects of neonicotinoids on aromatase activity and hormone production. Toxicol Appl Pharmacol 332:15-24.

CDPR (California Department of Pesticide Regulation). 2006. Imidacloprid. Risk Characterization Document (RCD) Dietary and Drinking Water Exposure.

CDPR (California Department of Pesticide Regulation). 2013. Imidacloprid., Summary of Toxicological Data.

CDPR (California Pesticide Information Portal (CALPIP) Application). 2017. Version 2019.04. Pesticide Use Reporting (PUR) Data Update. Available at: Https://calpip.Cdpr.Ca.Gov/main.Cfm. 2017..

Gawade L, Dadarkar SS, Husain R, Gatne M. 2013. A detailed study of developmental immunotoxicity of imidacloprid in wistar rats. Food Chem Toxicol 51:61-70.

Gu YH, Li Y, Huang XF, Zheng JF, Yang J, Diao H, et al. 2013. Reproductive effects of two neonicotinoid insecticides on mouse sperm function and early embryonic development *in vitro*. PLoS One 8:e70112.

Hafez EM, Sahar YI, Maha KA-M, Karem TI, Safaa MAR. 2016. The neonicotinoid insecticide imidacloprid: A male reproductive system toxicity inducer-human and experimental study. Toxicology: Open Access 2:1-8.

Ishikawa S, Hiraga K, Hiradate Y, Tanemura K. 2015. The effects analysis of two neonicotinoid insecticides on *in vitro* maturation of porcine oocytes using hanging drop monoculture method. J Vet Med Sci 77:725-728.

Kapoor U, Srivastava MK, Srivastava LP. 2011. Toxicological impact of technical imidacloprid on ovarian morphology, hormones and antioxidant enzymes in female rats. Food Chem Toxicol 49:3086-3089.

Kara M, Yumrutas O, Demir CF, Ozdemir HH, Bozgeyik I, Coskun S, et al. 2015. Insecticide imidacloprid influences cognitive functions and alters learning performance and related gene expression in a rat model. Int J Exp Pathol 96:332-337.

Keil AP, Daniels JL, Hertz-Picciotto I. 2014. Autism spectrum disorder, flea and tick medication, and adjustments for exposure misclassification: The charge (childhood autism risks from genetics and environment) case-control study. Environ Health 13:3.

Kimura-Kuroda J, Komuta Y, Kuroda Y, Hayashi M, Kawano H. 2012. Nicotine-like effects of the neonicotinoid insecticides acetamiprid and imidacloprid on cerebellar neurons from neonatal rats. PLoS One 7:e32432.

Lonare M, Kumar M, Raut S, More A, Doltade S, Badgujar P, et al. 2016. Evaluation of ameliorative effect of curcumin on imidacloprid-induced male reproductive toxicity in Wistar rats. Environ Toxicol 31:1250-1263.

Mahajan L, Verma PK, Raina R, Sood S. 2018. Potentiating effect of imidacloprid on arsenic-induced testicular toxicity in Wistar rats. BMC Pharmacol Toxicol 19:48.

Memon S, Memon N, Mal B, Shaikh SA, MA. S. 2014. Histopathological changes in the gonads of male rabbits (*Oryctolagus cuniculus*) on exposure to imidacloprid insecticide. J Entomol Zool Stud 2:159-163.

Mikolić A, Karačonji IB. 2018. Imidacloprid as reproductive toxicant and endocrine disruptor: Investigations in laboratory animals. Arh Hig Rada Toksikol 69:103-108.

Mzid M, Ghlissi Z, Salem MB, Khedir SB, Chaabouni K, Ayedi F, et al. 2018. Chemoprotective role of ethanol extract of *Urtica urens* l. Against the toxicity of imidacloprid on endocrine disruption and ovarian morphometric in female rats, GC/MS analysis. Biomed Pharmacother 97:518-527.

Najafi G, Mazdak Razi, Aref Hoshyar, Simineh Shahmohamadloo, Feyzi S. 2010. The effect of chronic exposure with imidaclopridinsecticide on fertility in mature male rats International Journal of Fertility and Sterility 4:9-16.

Nakayama A, Yoshida M, Kagawa N, Nagao T. 2019. The neonicotinoids acetamiprid and imidacloprid impair neurogenesis and alter the microglial profile in the hippocampal dentate gyrus of mouse neonates. J Appl Toxicol 39:877-887.

Ndonwi EN, Atogho-Tiedeu B, Lontchi-Yimagou E, Shinkafi TS, Nanfa D, Balti EV, et al. 2020. Metabolic effects of exposure to pesticides during gestation in female Wistar rats and their offspring: A risk factor for diabetes? Toxicol Res 36:249-256.

NTP (National Toxicology Program). 2020. Research Report on the Scoping Review of Potential Human Health Effects Associated with Exposures to Neonicotinoid Pesticides. Available: https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/rr15_508.pdf [accessed 25 September 2020].

Shaw GM, Yang W, Roberts E, Kegley SE, Padula A, English PB, et al. 2014. Early pregnancy agricultural pesticide exposures and risk of gastroschisis among offspring in the san joaquin valley of california. Birth Defects Research Part A: Clinical and Molecular Teratology 100:686-694.

Sheets LP, Li AA, Minnema DJ, Collier RH, Creek MR, Peffer RC. 2016. A critical review of neonicotinoid insecticides for developmental neurotoxicity. Crit Rev Toxicol 46:153-190.

Tetsatsi ACM, Nkeng-Effouet PA, Alumeti DM, Bonsou GRF, Kamanyi A, Watcho P. 2019. Colibri® insecticide induces male reproductive toxicity: Alleviating effects of lannea acida (anacardiaceae) in rats. Basic Clin Androl 29:16.

Vignet C, Cappello T, Fu Q, Lajoie K, De Marco G, Clérandeau C, et al. 2019. Imidacloprid induces adverse effects on fish early life stages that are more severe in Japanese medaka (*Oryzias latipes*) than in zebrafish (*Danio rerio*). Chemosphere 225:470-478.

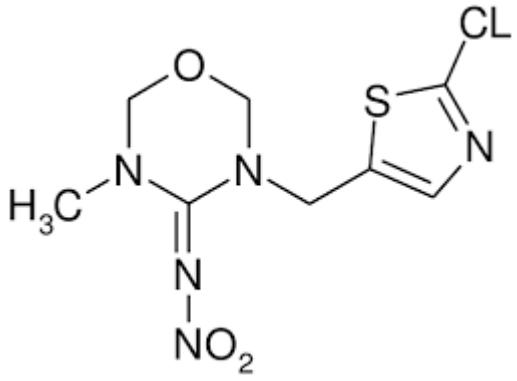
Vohra P, Khera K. 2016. Effect of imidacloprid on reproduction of female albino rats in three generation study. Journal of Veterinary Science & Technology 7:1-7.

Yang W, Carmichael SL, Roberts EM, Kegley SE, Padula AM, English PB, et al. 2014. Residential agricultural pesticide exposures and risk of neural tube defects and orofacial clefts among offspring in the San Joaquin Valley of California. Am J Epidemiol 179:740-748.

Yuan X, Shen J, Zhang X, Tu W, Fu Z, Jin Y. 2020. Imidacloprid disrupts the endocrine system by interacting with androgen receptor in male mice. Sci Total Environ 708:135163.

Thiamethoxam

(CAS No. 153719-23-4)



Thiamethoxam is a systemic neonicotinoid insecticide used to control sucking insects, such as thrips and aphids, and soil insects, such as beetles. Thiamethoxam is used on a variety of crops, including cucumbers, onions, wine grapes, and cotton, and for structural pest control (US EPA 2020). According to the California Department of Pesticide Regulation (DPR), 46,879 pounds of thiamethoxam were used in California in 2017 (CDPR 2017).

Thiamethoxam passed the animal data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

No human studies reporting developmental and reproductive toxicity (DART)-related effects of thiamethoxam were identified.

Animal studies

Findings reported in whole animal studies examining possible DART effects of exposure to thiamethoxam are summarized here.

Maternal and developmental effects

- Rat: In a guideline developmental (teratology) study with exposure from gestation day (GD) 6-15, maternal body weight gain and food consumption were

decreased at the mid- and high doses, and a reduction in fetal body weights and an increase in skeletal anomalies and variations (related to delayed ossification) were observed at the high dose (750 mg/kg-day) (CDPR 2008).

- Rat: In a review describing guideline reproduction studies, maternal exposure from GD 6 to lactation day 21 decreased pup body weight and body weight gain and delayed sexual maturation (delay in onset of preputial separation) in F1 animals at the high dose (4000 ppm) (Sheets et al. 2016).
- Rat: In a guideline developmental neurotoxicity study, dams at the high dose (4000 ppm; 299 mg/kg-day) exposed from GD 7 through postnatal day (PND) 22 had reduced food consumption along with reduced maternal body weights; reduced body weights of high dose pups were noted from PND 1 through study termination at PND 63 (US EPA 2005) (CDPR 2008).
- Rabbit: In a guideline developmental (teratology) study with exposure on GD 7-19, reduction in maternal body weight gain at the two high dose levels was observed. Dose-related increases in post implantation losses resulting from increased early resorptions were noted at the high dose (150 mg/kg-day), along with reduced mean fetal weights and increased skeletal anomalies and variations at the high dose (CDPR 2008).
- Zebrafish: Exposure beginning at the embryo stage did not affect embryo survival within 48 hours, cause morphological alterations or delays in embryo/larvae development, or alter expression of the key developmental genes *ntl*, *krox20*, and *shh* (Liu et al. 2018).

Neurodevelopmental effects

- Rat: In a guideline developmental neurotoxicity study, maternal exposure from GD 7 – PND 22 had effects at the high dose (4000 ppm or 299 mg/kg-day) on maternal feed consumption, body weight and pup body weight (see above under Maternal and Developmental Effects), while no effects were observed on offspring relative brain weights, histopathology or functional or neurobehavioral parameters (as assessed by the functional observational battery, motor activity, acoustic startle response and learning and memory tests) at any dose (US EPA 2005) (CDPR 2008) (Sheets et al. 2016).
- Zebrafish: Exposure beginning at the embryo stage altered locomotor activity in larvae at concentrations as low as 0.1 mg/L (Liu et al. 2018).

Male reproductive effects

- Rat: In a two generation reproduction guideline study with exposure via diet, germ cell loss/disorganization with Sertoli cell vacuolation was observed in high dose (1500 ppm) F1 males, and higher percentages of sperm with detached heads were observed in three high dose males (two F0 males and one F1 male) (CDPR 2008).

- Rat: In a two generation reproduction guideline study with exposure via diet, an increased incidence of diffuse tubular atrophy was observed in the testes of F0 and F1 males at the 1000 ppm but not at 2500 ppm (CDPR 2008).
- Dog: In a 12-month chronic dietary toxicity guideline study, along with reduced body weight gain in high-dose (1500 ppm) males, absolute and relative testis weights were reduced in two of four dogs and this decrease in testis weight was associated with a slight increase in the incidence and severity of atrophy of seminiferous tubules (CDPR 2008).

Mechanistic, in vitro, and other relevant data

- Pig: In porcine embryos exposed *in vitro*, expansion and hatching of blastocysts was decreased, and expanded blastocysts had decreased cell proliferation, increased reactive oxygen species (ROS) and γH2Ax levels, altered gene expression of antioxidant enzymes (increased *Sod1* and decreased *Mnsod*, *Gpx1, Igta5, Cox2*), and decreased activity of maturation-promoting factor (Nie et al. 2019b).
- Cattle: *In vitro* exposure of bovine oocytes delayed oocyte progression to the metaphase I stage, blocked development at this stage, triggered disordered chromosomes and spindles at the metaphase II stage, impaired cleavage of metaphase II oocyte and inhibited development to morulae and blastocysts (Nie et al. 2019a).
- Mouse: Exposure of 2-cell stage embryos for 72 h to concentrations of 10 μM and above decreased the number of embryos reaching the blastocyst stage and the average number of cells per blastocyst (Babeľová et al. 2017).

References cited in "Thiamethoxam"

Babeľová J, Šefčíková Z, Čikoš Š, Špirková A, Kovaříková V, Koppel J, et al. 2017. Exposure to neonicotinoid insecticides induces embryotoxicity in mice and rabbits. Toxicology 392:71-80.

CDPR. 2008. Thiamethoxam California Department of Pesticide Regulation, Summary of Toxicology Data.

CDPR California pesticide information portal (calpip) application. 2017. Version 2019.04.Pesticide Use Reporting (PUR) Data Update. Available at: Https://calpip.Cdpr.Ca.Gov/main.Cfm..

Liu X, Zhang Q, Li S, Mi P, Chen D, Zhao X, et al. 2018. Developmental toxicity and neurotoxicity of synthetic organic insecticides in zebrafish (*Danio rerio*): A comparative study of deltamethrin, acephate, and thiamethoxam. Chemosphere 199:16-25.

Nie ZW, Niu YJ, Zhou W, Kim JY, Ock SA, Cui XS. 2019a. Thiamethoxam induces meiotic arrest and reduces the quality of oocytes in cattle. Toxicol In Vitro 61:104635.

Nie ZW, Niu YJ, Zhou W, Kim YH, Shin KT, Cui XS. 2019b. Thiamethoxam inhibits blastocyst expansion and hatching via reactive-oxygen species-induced g2 checkpoint activation in pigs. Cell Signal 53:294-303.

Sheets LP, Li AA, Minnema DJ, Collier RH, Creek MR, Peffer RC. 2016. A critical review of neonicotinoid insecticides for developmental neurotoxicity. Crit Rev Toxicol 46:153-190.

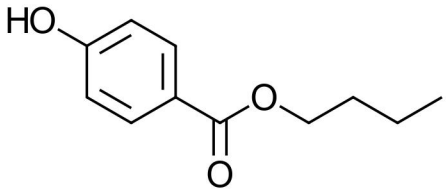
US EPA (US Environmental Protection Agency). 2005. Thiamethoxam - Developmental Neurotoxicity Study, Data Evaluation Record (DER). MRID 46028202.

US EPA (US Environmental Protection Agency). 2020. Clothianidin and thiamethoxam Proposed Interim Registration Review Decision. Docket Numbers EPA-HQ-OPP-2011-0865 and EPA-HQ-OPP-2011-0581.

Parabens:

Butyl paraben

(Butyl p-hydroxybenzoate, butyl 4-hydroxybenzoate, CAS No. 94-26-8)



Butyl paraben is a member of the class of parabens. It has been widely used as an antimicrobial preservative in cosmetics and medication suspensions. It is now found in more than 20,000 cosmetic products including eye shadow, facial moisturizer/treatment, anti-aging cream, foundation, and sunscreen. It is also used as a preservative in some foods and drugs (Health Canada 2020; US FDA 2020).

Butyl paraben passed the human and animal data screens, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Numerous human studies reporting developmental and reproductive toxicity (DART)-related effects associated with butyl paraben were identified in the recent literature. A number of DART findings reported in epidemiologic studies published within the last seven years are summarized here. The findings are organized by groups of outcomes.

Developmental effects

- Increased concentrations of butyl paraben in urine samples from pregnant women in the first trimester were associated with reduced head circumference in girls at birth and increased birth weight in boys (prospective cohort study) (Jamal et al. 2020).
- Maternal exposure was positively associated with the child being overweight within the first eight years of life, with a stronger trend in girls (prospective cohort study) (Leppert et al. 2020).

Female reproductive effects

- Exposure was associated with decreased sex hormone binding globulin (SHBG) in pregnant women (cross-sectional study) (Aker et al. 2019).
- In pregnant women, exposure was associated with decreased serum levels of total triiodothyronine (T_3) (cross-sectional study) (Aker et al. 2018).
- Exposure was positively associated with blood glucose levels, for both the 1st trimester and 2nd trimester (cross-sectional study) (Bellavia et al. 2019).
- No association of exposure and gestational diabetes mellitus (cross-sectional study) (Li et al. 2019).
- Urinary butyl paraben concentrations were not associated with *in vitro* fertilization outcomes, including total and mature oocyte counts, proportion of high embryo quality, and rates of fertilization, implantation, clinical pregnancy, and live birth (prospective cohort study) (Minguez-Alarcon et al. 2016).
- Butyl paraben concentrations were associated with shortened menstrual cycle length (cross-sectional study) (Nishihama et al. 2016).
- No consistent associations of urinary butyl paraben with day-3 follicle-stimulating hormone, antral follicle count, or ovarian volume in women seeking fertility treatment (prospective cohort study) (Smith et al. 2013).

Male reproductive effects

- Urinary levels of butyl paraben were positively associated with sperm XY18 disomy (cross-sectional study) (Jurewicz et al. 2017).
- No association of urinary butyl paraben with semen volume, sperm concentration or sperm motility in male partners of couples seeking infertility consultation (cross-sectional study) (Nishihama et al. 2017).
- Urinary levels of butyl paraben were associated with diminished sperm count and poor sperm motility (prospective cohort study) (Smarr et al. 2018).

Animal studies

Numerous whole animal studies examining possible DART effects of exposure to butyl paraben were identified. Findings reported in these studies are summarized here.

Developmental effects, prenatal exposure only

- Rat: Exposure via gavage on gestation days (GD) 6-19 at doses up to 1,000 mg/kg/day had no effect on developmental parameters, including embryo/fetal viability, fetal weight, malformations, and variations (Daston 2004).
- Rat: Maternal treatment on GD 7 - 21 had no effect on anogenital distance in GD 21 fetuses, decreased mRNA expression levels of estradiol receptor-beta in fetal ovaries and mRNA levels of the steroidogenic acute regulatory (StAR) and

peripheral benzodiazepine receptor (PBR) genes in female fetal adrenal glands, and had no effect on fetal testicular histopathology, or testosterone levels or production (Taxvig et al. 2008).

- Mouse: Butyl paraben administered subcutaneously on days 1-4 of pregnancy did not affect litter size, the number of pups born, postnatal day (PND) 3 litter weights, or the number of pups surviving to PND 5 (Shaw and deCatanzaro 2009).

Developmental effects, prenatal and postnatal exposure

- Rat: Perinatal exposure to butyl paraben caused deficits in social, learning and memory behaviors that are similar to some of the neurodevelopmental disorders observed in the valproic acid model of autism. Butyl paraben also caused abnormal changes in levels of monoamines, amino acids and brain-derived neurotrophic factor (BDNF) in the brain tissues of the offspring at PND 24 (Ali and Elgoly 2013).
- Rat: Exposure from GD 7 – PND 22 reduced anogenital distance in newborn male and female offspring, reduced ovary weights and increased mammary gland growth in prepubertal females, and significantly reduced sperm count at doses of 10 mg/kg bw/d or higher in males (Boberg et al. 2016).
- Rat: Butyl paraben at doses of 10, 100, or 200 mg/kg from GD 12 – PND 21 did not show estrogenic activity and did not impair sexual development or fertility capacity in females (Guerra et al. 2017a).
- Rat: Exposure from GD 12 – PND 20 adversely affected spermatogenesis kinetics at doses of 10 and 200 mg/kg, impaired sperm motility, and increased sperm head abnormalities (Guerra et al. 2017b).
- Rat: Exposure from GD 6 – PND 20 reduced the proportion of pups born alive, the proportion of pups surviving to weaning, the body weights of female offspring, the weights of testes, seminal vesicles and prostate glands, sperm count and sperm motile activity in the epididymis (Kang et al. 2002).
- Rat: Exposure via subcutaneous injection from GD 6 – PND 21 delayed testicular descent and preputial separation, and decreased sperm count, motility and daily sperm production in males. Females were sub-fertile, with increased pre- and post-implantation loss (Maske et al. 2020).
- Rat: Exposure from GD 6 – PND 21 resulted in impaired steroidogenesis and folliculogenesis in females (Maske et al. 2018).
- Rat: Oral exposure to pregnant Wistar rats from GD 7 – PND 21 reduced anogenital distance, delayed preputial separation, reduced weights of testes, epididymides, and seminal vesicles, decreased serum testosterone, luteinizing hormone, and follicle–stimulating hormone, increased serum 17β-estradiol, and reduced in a dose-dependent manner epididymal cauda sperm counts and daily sperm production in males (Zhang et al. 2014).

Female reproductive effects

- Rat: Butyl paraben caused myometrial hypertrophy in females (Vo et al. 2010).
- Rat: Butyl paraben significantly increased uterus weight in an uterotrophic assay (Vo and Jeung 2009).
- Mouse: Butyl paraben had no effect on uterine wet or dry mass in ovariectomized CF-1 and CD-1 mice (Shaw and deCatanzaro 2009).

Male reproductive effects

- Rat: Reduced weights of the testis and epididymis, reduced total epididymal sperm count at high dose (40,000 ppm, approximately 2500 mg/kg-day) (NTP 2020).
- Rat: A single oral dose of 1,000 mg/kg butyl paraben to three-week-old male rats caused a gradual collapse of Sertoli cell vimentin filaments and decreased actin staining intensity. Spermatogenic cells became separated from the basement membrane and sloughed into the lumen in treated rats (Alam and Kurohmaru 2014).
- Rat: A single oral dose of 1,000 mg/kg butyl paraben to three-week-old male rats caused progressive detachment and sloughing of spermatogenic cells into the lumen of the seminiferous tubules at 3 h; this effect was enhanced at 6 h. Thin seminiferous epithelia and wide tubular lumina were seen at 24 h. A significant increase in the number of apoptotic spermatogenic cells in treated animals (Alam et al. 2014).
- Rat: Repeated subcutaneous injection of butyl paraben for one complete spermatogenic cycle caused dose-dependent increases in prostate relative weights, sperm with abnormal morphology, and histopathological changes in sexual organs (Garcia et al. 2017).
- Rat: Butyl paraben treatment via diet did not cause changes in organ weights, histopathology of reproductive tissues, sperm production, motility, morphology or reproductive hormone levels (Hoberman et al. 2008).
- Rat: Treatment of 3-week-old Wistar rats decreased the absolute and relative weights of epididymides, the cauda epididymal sperm reserve, sperm count, and daily sperm production, and decreased serum testosterone levels in a dose-dependent manner (Oishi 2001).
- Rat: In a repeated 28-day oral toxicity study (OECD TG407 protocol) butyl paraben caused DNA hypermethylation in germ cells from the mitotic through post-meiotic stage in adult rat testes (Park et al. 2012).
- Mouse: Treatment of 4-week-old mice for 10 weeks increased the absolute and relative weights of the epididymides, and decreased in a dose-dependent manner both round and elongated spermatid counts in stage VII-VIII seminiferous tubules, and serum testosterone levels (Oishi 2002).

Mechanistic, in vitro, and other relevant data

- Human semen culture: Butyl paraben reduced sperm motility and viability in human semen samples incubated *in vitro* (Li et al. 2017).
- Human adrenocortical carcinoma cell line (H295R cells): In the steroid synthesis assay, butyl paraben increased progesterone production and had no effect on testosterone or estradiol production (Taxvig et al. 2008).
- Human: Butyl paraben activated pregnane X receptor (PXR) in a reporter gene assay that detects transcriptional activation (Fujino et al. 2019)
- Human: In the MCF-7 breast cancer cell line, treatment with butyl paraben or its metabolite, 3-hydroxy *n*-butyl 4-hydroxybenzoate (3OH), induced cell proliferation, expression of the pro-proliferative, estrogen-inducible gene GREB1, and promoted estrogen receptor (ER)-dependent transcriptional activity of an estrogen response element (ERE) reporter gene. Computational docking studies predict that both butyl paraben and 3OH can be docked within the ligand-binding pocket of ERα (Gonzalez et al. 2018).
- Human: The relative binding affinities to human recombinant estrogen receptors alpha and beta, assessed in a cell free competitive binding assay, were: iso-butylparaben > butyl paraben > isopropylparaben = propylparaben > ethylparaben (Vo et al. 2010).
- Rat Sertoli cell culture: Butyl paraben caused an increased number and size of vacuoles in the cytoplasm of rat Sertoli cells cultured *in vitro* (Alam and Kurohmaru 2014).
- Rat: Butyl paraben activated PXR and constitutive androstane receptor (CAR) in reporter gene assays detecting transcriptional activation (Fujino et al. 2019).
- Rat: *In vivo* exposure increased Amh mRNA levels but had no effect on Foxl2 and Kitlg mRNA levels in primordial follicles, indicating the influence of butyl paraben on ovarian folliculogenesis and steroidogenesis (Lee et al. 2017).
- Rat pituitary cell line (GH3 cells): Butyl paraben had weak thyroid receptor agonist activity, inducing cell proliferation in the T screen assay (Taxvig et al. 2008).

References cited in "Butyl paraben"

Aker AM, Johns L, McElrath TF, Cantonwine DE, Mukherjee B, Meeker JD. 2018. Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: A repeated measures study. Environ Int 113:341-349.

Aker AM, Ferguson KK, Rosario ZY, Mukherjee B, Alshawabkeh AN, Calafat AM, et al. 2019. A repeated measures study of phenol, paraben and triclocarban urinary biomarkers and circulating maternal hormones during gestation in the Puerto Rico protect cohort. Environ Health 18:28.

Alam MS, Kurohmaru M. 2014. Disruption of sertoli cell vimentin filaments in prepubertal rats: An acute effect of butylparaben *in vivo* and *in vitro*. Acta Histochem 116:682-687.

Alam MS, Ohsako S, Kanai Y, Kurohmaru M. 2014. Single administration of butylparaben induces spermatogenic cell apoptosis in prepubertal rats. Acta Histochem 116:474-480.

Ali EH, Elgoly AH. 2013. Combined prenatal and postnatal butyl paraben exposure produces autism-like symptoms in offspring: Comparison with valproic acid autistic model. Pharmacol Biochem Behav 111:102-110.

Bellavia A, Chiu YH, Brown FM, Mínguez-Alarcón L, Ford JB, Keller M, et al. 2019. Urinary concentrations of parabens mixture and pregnancy glucose levels among women from a fertility clinic. Environ Res 168:389-396.

Boberg J, Axelstad M, Svingen T, Mandrup K, Christiansen S, Vinggaard AM, et al. 2016. Multiple endocrine disrupting effects in rats perinatally exposed to butylparaben. Toxicol Sci 152:244-256.

Daston GP. 2004. Developmental toxicity evaluation of butylparaben in Sprague-Dawley rats. Birth Defects Res B Dev Reprod Toxicol 71:296-302.

Fujino C, Watanabe Y, Sanoh S, Hattori S, Nakajima H, Uramaru N, et al. 2019. Comparative study of the effect of 17 parabens on PXR-, CAR- and PPARalpha-mediated transcriptional activation. Food Chem Toxicol 133:110792.

Garcia T, Schreiber E, Kumar V, Prasad R, Sirvent JJ, Domingo JL, et al. 2017. Effects on the reproductive system of young male rats of subcutaneous exposure to n-butylparaben. Food Chem Toxicol 106:47-57.

Gonzalez TL, Moos RK, Gersch CL, Johnson MD, Richardson RJ, Koch HM, et al. 2018. Metabolites of n-butylparaben and iso-butylparaben exhibit estrogenic properties in mcf-7 and t47d human breast cancer cell lines. Toxicol Sci 164:50-59.

Guerra MT, Sanabria M, Cagliarani SV, Leite GA, Borges CD, De Grava Kempinas W. 2017a. Long-term effects of *in utero* and lactational exposure to butyl paraben in female rats. Environ Toxicol 32:776-788.

Guerra MT, Sanabria M, Leite GA, Borges CS, Cucielo MS, Anselmo-Franci JA, et al. 2017b. Maternal exposure to butyl paraben impairs testicular structure and sperm quality on male rats. Environ Toxicol 32:1273-1289.

Health Canada H. 2020. Draft Screening Assessment Parabens Group. Available: https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/draft-screening-assessment-parabens-group.html.[accessed 10 September 2020]

Hoberman AM, Schreur DK, Leazer T, Daston GP, Carthew P, Re T, et al. 2008. Lack of effect of butylparaben and methylparaben on the reproductive system in male rats. Birth Defects Res B Dev Reprod Toxicol 83:123-133.

Jamal A, Rastkari N, Dehghaniathar R, Nodehi RN, Nasseri S, Kashani H, et al. 2020. Prenatal urinary concentrations of environmental phenols and birth outcomes in the mother-infant pairs of Tehran environment and neurodevelopmental disorders (TEND) cohort study. Environ Res 184:109331.

Jurewicz J, Radwan M, Wielgomas B, Klimowska A, Kaluzny P, Radwan P, et al. 2017. Environmental exposure to parabens and sperm chromosome disomy. Int J Environ Health Res 27:332-343.

Kang KS, Che JH, Ryu DY, Kim TW, Li GX, Lee YS. 2002. Decreased sperm number and motile activity on the f1 offspring maternally exposed to butyl p-hydroxybenzoic acid (butyl paraben). J Vet Med Sci 64:227-235.

Lee JH, Lee M, Ahn C, Kang HY, Tran DN, Jeung EB. 2017. Parabens accelerate ovarian dysfunction in a 4-vinylcyclohexene diepoxide-induced ovarian failure model. Int J Environ Res Public Health 14.

Leppert B, Strunz S, Seiwert B, Schlittenbauer L, Schlichting R, Pfeiffer C, et al. 2020. Maternal paraben exposure triggers childhood overweight development. Nat Commun 11:561.

Li D, Yuan D, Zhang L, Qiao P, Liang X, Chang B. 2017. [increase of apoptosis and decrease of sperm motility induced by oxidative stress after exposed to butyl p-hydroxybenzoate]. Wei Sheng Yan Jiu 46:196-200.

Li Y, Xu S, Li Y, Zhang B, Huo W, Zhu Y, et al. 2019. Association between urinary parabens and gestational diabetes mellitus across prepregnancy body mass index categories. Environ Res 170:151-159.

Maske P, Dighe V, Vanage G. 2018. N-butylparaben exposure during perinatal period impairs fertility of the F1 generation female rats. Chemosphere 213:114-123.

Maske P, Dighe V, Mote C, Vanage G. 2020. N-butylparaben exposure through gestation and lactation impairs spermatogenesis and steroidogenesis causing reduced fertility in the F1 generation male rats. Environ Pollut 256:112957.

Minguez-Alarcon L, Chiu YH, Messerlian C, Williams PL, Sabatini ME, Toth TL, et al. 2016. Urinary paraben concentrations and *in vitro* fertilization outcomes among women from a fertility clinic. Fertil Steril 105:714-721.

Nishihama Y, Yoshinaga J, Iida A, Konishi S, Imai H, Yoneyama M, et al. 2016. Association between paraben exposure and menstrual cycle in female university students in japan. Reprod Toxicol 63:107-113.

Nishihama Y, Toshima H, Yoshinaga J, Mizumoto Y, Yoneyama M, Nakajima D, et al. 2017. Paraben exposure and semen quality of Japanese male partners of subfertile couples. Environ Health Prev Med 22:5.

NTP (National Toxicology Program). 2020. NTP study on butylparaben (study no. M88007).

Oishi S. 2001. Effects of butylparaben on the male reproductive system in rats. Toxicol Ind Health 17:31-39.

Oishi S. 2002. Effects of butyl paraben on the male reproductive system in mice. Arch Toxicol 76:423-429.

Park CJ, Nah WH, Lee JE, Oh YS, Gye MC. 2012. Butyl paraben-induced changes in DNA methylation in rat epididymal spermatozoa. Andrologia 44 Suppl 1:187-193.

Shaw J, deCatanzaro D. 2009. Estrogenicity of parabens revisited: Impact of parabens on early pregnancy and an uterotrophic assay in mice. Reprod Toxicol 28:26-31.

Smarr MM, Honda M, Kannan K, Chen Z, Kim S, Louis GMB. 2018. Male urinary biomarkers of antimicrobial exposure and bi-directional associations with semen quality parameters. Reprod Toxicol 77:103-108.

Smith KW, Souter I, Dimitriadis I, Ehrlich S, Williams PL, Calafat AM, et al. 2013. Urinary paraben concentrations and ovarian aging among women from a fertility center. Environ Health Perspect 121:1299-1305.

Taxvig C, Vinggaard AM, Hass U, Axelstad M, Boberg J, Hansen PR, et al. 2008. Do parabens have the ability to interfere with steroidogenesis? Toxicol Sci 106:206-213.

US FDA (Food and Drug Administration). 2020. Voluntary Cosmetic Registration Program. Available: https://www.fda.gov/cosmetics/voluntary-cosmetic-registration-program [accessed 2 September 2020].

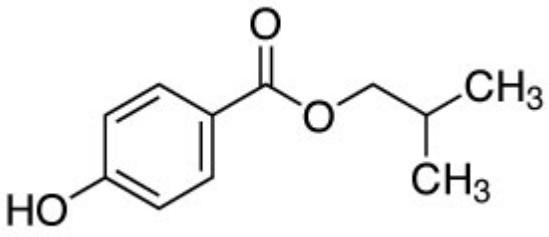
Vo TT, Jeung EB. 2009. An evaluation of estrogenic activity of parabens using uterine calbindin-d9k gene in an immature rat model. Toxicol Sci 112:68-77.

Vo TT, Yoo YM, Choi KC, Jeung EB. 2010. Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model. Reprod Toxicol 29:306-316.

Zhang L, Dong L, Ding S, Qiao P, Wang C, Zhang M, et al. 2014. Effects of n-butylparaben on steroidogenesis and spermatogenesis through changed e_2 levels in male rat offspring. Environ Toxicol Pharmacol 37:705-717.

Isobutyl paraben

[Isobutyl p-hydroxybenzoate, isobutyl 4-hydroxybenzoate, CAS No. 4247-02-3]



Isobutyl paraben is a member of the class of parabens. It has been used as an antimicrobial preservative in cosmetics and medication suspensions. Isobutyl paraben is frequently used in combination with other parabens. There are no data on the total quantities of this paraben in production or use (Health Canada 2020; US FDA 2020).

Isobutyl paraben passed the animal data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

No human epidemiologic studies reporting developmental and reproductive toxicity (DART)-related effects associated with isobutyl paraben were identified.

Animal studies

Findings reported in whole animal studies examining possible DART effects of exposure to isobutyl paraben are summarized here.

Developmental effects

- Rat: Exposure during gestation and lactation decreased plasma corticosterone concentration and increased the uterus weight in dams at weaning, and increased uterine sensitivity to estrogen in adult ovariectomized female offspring. No effects were observed on other parameters assessed, including organ weights (other than the uterus) in dams, ratio of male pups, anogenital distance, organ weights and plasma hormone concentrations in offspring, puberty, estrous cycle and response of organ weight (other than the uterus) and plasma hormone concentrations to estrogen in adult female offspring, and reproductive and adrenal function in adult male offspring (Kawaguchi et al. 2009b).

- Rat: Exposure during gestation and lactation reduced epididymal sperm count and motility in adult male offspring (Yang et al. 2016).

Neurodevelopmental effects

- Rat: Exposure during gestation and lactation reduced time spent in the open arms of the elevated plus maze (interpreted as increased anxiety) and impaired performance in the passive avoidance test (impaired learning ability) in male offspring at 5-6 weeks of age (Kawaguchi et al. 2009a).
- Rat: Exposure during gestation and lactation impaired social recognition in an intruder test in 16-week-old ovariectomized female offspring (Kawaguchi et al. 2010).

Reproductive effects

- Rat: Isobutyl paraben caused myometrial hypertrophy in female rats (Vo et al. 2010).
- Rat: Isobutyl paraben significantly increased uterus weight in a uterotrophic assay (Vo and Jeung 2009)

Mechanistic, in vitro, and other relevant data

- Human: In the MCF-7 breast cancer cell line, treatment with isobutyl paraben or its metabolite, 2-hydroxy iso-butyl 4-hydroxybenzoate (abbreviated as 2OH), induced cell proliferation. 2OH also induced cell proliferation in a second breast cancer cell line, T47D. In MCR-7 cells, isobutyl paraben and 2OH each induced expression of the pro-proliferative, estrogen-inducible gene GREB1, and 2OH promoted estrogen receptor (ER)-dependent transcriptional activity of an estrogen response element (ERE) reporter gene. Computational docking studies predict that isobutyl paraben and its metabolite 2OH can be docked within the ligand-binding pocket of ERα (Gonzalez et al. 2018).
- Rat: In pituitary cancer GH3 cells transfected with an ERE reporter gene, isobutyl paraben increased ERE activity and both mRNA and protein expression of progesterone receptor and calbindin-D_{9k} (Kim et al. 2012).
- Rat: Isobutyl paraben activated constitutive androstane receptor (CAR) in reporter gene assays detecting transcriptional activation (Fujino et al. 2019).
- Human: The relative binding affinities to human recombinant estrogen receptors alpha and beta, assessed in a cell free competitive binding assay, were: iso-butylparaben > butyl paraben > isopropylparaben = propylparaben > ethylparaben (Vo et al. 2010).

Referenes cited in "Isobutyl paraben"

Fujino C, Watanabe Y, Sanoh S, Hattori S, Nakajima H, Uramaru N, et al. 2019. Comparative study of the effect of 17 parabens on pxr-, car- and pparalpha-mediated transcriptional activation. Food Chem Toxicol 133:110792.

Gonzalez TL, Moos RK, Gersch CL, Johnson MD, Richardson RJ, Koch HM, et al. 2018. Metabolites of n-butylparaben and iso-butylparaben exhibit estrogenic properties in mcf-7 and t47d human breast cancer cell lines. Toxicol Sci 164:50-59.

Health Canada. 2020. Draft Screening Assessment Parabens Group. Available: https://www.canada.ca/content/dam/eccc/documents/pdf/pded/parabens/Draft-screening-assessment-parabens-group.pdf. [accessed 13 September 2020].

Kawaguchi M, Irie K, Morohoshi K, Watanabe G, Taya K, Morita M, et al. 2009a. Maternal isobutyl-paraben exposure alters anxiety and passive avoidance test performance in adult male rats. Neurosci Res 65:136-140.

Kawaguchi M, Morohoshi K, Masuda J, Watanabe G, Morita M, Imai H, et al. 2009b. Maternal isobutyl-paraben exposure decreases the plasma corticosterone level in dams and sensitivity to estrogen in female offspring rats. J Vet Med Sci 71:1027-1033.

Kawaguchi M, Morohoshi K, Imai H, Morita M, Kato N, Himi T. 2010. Maternal exposure to isobutyl-paraben impairs social recognition in adult female rats. Exp Anim 59:631-635.

Kim SM, Jung EM, An BS, Hwang I, Vo TT, Kim SR, et al. 2012. Additional effects of bisphenol a and paraben on the induction of calbindin-D_{9k} and progesterone receptor via an estrogen receptor pathway in rat pituitary GH3 cells. J Physiol Pharmacol 63:445-455.

US FDA (Food and Drug Administration). 2020. Voluntary Cosmetic Registration Program. Available: https://www.fda.gov/cosmetics/voluntary-cosmetic-registration-program [accessed 2 September 2020].

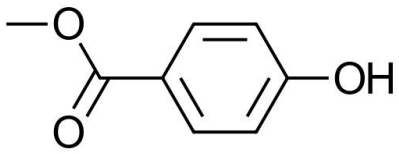
Vo TT, Jeung EB. 2009. An evaluation of estrogenic activity of parabens using uterine calbindin-d9k gene in an immature rat model. Toxicol Sci 112:68-77.

Vo TT, Yoo YM, Choi KC, Jeung EB. 2010. Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model. Reprod Toxicol 29:306-316.

Yang YJ, Hong YP, Chae SA. 2016. Reduction in semen quality after mixed exposure to bisphenol a and isobutylparaben *in utero* and during lactation periods. Hum Exp Toxicol 35:902-911.

Methyl paraben

(Methyl p-hydroxybenzoate, methyl 4-hydroxybenzoate, CAS No. 99-76-3)



Methyl paraben is a member of the class of parabens. It is an anti-fungal agent approved by the US Environmental Protection Agency (US EPA) as a preservative in pesticides, food, beverages, cosmetics, personal care products, topical preparations, and parenteral solutions (US EPA 2005). According to the report by Cherian et al. (2020), in 2019 the US Food and Drug Administration Voluntary Cosmetic Registration Program listed a total of 11,739 cosmetic products containing methyl paraben, 9,347 of which are "leave-on" formulations (products left on skin or hair until next wash).

Methyl paraben passed the human and animal data screens, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies that were identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Numerous human studies reporting developmental and reproductive toxicity (DART)-related effects associated with methyl paraben were identified in the recent literature. A number of DART findings reported in epidemiologic studies published within the last nine years are summarized here. The findings are organized by groups of outcomes.

Developmental effects

In the studies below, developmental effects, including birth outcomes, are associated with prenatal exposures unless otherwise specified.

- Methyl paraben concentrations were associated with an increase in gestational age (prospective cohort study) (Aker et al. 2019b).
- Meconium methyl paraben was associated with preterm birth, decreased gestational age and birthweight, and child attention-deficit hyperactivity disorder (ADHD) at age 6-7 years (cross-sectional and prospective cohort study) (Baker et al. 2020).

- Maternal methyl paraben levels were associated with lower T helper 1 cells (Th1) and marginally lower T helper 2 cells (Th2) (prospective cohort study) (Berger et al. 2018).
- Pregnant women in the group with methyl paraben levels above the third quartile had neonates with lower body weight than those with lower methyl paraben levels (prospective cohort study) (Chang et al. 2019).
- One log-unit increase of maternal methyl paraben led to a marginally significant increase in head circumference at birth (prospective cohort study) (Jamal et al. 2020).
- Each 2-fold increase in average prenatal methyl paraben concentration was associated with lower Major Depression Inventory (MDI) scores among girls (prospective cohort study) (Jiang et al. 2019).
- Higher maternal urinary levels of methyl paraben were positively associated with birth length in boys; no significant associations with birth length were observed in girls (prospective cohort study) (Wu et al. 2017).

Female reproductive effects

- Methyl paraben was marginally associated with decreased sex hormone binding globulin (SHBG) in pregnant women (cross-sectional study) (Aker et al. 2019a).
- Meconium methyl paraben was associated with lower maternal thyroid stimulating hormone and T_3, and increased total thyroxine (T_4) (cross-sectional study) (Baker et al. 2020).
- In pregnant women, urinary methyl paraben was associated with increased T_3 and marginally associated with increased T_4. Gestational age-specific multivariate regression analyses showed that the magnitude and direction of some of the observed associations were dependent on the timing of exposure (cross-sectional study) (Aker et al. 2018).
- Earlier breast development, pubic hair development and menarche were associated with higher prenatal methyl paraben exposures (prospective cohort study) (Harley et al. 2019).
- Urinary methyl paraben concentrations were not associated with IVF outcomes, specifically total and mature oocyte counts, proportion of high embryo quality, and rates of fertilization, implantation, pregnancy, and live birth (prospective cohort study) (Minguez-Alarcon et al. 2016).
- The highest quartile urinary methyl paraben concentrations were associated with reduction in fecundity (prospective cohort study) (Smarr et al. 2017).
- Urinary concentrations of methyl paraben in the first trimester of pregnancy were associated with increased gestational weight gain rate, and this association was stronger than those of the second or third trimesters (prospective cohort study) (Wen et al. 2020).

Male reproductive effects

- Compared to the lowest quartile of paternal methyl paraben concentration, concentrations of methyl paraben in the second quartile were associated with decreased odds of live birth following intrauterine insemination (prospective cohort study) (Dodge et al. 2015).
- No significant association of methyl paraben and sperm chromosome disomy (cross-sectional study) (Jurewicz et al. 2017).
- Cord blood levels of methyl paraben were inversely associated with testosterone levels (cross-sectional study) (Kolatorova et al. 2018).
- No significant associations between methyl paraben and serum hormone levels, semen quality parameters, or sperm DNA damage (cross-sectional study) (Meeker et al. 2011).
- No significant association was found between methyl paraben and semen parameters (cross-sectional study) (Nishihama et al. 2017).
- Methyl paraben was associated with diminished sperm count and several sperm motility parameters (prospective cohort study) (Smarr et al. 2018).

Animal studies

Relevant whole animal studies examining possible DART effects of exposure to methyl paraben were identified. Findings reported in these studies are summarized here.

Developmental effects

- Zebrafish embryos: Decreased heart rate and hatching rate and defects including pericardial edema blood cell accumulation and bent spine (Dambal et al. 2017).
- Zebrafish embryos: Alterations in developmental landmarks such as heart rate and hatching percentage (Luzeena Raja et al. 2019).

Female reproductive effects

- Rat: Perinatal exposure to methyl paraben induced measurable changes in both mammary histology (by Masson's Trichrome Stain) and transcriptome (by microarrays) (Gopalakrishnan et al. 2017).
- Rat: Postnatal exposure to methyl paraben caused morphological/histological changes in mammary glands (Manservisi et al. 2015).
- Rat: Methyl paraben administered to 8-week-old female rats caused increased diestrus phase in treated animal and increased mRNA levels of Amh, Star and Cyp11a1 genes in primordial follicles. Methyl paraben also induced an increase in follicle-stimulating hormone (FSH) levels in serum and significantly decreased the total number of follicles (Lee et al. 2017).

- Rat: A high dose of methyl paraben (1000 mg/kg body weight/day) to prepubertal female rats resulted in a significant delay in the date of vaginal opening and a decrease in length of the estrous cycle following oral treatment from postnatal day 21-40 (Vo et al. 2010).
- Gerbil: Exposure of adult female gerbils resulted in morphological changes in the Skene's paraurethral glands, which are the female counterpart to the male prostate gland, including epithelial hyperplasia, increased cell proliferation, a higher frequency of androgen receptor (AR)-positive cells, stromal inflammatory infiltration, and intraepithelial neoplasia foci (Costa et al. 2017).

Male reproductive effects

- Rat: Exposure of 22-day-old rats to methyl paraben in diets for 8 weeks had no effect on histopathology or organ weights of reproductive tissues, sperm production, motility, morphology or reproductive hormone levels (Hoberman et al. 2008).
- Rat: Dietary exposure for 8 weeks did not cause any treatment-related effects on the male reproductive system (Oishi 2004).
- Gerbil: Exposure of adult male gerbils resulted in morphological changes in the prostate tissues, including epithelial hyperplasia, increased cell proliferation, and a higher frequency of androgen receptor (AR)-positive cells (Costa et al. 2017).

Mechanistic, in vitro, and other relevant data

- Human primary granulosa cell cultures: No effect on progesterone production or the expression of genes controlling steroid production (Herrera-Cogco et al. 2020).

References cited in "Methyl paraben"

Aker AM, Johns L, McElrath TF, Cantonwine DE, Mukherjee B, Meeker JD. 2018. Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: A repeated measures study. Environ Int 113:341-349.

Aker AM, Ferguson KK, Rosario ZY, Mukherjee B, Alshawabkeh AN, Calafat AM, et al. 2019a. A repeated measures study of phenol, paraben and triclocarban urinary biomarkers and circulating maternal hormones during gestation in the Puerto Rico PROTECT cohort. Environ Health 18:28.

Aker AM, Ferguson KK, Rosario ZY, Mukherjee B, Alshawabkeh AN, Cordero JF, et al. 2019b. The associations between prenatal exposure to triclocarban, phenols and parabens with gestational age and birth weight in northern Puerto Rico. Environ Res 169:41-51.

Baker BH, Wu H, Laue HE, Boivin A, Gillet V, Langlois MF, et al. 2020. Methylparaben in meconium and risk of maternal thyroid dysfunction, adverse birth outcomes, and attention-deficit hyperactivity disorder (ADHD). Environ Int 139:105716.

Berger K, Eskenazi B, Balmes J, Holland N, Calafat AM, Harley KG. 2018. Associations between prenatal maternal urinary concentrations of personal care product chemical biomarkers and childhood respiratory and allergic outcomes in the CHAMACOS study. Environ Int 121:538-549.

Chang CH, Wang PW, Liang HW, Huang YF, Huang LW, Chen HC, et al. 2019. The sex-specific association between maternal paraben exposure and size at birth. Int J Hyg Environ Health 222:955-964.

Cherian P, Zhu J, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, et al. 2020. Amended safety assessment of parabens as used in cosmetics. Int J Toxicol 39:5s-97s.

Costa JR, Campos MS, Lima RF, Gomes LS, Marques MR, Taboga SR, et al. 2017. Endocrine-disrupting effects of methylparaben on the adult gerbil prostate. Environ Toxicol 32:1801-1812.

Dambal VY, Selvan KP, Lite C, Barathi S, Santosh W. 2017. Developmental toxicity and induction of vitellogenin in embryo-larval stages of zebrafish (*Danio rerio*) exposed to methyl paraben. Ecotoxicol Environ Saf 141:113-118.

Dodge LE, Williams PL, Williams MA, Missmer SA, Toth TL, Calafat AM, et al. 2015. Paternal urinary concentrations of parabens and other phenols in relation to reproductive outcomes among couples from a fertility clinic. Environ Health Perspect 123:665-671.

Gopalakrishnan K, Teitelbaum SL, Lambertini L, Wetmur J, Manservisi F, Falcioni L, et al. 2017. Changes in mammary histology and transcriptome profiles by low-dose exposure to environmental phenols at critical windows of development. Environ Res 152:233-243.

Harley KG, Berger KP, Kogut K, Parra K, Lustig RH, Greenspan LC, et al. 2019. Association of phthalates, parabens and phenols found in personal care products with pubertal timing in girls and boys. Hum Reprod 34:109-117.

Herrera-Cogco E, Lopez-Bayghen B, Hernandez-Melchor D, Lopez-Luna A, Palafox-Gomez C, Ramirez-Martinez L, et al. 2020. Paraben concentrations found in human body fluids do not exert steroidogenic effects in human granulosa primary cell cultures. Toxicol Mech Methods 30:336-349.

Hoberman AM, Schreur DK, Leazer T, Daston GP, Carthew P, Re T, et al. 2008. Lack of effect of butylparaben and methylparaben on the reproductive system in male rats. Birth Defects Res B Dev Reprod Toxicol 83:123-133.

Jamal A, Rastkari N, Dehghaniathar R, Nodehi RN, Nasseri S, Kashani H, et al. 2020. Prenatal urinary concentrations of environmental phenols and birth outcomes in the mother-infant pairs of Tehran environment and neurodevelopmental disorders (TEND) cohort study. Environ Res 184:109331.

Jiang Y, Zhao H, Xia W, Li Y, Liu H, Hao K, et al. 2019. Prenatal exposure to benzophenones, parabens and triclosan and neurocognitive development at 2 years. Environ Int 126:413-421.

Jurewicz J, Radwan M, Wielgomas B, Klimowska A, Kaluzny P, Radwan P, et al. 2017. Environmental exposure to parabens and sperm chromosome disomy. Int J Environ Health Res 27:332-343.

Kolatorova L, Vitku J, Hampl R, Adamcova K, Skodova T, Simkova M, et al. 2018. Exposure to bisphenols and parabens during pregnancy and relations to steroid changes. Environ Res 163:115-122.

Lee JH, Lee M, Ahn C, Kang HY, Tran DN, Jeung EB. 2017. Parabens accelerate ovarian dysfunction in a 4-vinylcyclohexene diepoxide-induced ovarian failure model. Int J Environ Res Public Health 14.

Luzeena Raja G, Divya Subhashree K, Lite C, Santosh W, Barathi S. 2019. Transient exposure of methylparaben to zebrafish (*Danio rerio*) embryos altered cortisol level, acetylcholinesterase activity and induced anxiety-like behaviour. Gen Comp Endocrinol 279:53-59.

Manservisi F, Gopalakrishnan K, Tibaldi E, Hysi A, Iezzi M, Lambertini L, et al. 2015. Effect of maternal exposure to endocrine disrupting chemicals on reproduction and mammary gland development in female Sprague-Dawley rats. Reprod Toxicol 54:110-119.

Meeker JD, Yang T, Ye X, Calafat AM, Hauser R. 2011. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. Environ Health Perspect 119:252-257.

Minguez-Alarcon L, Chiu YH, Messerlian C, Williams PL, Sabatini ME, Toth TL, et al. 2016. Urinary paraben concentrations and *in vitro* fertilization outcomes among women from a fertility clinic. Fertil Steril 105:714-721.

Nishihama Y, Toshima H, Yoshinaga J, Mizumoto Y, Yoneyama M, Nakajima D, et al. 2017. Paraben exposure and semen quality of japanese male partners of subfertile couples. Environ Health Prev Med 22:5.

Oishi S. 2004. Lack of spermatotoxic effects of methyl and ethyl esters of p-hydroxybenzoic acid in rats. Food Chem Toxicol 42:1845-1849.

Smarr MM, Sundaram R, Honda M, Kannan K, Louis GM. 2017. Urinary concentrations of parabens and other antimicrobial chemicals and their association with couples' fecundity. Environ Health Perspect 125:730-736.

Smarr MM, Honda M, Kannan K, Chen Z, Kim S, Louis GMB. 2018. Male urinary biomarkers of antimicrobial exposure and bi-directional associations with semen quality parameters. Reprod Toxicol 77:103-108.

US EPA (US Environmental Protection Agency). 2005. Inert Reassessment of Methyl p-hydroxybenzoate. Available: https://www.epa.gov/ingredients-used-pesticide-products/inert-reassessment-document-methyl-p-hydroxybenzoate-cas-no-99 [accessed 2 September 2020].

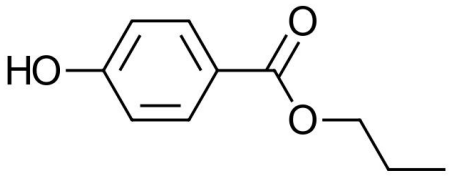
Vo TT, Yoo YM, Choi KC, Jeung EB. 2010. Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model. Reprod Toxicol 29:306-316.

Wen Q, Zhou Y, Wang Y, Li J, Zhao H, Liao J, et al. 2020. Association between urinary paraben concentrations and gestational weight gain during pregnancy. J Expo Sci Environ Epidemiol.

Wu C, Huo W, Li Y, Zhang B, Wan Y, Zheng T, et al. 2017. Maternal urinary paraben levels and offspring size at birth from a chinese birth cohort. Chemosphere 172:29-36.

Propyl paraben

(Propyl p-hydroxybenzoate, propyl 4-hydroxybenzoate, CAS No. 94-13-3)



Propyl paraben is a member of the class of parabens. It occurs as a natural substance found in many plants and some insects, and is also manufactured synthetically for use in cosmetics and pharmaceuticals (Health Canada 2020; US FDA 2020). According to the report by Cherian et al. (2020), in 2019 the US Food and Drug Administration Voluntary Cosmetic Registration Program listed a total of 9,034 cosmetic products containing propyl paraben, 7,520 of which are leave-on formulations (products left on skin or hair until next wash), an increase from 7,118 products reported in 2006.

Propyl paraben passed the human data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Numerous human studies reporting developmental and reproductive toxicity (DART)-related effects associated with propyl parabenwere identified in the recent literature. A number of DART findings reported in epidemiologic studies are summarized here, with an emphasis on those published within the last five years. The findings are organized by groups of outcomes.

Developmental effects

In the studies below, developmental effects, including birth outcomes, are associated with prenatal exposures unless otherwise specified.

- Maternal urinary concentrations of propyl paraben were associated with an increase in gestational age (prospective cohort study) (Aker et al. 2019b).
- Maternal propyl paraben exposure was associated with decreased body length (prospective cohort study) (Geer et al. 2017).

- Earlier genital development in boys was associated with maternal propyl paraben exposure (prospective cohort study) (Harley et al. 2019).
- In cord blood samples, propyl paraben levels were inversely associated with testosterone levels (cross-sectional study) (Kolatorova et al. 2018).
- Significant association between propyl paraben levels in placenta and the risk of male genital malformations (case-control study) (Fernández et al. 2016).
- Propyl paraben in maternal serum was associated with shorter anogenital distance in male infants, independent of body size and other putative confounders (case-control study) (Fisher et al. 2020).
- Maternal propyl paraben exposure was associated with decreased odds of probable asthma in children at age seven (prospective cohort study) (Berger et al. 2018a).

Female reproductive effects

- Propyl paraben was marginally associated with decreased sex hormone binding globulin in pregnant women (cross-sectional study) (Aker et al. 2019a).
- In pregnant women, urinary concentrations of propyl paraben were associated with decreased serum levels of free thyroxine (FT_4) (cross-sectional study) (Aker et al. 2018).
- In pregnant women, urinary concentrations of propyl paraben were associated with decreased serum levels of thyroid-stimulating hormone (prospective cohort study) (Berger et al. 2018b).
- Urinary concentrations of propyl paraben were associated with decreased antral follicle counts and estradiol levels, and increased serum concentrations of follicle-stimulating hormone (FSH) (prospective cohort study) (Jurewicz et al. 2020).
- Non-linear associations of propyl paraben with gestational diabetes mellitus in women who were overweight/obese before pregnancy (cross-sectional study) (Li et al. 2019).
- Urinary propyl paraben concentrations were not associated with *in vitro* fertilization (IVF) outcomes, including total and mature oocyte counts, proportion of high embryo quality, and fertilization rates. No significant associations were found between urinary paraben concentrations and rates of implantation, clinical pregnancy, and live births (prospective cohort study) (Minguez-Alarcon et al. 2016).
- Suggestive trend but marginal association of propyl paraben exposure with lower antral follicle count (prospective cohort study) (Smith et al. 2013).
- Maternal urinary levels of propyl paraben in the first trimester were associated with an increased first-trimester gestational weight gain rate. This association

was stronger than those of the second or third trimesters and stronger among overweight/obese women (prospective cohort study) (Wen et al. 2020).

Male reproductive effects

- Positive association between urinary level of propyl paraben and disomy of chromosome 13 (cross-sectional study) (Jurewicz et al. 2017).
- No associations between propyl paraben and markers of male reproductive health, including serum hormone levels, semen quality parameters, and sperm DNA damage (cross-sectional study) (Meeker et al. 2011).
- No association between urinary propyl paraben concentrations and semen parameters among male partners of couples who visited a gynecology clinic for infertility consultation (cross-sectional study) (Nishihama et al. 2017).

Animal studies

Whole animal studies examining possible DART effects of exposure to propyl paraben were identified. Findings reported in these studies are summarized here.

Developmental effects

- Mouse: Exposure on gestational days 1-4 had no impact on the number of implantation sites observed (Shaw and deCatanzaro 2009).
- Zebrafish: Propyl paraben was not toxic to zebrafish embryos at concentrations up to 1000 µg/L (Torres et al. 2016).

Female reproductive effects

- Rat: Propyl paraben administered to 8-week-old females caused increased duration of diestrus phases, increased FSH levels in serum and decreased total number of ovarian follicles. It also increased the mRNA level of Amh gene but had no effect on the mRNA levels of Foxl2 and Kitlg genes in primordial follicles. (Lee et al. 2017).
- Rat: Exposure to propyl paraben at doses up to 1000 mg/kg-day from PND 21-40 caused myometrial hypertrophy in females in a dose-dependent manner (Vo et al. 2010).
- Rat: Exposure of females at doses up to 1000 mg/kg-day on postnatal days 4-90 had no effect on reproductive development or function (Sivaraman et al. 2018).

Male reproductive effects

- Rat: In a Hershberger bioassay, propyl paraben decreased the organ weights of all accessory sex organs, increased serum levels of luteinizing hormone, reduced serum levels of FSH, and cause histopathological changes such atrophy, hyalinization, and anastomosis in androgenic tissues (Ozdemir et al. 2018).

References cited in "Propyl paraben"

Aker AM, Johns L, McElrath TF, Cantonwine DE, Mukherjee B, Meeker JD. 2018. Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: A repeated measures study. Environ Int 113:341-349.

Aker AM, Ferguson KK, Rosario ZY, Mukherjee B, Alshawabkeh AN, Calafat AM, et al. 2019a. A repeated measures study of phenol, paraben and triclocarban urinary biomarkers and circulating maternal hormones during gestation in the Puerto Rico protect cohort. Environ Health 18:28.

Aker AM, Ferguson KK, Rosario ZY, Mukherjee B, Alshawabkeh AN, Cordero JF, et al. 2019b. The associations between prenatal exposure to triclocarban, phenols and parabens with gestational age and birth weight in northern Puerto Rico. Environ Res 169:41-51.

Berger K, Eskenazi B, Balmes J, Holland N, Calafat AM, Harley KG. 2018a. Associations between prenatal maternal urinary concentrations of personal care product chemical biomarkers and childhood respiratory and allergic outcomes in the chamacos study. Environ Int 121:538-549.

Berger K, Gunier RB, Chevrier J, Calafat AM, Ye X, Eskenazi B, et al. 2018b. Associations of maternal exposure to triclosan, parabens, and other phenols with prenatal maternal and neonatal thyroid hormone levels. Environ Res 165:379-386.

Cherian P, Zhu J, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, et al. 2020. Amended safety assessment of parabens as used in cosmetics. Int J Toxicol 39:5s-97s.

Fernández MF, Arrebola JP, Jiménez-Díaz I, Sáenz JM, Molina-Molina JM, Ballesteros O, et al. 2016. Bisphenol a and other phenols in human placenta from children with cryptorchidism or hypospadias. Reprod Toxicol 59:89-95.

Fisher BG, Thankamony A, Mendiola J, Petry CJ, Frederiksen H, Andersson AM, et al. 2020. Maternal serum concentrations of bisphenol a and propyl paraben in early pregnancy are associated with male infant genital development. Hum Reprod 35:913-928.

Geer LA, Pycke BFG, Waxenbaum J, Sherer DM, Abulafia O, Halden RU. 2017. Association of birth outcomes with fetal exposure to parabens, triclosan and triclocarban in an immigrant population in Brooklyn, New York. J Hazard Mater 323:177-183.

Harley KG, Berger KP, Kogut K, Parra K, Lustig RH, Greenspan LC, et al. 2019. Association of phthalates, parabens and phenols found in personal care products with pubertal timing in girls and boys. Hum Reprod 34:109-117.

Health Canada H. 2020. Draft Screening Assessment Parabens Group. Available: https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/draft-screening-assessment-parabens-group.html. [accessed 10 September 2020]

Jurewicz J, Radwan M, Wielgomas B, Klimowska A, Kaluzny P, Radwan P, et al. 2017. Environmental exposure to parabens and sperm chromosome disomy. Int J Environ Health Res 27:332-343.

Jurewicz J, Radwan M, Wielgomas B, Karwacka A, Klimowska A, Kaluzny P, et al. 2020. Parameters of ovarian reserve in relation to urinary concentrations of parabens. Environ Health 19:26.

Kolatorova L, Vitku J, Hampl R, Adamcova K, Skodova T, Simkova M, et al. 2018. Exposure to bisphenols and parabens during pregnancy and relations to steroid changes. Environ Res 163:115-122.

Lee JH, Lee M, Ahn C, Kang HY, Tran DN, Jeung EB. 2017. Parabens accelerate ovarian dysfunction in a 4-vinylcyclohexene diepoxide-induced ovarian failure model. Int J Environ Res Public Health 14.

Li Y, Xu S, Li Y, Zhang B, Huo W, Zhu Y, et al. 2019. Association between urinary parabens and gestational diabetes mellitus across prepregnancy body mass index categories. Environ Res 170:151-159.

Meeker JD, Yang T, Ye X, Calafat AM, Hauser R. 2011. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. Environ Health Perspect 119:252-257.

Minguez-Alarcon L, Chiu YH, Messerlian C, Williams PL, Sabatini ME, Toth TL, et al. 2016. Urinary paraben concentrations and *in vitro* fertilization outcomes among women from a fertility clinic. Fertil Steril 105:714-721.

Nishihama Y, Toshima H, Yoshinaga J, Mizumoto Y, Yoneyama M, Nakajima D, et al. 2017. Paraben exposure and semen quality of japanese male partners of subfertile couples. Environ Health Prev Med 22:5.

Ozdemir E, Barlas N, Cetinkaya MA. 2018. Assessing the antiandrogenic properties of propyl paraben using the Hershberger bioassay. Toxicol Res (Camb) 7:235-243.

Shaw J, deCatanzaro D. 2009. Estrogenicity of parabens revisited: Impact of parabens on early pregnancy and an uterotrophic assay in mice. Reprod Toxicol 28:26-31.

Sivaraman L, Pouliot L, Wang B, Brodie T, Graziano M, McNerney ME. 2018. Safety assessment of propylparaben in juvenile rats. Regul Toxicol Pharmacol 92:370-381.

Smith KW, Souter I, Dimitriadis I, Ehrlich S, Williams PL, Calafat AM, et al. 2013. Urinary paraben concentrations and ovarian aging among women from a fertility center. Environ Health Perspect 121:1299-1305.

Torres T, Cunha I, Martins R, Santos MM. 2016. Screening the toxicity of selected personal care products using embryo bioassays: 4-mbc, propylparaben and triclocarban. Int J Mol Sci 17.

US FDA (Food and Drug Administration). 2020. Voluntary Cosmetic Registration Program. Available: https://www.fda.gov/cosmetics/voluntary-cosmetic-registration-program. [accessed 2 September 2020].

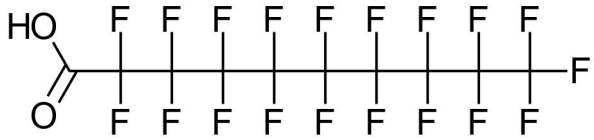
Vo TT, Yoo YM, Choi KC, Jeung EB. 2010. Potential estrogenic effect(s) of parabens at the prepubertal stage of a postnatal female rat model. Reprod Toxicol 29:306-316.

Wen Q, Zhou Y, Wang Y, Li J, Zhao H, Liao J, et al. 2020. Association between urinary paraben concentrations and gestational weight gain during pregnancy. J Expo Sci Environ Epidemiol 30:845–855.

Per- and polyfluorinated substances (PFASs):

Perfluorodecanoic acid (PFDA)

(Nonadecafluorodecanoic acid, CAS No. 335-76-2)



PFDA is a perfluorinated organic compound with surfactant properties, and a member of a large group of substances collectively called per- and polyfluorinated substances (PFASs). PFASs are commonly used to make products resistant to stains, grease, soil and water, and are used in various industries. PFASs are global pollutants of air, water, soil and wildlife, and are very persistent in the environment.

Human biomonitoring studies indicate that exposure to PFDA is widespread. For example, Table 4 below summarizes data on serum concentrations of PFDA (geometric mean and 95% confidence interval [CI]) measured in studies conducted by the Biomonitoring California Program between 2010 and 2018 (Biomonitoring California 2020).

Table 4. PFDA serum concentrations (ng/ml) in studies of California residents. Data from Biomonitoring California (https://biomonitoring.ca.gov/) (Biomonitoring California 2020).

Project	Sample Year	Geometric mean (ng/ml)	95% Lower CI	95% Upper CI	N	Detection Frequency
California Teachers Study (CTS)	2011	0.22	0.21	0.23	1759	94.70%
Firefighter Occupational Exposures (FOX) Project	2010 to 2011	0.899	0.783	1.03	101	100%
Measuring Analytes in Maternal Archived Samples (MAMAS)	2012 to 2015	0.198	0.174	0.226	200	83%
Biomonitoring Exposures Study (BEST) - 1.Pilot	2011 to 2012	0.245	0.216	0.278	110	100%
Biomonitoring Exposures Study (BEST) - 2.Expanded	2013	0.188	0.173	0.205	337	82.50%
Asian/Pacific Islander Community Exposures (ACE) Project - ACE 1	2016	0.477	0.406	0.559	96	80.20%
Asian/Pacific Islander Community Exposures (ACE) Project - ACE 2	2017	0.559	0.49	0.636	99	87.90%
California Regional Exposure Study, Los Angeles County (CARE-LA)	2018	0.0967	0.0894	0.105	425	69.20%

Reports from other human biomonitoring studies of PFDA include:

- Serum concentration level (μg/L; geometric mean and 95% CI) reported by the National Health and Nutrition Examination Survey (NHANES) in 2009 to 2010 for males aged 20 years or older was 0.30 (0.28, 0.34) (Dobraca et al. 2015).
- The unadjusted geometric mean and 95% CI for PFDA serum levels (ng/mL) in US children aged 3–11 years, from data reported by NHANES for 2013 to 2014, was 0.09 (0.08 − 0.1) (Jain 2018).
- PFDA was detected in the serum of 68% of mothers in the Northern California CHARGE (CHildhood Autism Risk from Genetics and Environment) case-control study (serum collected 2009 – 2016) (Kim et al. 2020).

PFDA passed the human data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Numerous human studies reporting developmental and reproductive toxicity (DART)-related effects associated with PFDA were identified in the recent literature. DART findings reported in epidemiologic studies published within the last six years are summarized here. The findings are organized by groups of outcomes.

Gestation duration

- Increased risk of preterm birth (the Danish national birth cohort study) (Meng et al. 2018).

Indicators of fetal growth

- Inversely associated with birth weight (prospective cohort study) (Kashino et al. 2020; Wang et al. 2016); (longitudinal cohort) (Gyllenhammar et al. 2018; Wikström et al. 2020); and (retrospective cohort study) (Kwon et al. 2016).
- Elevated odds of small for gestational age; with lower average childhood height z-score in girls (prospective study) (Wang et al. 2016).
- Associated with lower birth weight for gestational age, and small for gestational age at birth (Swedish longitudinal cohort) (Wikström et al. 2020).

Neurodevelopmental effects

- Prenatal levels of 19 PFASs were measured in maternal blood at week 17 of gestation. No associations with attention-deficit/hyperactivity disorder symptoms, language skills or intelligence quotient (IQ). Positive associations between

verbal working memory and increasing quintiles of PFDA (prospective study) (Skogheim et al. 2020).

- PFDA was measured in maternal serum and in serum from children at ages 5 and 7 years. No associations between prenatal PFAS (including PFDA) concentrations and strengths and difficulties questionnaire (SDQ) scores, while a two-fold increase in 5 year-old serum PFDA concentrations was associated with increases in total SDQ (birth cohort in the Faroe Islands study) (Oulhote et al. 2016).
- PFDA maternal plasma concentration measured once between GWs 12 to 16 was associated with increased risk of developmental problems in personal-social skills (birth cohort) (Niu et al. 2019).

Anogenital distance, prenatal exposure

- Inversely associated with anogenital distance at birth (prospective cohort study) (Tian et al. 2019).
- Associated with a decreased anogenital distance in three months-old girls (p-value for trend <0.05) after adjusting for age and weight-for-age standard deviation score (Odense child cohort) (Lind et al. 2017).

Endocrine effects

- PFAS levels and three thyroid hormones (THs) were measured in cord blood. Thyroid stimulating hormone (TSH) levels decreased with increasing concentrations of PFDA (cross-sectional study) (Aimuzi et al. 2019).
- Levels of PFDA and THs were measured in maternal blood samples collected between gestational weeks (GWs) 5 and 19. Higher PFDA levels were associated with higher TSH levels before GW 10 and with lower TSH levels at or after GW 10. PFDA was correlated with lower free thyroxine levels before GW 8 and high free thyoxine levels thereafter (cross-sectional study) (Inoue et al. 2019).
- PFDA was measured in the third trimester and THs levels in cord serum in 116 neonates. PFDA was associated with lower cord total triiodothyronine (T_3) (prospective birth cohort study) (Wang et al. 2014).
- PFDA and THs were measured in serum from pregnant women in their second trimester and 3 days and 6 weeks after delivery (Berg et al. 2015) and in addition, TSH concentration was analyzed in newborns in heel-prick samples at the two visits after delivery (Berg et al. 2017). PFDA was inversely associated with mean maternal T_3 and free T_3 (prospective study) (Berg et al. 2015; Berg et al. 2017).
- Thyroid hormones were analyzed in paired maternal and cord serum samples collected around delivery. Maternal PFDA negatively correlates with maternal TSH (cross sectional study in Beijing) (Yang et al. 2016).

- PFDA in cord blood was associated with higher placental aromatase levels (Chinese prospective birth cohort) (Yao et al. 2019).
- Maternal serum PFDA was measured once between gestational weeks 10 and 15. Serum concentrations of dehydroepiandrosterone (DHEA), dehydroepiandrosterone-sulfate, androstenedione, 17-hydroxyprogesterone, testosterone, luteinizing hormone and follicle stimulating hormone were measured in children (44% girls; 56% boys) at a mean age of 3.9 months. A two-fold increase in maternal PFDA concentration was associated with a 19.6% reduction in DHEA in daughters (prospective Odense child cohort) (Jensen et al. 2020).

Female reproductive effects

- Associated with longer menstrual cycles (prospective pregnancy cohort study) (Lum et al. 2017).
- Associated with a greater risk of miscarriage (nested case-control in the Odense child cohort study) (Jensen et al. 2015).

Male reproductive effects

- Associated with lower testosterone levels (cross-sectional study) (Zhou et al. 2016).

Other DART effects

Puberty

- Prenatal exposure to PFDA was associated with a lower mean age of puberty onset in girls, and a higher mean age of puberty onset in boys (puberty cohort, nested within the Danish National birth cohort) (Ernst et al. 2019).

Atopic dermatitis

- The highest quartile of cord plasma (at parturition) of PFDA was associated with with atopic dermatitis (prospective birth cohort study) (Chen et al. 2018).

Epigenetic

- PFDA, telomere length at birth and reactive oxygen species were measured in umbilical cord blood of 581 newborns. PFDA was associated with shorter telomere length and elevated reactive oxygen species levels (prospective cohort study) (Liu et al. 2018).

Animal studies

A limited number of whole animal studies examining possible DART effects of exposure to PFDA were identified. Findings reported in these studies are summarized here.

Developmental effects

- Mice: Decreased fetal viability and reduced fetal body weight (Harris and Birnbaum 1989).

Endocrine effects

- Rats: Exposure of sexually mature males resulted in decreased plasma testosterone and 5α-dihydrotestosterone in males. Secondary to the decrease in plasma androgen concentrations were dose-related decreases in the weights and epithelial heights of accessory sex organs. Plasma luteinizing hormone concentrations were not significantly altered by PFDA treatment (Bookstaff et al. 1990).
- Zebrafish: Fertilized zebrafish eggs were exposed to PFDA until hatching. Steroid hormones were measured in whole blood. There was an increased ratio of estradiol to testosterone and estradiol to 11-ketotestosterone in males (Jo et al. 2014).

Mechanistic, in vitro, and other relevant data

- Mouse: In hepatocytes exposed *in vitro*, PFDA increased DNA strand breaks and DNA oxidative damage, as assessed by the comet assay (Xu et al. 2019).
- Pig: Negatively impacted oocyte viability and maturation *in vitro*; higher levels of intracellular calcium relative to control oocytes (Domínguez et al. 2019).
- Rat: In males exposed *in vivo* it was reported that PFDA displaced radiolabeled thyroxine from albumin with an affinity similar to thyroxine (Gutshall et al. 1989).
- Chinese hamster ovary cell line: Concentration-dependent androgen receptor antagonist inhibitory concentration $[IC]_{85} = 2.4x10^{-5}$ M (Kjeldsen and Bonefeld-Jørgensen 2013).
- Human choriocarcinoma, JEG-3 cell line: Slight decrease in aromatase activity at 10^{-5} M (Kjeldsen and Bonefeld-Jørgensen 2013).

References cited in "PFDA"

Aimuzi R, Luo K, Chen Q, Wang H, Feng L, Ouyang F, et al. 2019. Perfluoroalkyl and polyfluoroalkyl substances and fetal thyroid hormone levels in umbilical cord blood among newborns by prelabor caesarean delivery. Environ Int 130:104929.

Berg V, Nøst TH, Hansen S, Elverland A, Veyhe AS, Jorde R, et al. 2015. Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach. Environ Int 77:63-69.

Berg V, Nøst TH, Pettersen RD, Hansen S, Veyhe AS, Jorde R, et al. 2017. Persistent organic pollutants and the association with maternal and infant thyroid homeostasis: A multipollutant assessment. Environ Health Perspect 125:127-133.

Biomonitoring California. 2020. Available: https://biomonitoring.ca.gov/results/chemical/2183?field_chemical_name_target_id_selective%5B%5D=158&field_chemical_name_target_id_selective%5B%5D=161&field_chemical_name_target_id_selective%5B%5D=162&field_chemical_name_target_id_selective%5B%5D=1345 [accessed 28 August 2020].

Bookstaff RC, Moore RW, Ingall GB, Peterson RE. 1990. Androgenic deficiency in male rats treated with perfluorodecanoic acid. Toxicology and Applied Pharmacology 104:322-333.

Chen Q, Huang R, Hua L, Guo Y, Huang L, Zhao Y, et al. 2018. Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and childhood atopic dermatitis: A prospective birth cohort study. Environ Health 17:8.

Dobraca D, Israel L, McNeel S, Voss R, Wang M, Gajek R, et al. 2015. Biomonitoring in California firefighters: Metals and perfluorinated chemicals. J Occup Environ Med 57:88-97.

Domínguez A, Salazar Z, Betancourt M, Ducolomb Y, Casas E, Fernández F, et al. 2019. Effect of perfluorodecanoic acid on pig oocyte viability, intracellular calcium levels and gap junction intercellular communication during oocyte maturation *in vitro*. Toxicol In Vitro 58:224-229.

Ernst A, Brix N, Lauridsen LLB, Olsen J, Parner ET, Liew Z, et al. 2019. Exposure to perfluoroalkyl substances during fetal life and pubertal development in boys and girls from the Danish national birth cohort. Environ Health Perspect 127:17004.

Gutshall DM, Pilcher GD, Langley AE. 1989. Mechanism of the serum thyroid hormone lowering effect of perfluoro-n-decanoic acid (PFDA) in rats. J Toxicol Environ Health 28:53-65.

Gyllenhammar I, Diderholm B, Gustafsson J, Berger U, Ridefelt P, Benskin JP, et al. 2018. Perfluoroalkyl acid levels in first-time mothers in relation to offspring weight gain and growth. Environ Int 111:191-199.

Harris MW, Birnbaum LS. 1989. Developmental toxicity of perfluorodecanoic acid in C57BL/6N mice. Fundam Appl Toxicol 12:442-448.

Inoue K, Ritz B, Andersen SL, Ramlau-Hansen CH, Hoyer BB, Bech BH, et al. 2019. Perfluoroalkyl substances and maternal thyroid hormones in early pregnancy; findings in the Danish national birth cohort. Environ Health Perspect 127:117002.

Jain RB. 2018. Contribution of diet and other factors to the observed levels of selected perfluoroalkyl acids in serum among US children aged 3-11 years. Environ Res 161:268-275.

Jensen RC, Glintborg D, Gade Timmermann CA, Nielsen F, Kyhl HB, Frederiksen H, et al. 2020. Prenatal exposure to perfluorodecanoic acid is associated with lower circulating concentration of adrenal steroid metabolites during mini puberty in human female infants. The Odense child cohort. Environ Res 182:109101.

Jensen TK, Andersen LB, Kyhl HB, Nielsen F, Christesen HT, Grandjean P. 2015. Association between perfluorinated compound exposure and miscarriage in Danish pregnant women. PLoS One 10:e0123496.

Jo A, Ji K, Choi K. 2014. Endocrine disruption effects of long-term exposure to perfluorodecanoic acid (PFDA) and perfluorotridecanoic acid (pftrda) in zebrafish (*Danio rerio*) and related mechanisms. Chemosphere 108:360-366.

Kashino I, Sasaki S, Okada E, Matsuura H, Goudarzi H, Miyashita C, et al. 2020. Prenatal exposure to 11 perfluoroalkyl substances and fetal growth: A large-scale, prospective birth cohort study. Environ Int 136:105355.

Kim K, Bennett DH, Calafat AM, Hertz-Picciotto I, Shin HM. 2020. Temporal trends and determinants of serum concentrations of per- and polyfluoroalkyl substances among northern California mothers with a young child, 2009-2016. Environ Res 186:109491.

Kjeldsen LS, Bonefeld-Jørgensen EC. 2013. Perfluorinated compounds affect the function of sex hormone receptors. Environ Sci Pollut Res Int 20:8031-8044.

Kwon EJ, Shin JS, Kim BM, Shah-Kulkarni S, Park H, Kho YL, et al. 2016. Prenatal exposure to perfluorinated compounds affects birth weight through GSTM1 polymorphism. J Occup Environ Med 58:e198-205.

Lind DV, Priskorn L, Lassen TH, Nielsen F, Kyhl HB, Kristensen DM, et al. 2017. Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort. Reprod Toxicol 68:200-206.

Liu H, Chen Q, Lei L, Zhou W, Huang L, Zhang J, et al. 2018. Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances affects leukocyte telomere length in female newborns. Environ Pollut 235:446-452.

Lum KJ, Sundaram R, Barr DB, Louis TA, Buck Louis GM. 2017. Perfluoroalkyl chemicals, menstrual cycle length, and fecundity: Findings from a prospective pregnancy study. Epidemiology 28:90-98.

Meng Q, Inoue K, Ritz B, Olsen J, Liew Z. 2018. Prenatal exposure to perfluoroalkyl substances and birth outcomes; an updated analysis from the Danish national birth cohort. Int J Environ Res Public Health 15.

Niu J, Liang H, Tian Y, Yuan W, Xiao H, Hu H, et al. 2019. Prenatal plasma concentrations of perfluoroalkyl and polyfluoroalkyl substances and neuropsychological development in children at four years of age. Environ Health 18:53.

Oulhote Y, Steuerwald U, Debes F, Weihe P, Grandjean P. 2016. Behavioral difficulties in 7-year old children in relation to developmental exposure to perfluorinated alkyl substances. Environ Int 97:237-245.

Skogheim TS, Villanger GD, Weyde KVF, Engel SM, Surén P, Øie MG, et al. 2020. Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children. Int J Hyg Environ Health 223:80-92.

Tian Y, Liang H, Miao M, Yang F, Ji H, Cao W, et al. 2019. Maternal plasma concentrations of perfluoroalkyl and polyfluoroalkyl substances during pregnancy and anogenital distance in male infants. Hum Reprod 34:1356-1368.

Wang Y, Rogan WJ, Chen PC, Lien GW, Chen HY, Tseng YC, et al. 2014. Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environ Health Perspect 122:529-534.

Wang Y, Adgent M, Su PH, Chen HY, Chen PC, Hsiung CA, et al. 2016. Prenatal exposure to perfluorocarboxylic acids (PFCAs) and fetal and postnatal growth in the taiwan maternal and infant cohort study. Environ Health Perspect 124:1794-1800.

Wikström S, Lin PI, Lindh CH, Shu H, Bornehag CG. 2020. Maternal serum levels of perfluoroalkyl substances in early pregnancy and offspring birth weight. Pediatr Res 87:1093-1099.

Xu M, Zhang T, Lv C, Niu Q, Zong W, Tang J, et al. 2019. Perfluorodecanoic acid-induced oxidative stress and DNA damage investigated at the cellular and molecular levels. Ecotoxicol Environ Saf 185:109699.

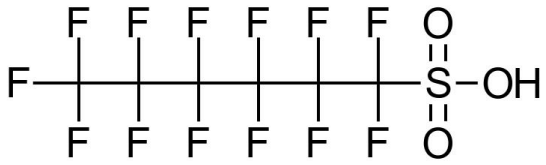
Yang L, Li J, Lai J, Luan H, Cai Z, Wang Y, et al. 2016. Placental transfer of perfluoroalkyl substances and associations with thyroid hormones: Beijing prenatal exposure study. Sci Rep 6:21699.

Yao Q, Shi R, Wang C, Han W, Gao Y, Zhang Y, et al. 2019. Cord blood per- and polyfluoroalkyl substances, placental steroidogenic enzyme, and cord blood reproductive hormone. Environ Int 129:573-582.

Zhou Y, Hu L-W, Qian Z, Chang J-J, King C, Paul G, et al. 2016. Association of perfluoroalkyl substances exposure with reproductive hormone levels in adolescents: By sex status. Environment international 94:189-195.

Perfluorohexanesulfonic acid (PFHxS)

(Tridecafluorohexane-1-sulfonic acid, CAS No. 355-46-4)



PFHxS is a perfluorinated organic compound with surfactant properties, and a member of a large group of substances collectively called per- and polyfluorinated substances (PFASs). PFASs are commonly used to make products resistant to stains, grease, soil and water, and are used in various industries. PFASs are global pollutants of air, water, soil and wildlife, and are very persistent in the environment.

Human biomonitoring studies indicate that exposure to PFHxS is widespread. For example, Table 5 below summarizes data on serum concentrations of PFHxS (geometric mean and 95% confidence interval [CI]) measured in studies conducted by the Biomonitoring California Program between 2010 and 2018 (Biomonitoring California 2020).

Table 5. PFHxS serum concentration (ng/ml) in studies in California residents. Data from Biomonitoring California (https://biomonitoring.ca.gov/) (Biomonitoring California 2020).

Project	Sample Year	Geometric mean (ng/ml)	95% Lower CI	95% Upper CI	N	Detection Frequency
California Teachers Study (CTS)	2011	1.62	1.56	1.68	1759	99.90%
Firefighter Occupational Exposures (FOX) Project	2010 to 2011	2.26	2	2.54	101	100%
Measuring Analytes in Maternal Archived Samples (MAMAS)	2012 to 2015	0.904	0.818	0.998	200	100%
Biomonitoring Exposures Study (BEST) - 1.Pilot	2011 to 2012	1.43	1.19	1.73	110	99.10%
Biomonitoring Exposures Study (BEST) - 2.Expanded	2013	1.03	0.937	1.13	337	99.10%
Asian/Pacific Islander Community Exposures (ACE) Project - ACE 1	2016	0.767	0.66	0.891	96	100%
Asian/Pacific Islander Community Exposures (ACE) Project - ACE 2	2017	1.29	1.14	1.45	99	100%
California Regional Exposure Study, Los Angeles County (CARE-LA)	2018	0.613	0.559	0.672	425	98.80%

Reports from other human biomonitoring studies of PFHxS include:

- Serum concentration level (*μ*g/L; geometric mean and 95% CI) reported by the National Health and Nutrition Examination Survey (NHANES) in 2009 to 2010 for males aged 20 years or older was 2.15 (1.93, 2.40) (Dobraca et al. 2015).
- The unadjusted geometric mean and 95% CI for PFHxS serum levels (ng/mL) in US children aged 3–11 years, from data reported by NHANES for 2013 to 2014, was 0.84 ng/mL (0.76 − 0.94) (Jain 2018).
- PFHxS was detected in the serum of 98% of mothers in the Northern California CHARGE (Childhood Autism Risk from Genetics and Environment) case-control study (serum collected 2009 – 2016) (Kim et al. 2020).

PFHxS passed the human data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies published within the last seven to eight years that were identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Numerous human studies reporting developmental and reproductive toxicity (DART)-related effects associated with PFHxS were identified in the recent literature. DART findings reported in epidemiologic studies published within the last eight years are summarized here. The findings are organized by groups of outcomes.

Indicators of fetal growth

- Association between increasing PFHxS concentration quartiles and decreased ponderal index at birth, but not birth weight or height (retrospective cohort study) (Alkhalawi et al. 2016).
- Increased odds of being <95% of optimal birth weight (cross sectional study) (Callan et al. 2016).
- Decreased birth length, but increased postnatal head circumference (average age at measurement: 19 months) (p value for trend=0.04) (cross sectional study) (Cao et al. 2018).
- Decreased birth weight (cohort study) (Hamm et al. 2010).
- No association with fetal growth in any trimester of pregnancy for the combined study population (three cohorts study) (Costa et al. 2019).
- No statistically significant associations with birth weight, birth length or ponderal index. In male infants a positive correlation with birth length was reported (cross sectional study) (Shi et al. 2017).

Neurodevelopmental effects

- Maternal plasma PFHxS, measured at gestation week (GW) 15, and child behavior analysis at 5 to 9 years of age. PFHxS was associated with higher Strength and Difficulties Questionnaire (SDQ)-total score (more problem behavior) in the Greenland cohort; no association observed in the Ukraine cohort (Birth cohorts from Greenland and Ukraine) (Høyer et al. 2018).
- Prenatal levels of 19 PFASs were measured in maternal blood at week 17 of gestation. Negative association between PFHxS and nonverbal working memory. No association with attention-deficit/hyperactivity disorder symptoms, language skills or intelligence quotient (IQ) (prospective study) (Skogheim et al. 2020).

Anogenital distance, prenatal exposure

- Maternal serum PFHxS at GW 5 to 12 was associated with decreased anogenital distance in girls at median age 3.5 months (p-trend<0.05) (Odense child cohort) (Lind et al. 2017).

Endocrine effects

- PFHxS levels and thyroid hormones were measured in maternal blood samples collected between GW 5 and 19. Higher PFHxS levels were associated with higher thyroid stimulating hormone levels before GW 10 (cross-sectional study) (Inoue et al. 2019).
- Maternal serum was collected around GW 11; higher PFHxS levels were associated with increased fasting glucose, fasting insulin and insulin resistance (based on a homeostatic assessment model) (prospective Odense child cohort) (Jensen et al. 2018).
- Positive association between cord blood PFHxS and cord blood estradiol levels. Higher cord blood PFHxS levels associated with higher levels of placental steroidogenic enzymes such as aromatase and 3β- and 17β-hydroxysteroid dehydrogenase. These associations were more pronounced in females than males (Chinese prospective birth cohort) (Yao et al. 2019).
- In patients with primary ovarian insufficiency, plasma levels of PFHxS were positively associated with plasma levels of follicle stimulating hormone and negatively associated with plasma levels of estradiol (case-control study) (Zhang et al. 2018).

Female reproductive effects

- First trimester plasma PFHxS was considered as a surrogate of preconception exposure. PFHxS was associated with a reduction in fecundability (cohort study) (Velez et al. 2015).
- Plasma PFHxS levels were positively associated with primary ovarian insufficiency (case-control study) (Zhang et al. 2018).
- Plasma PFHxS levels were associated with increased odds of self-reported history of irregular menstrual cycle and self-reported history of menorrhagia (Shanghai birth cohort study) (Zhou et al. 2017).
- Serum PFHxS levels were not associated with time to pregnancy in any region studied (cross-sectional multi region study from Greenland, Poland, and Ukraine) (Jorgensen et al. 2014).
- A non-significantly increased association of plasma PFHxS levels with miscarriage (OR and CI) 0.9 (0.7, 1.3) (case-control study) (Liew et al. 2020).

Male reproductive effects

- Increased serum PFHxS was associated with a 35% lower proportion of morphologically normal sperm (multi-region study from Greenland, Poland and Ukraine) (Toft et al. 2012).

Other DART effects

Atopic dermatitis

- The highest quartile of PFHxS in cord blood was associated with atopic dermatitis (prospective birth cohort study) (Chen et al. 2018).

Germ cell tumors

- Maternal PFHxS levels were significantly associated with pediatric germ cell tumors (case-control study) (Lin et al. 2020).

Metabolic effects

- Positive associations between maternal plasma PFHxS and increased triglycerides in the child at age 4. Prenatal PFAS concentrations were not associated with individual outcomes (cardiometabolic risk score and z-scores for weight gain, body mass index; waist circumference, blood pressure, high-density lipoprotein cholesterol; low-density lipoprotein cholesterol, total cholesterol, and triglycerides) or with the combined cardiometabolic-risk score (Spanish INMA birth cohort study) (Manzano-Salgado et al. 2017).
- Prenatal PFHxS serum samples were not associated with adiposity at 8 years of age (prospective cohort Study) (Braun et al. 2016).

- Maternal serum PFHxS levels were not associated with percent total body fat in girls (longitudinal study) (Hartman et al. 2017).

Puberty

- Prenatal exposure to PFHxS was associated with a lower mean age of puberty onset in girls and boys (puberty cohort, nested within the Danish national birth cohort) (Ernst et al. 2019).

Animal studies

Findings reported in whole animal studies examining possible DART effects of exposure to PFHxS published within the last seven years are summarized here.

Developmental effects

Developmental effects are associated with *in utero* exposures unless otherwise specified

- Mouse: Equivocal decrease in live litter size. Pup-born-to-implant ratio was unaffected. There were no treatment-related effects on postnatal survival, development, or onset of preputial separation or vaginal opening in F1 mice (Chang et al. 2018).
- Rat: Decreased male pup birth weight, low thyroxine levels in both dams and offspring (Ramhoj et al. 2018).
- Zebrafish: Morphometric effects in the larvae, specifically increased length and yolk sac area (Annunziato et al. 2019).

Neurodevelopmental effects

- Mouse: Alteration in neuroproteins involved in normal brain development after PFHxS exposure on postnatal day 10 (Lee and Viberg 2013).
- Zebrafish: At 14 days post-fertilization, treatment with PFHxS was associated with decreased activity in a behavioral assay (e.g., decreased distance traveled, and decreased travel through the center of the test “arena”) (Annunziato et al. 2019).

Mechanistic, in vitro, and other relevant data

- Chinese hamster ovary cell line: Concentration-dependent androgen receptor antagonism, starting at $5x10^{-5}$M (Kjeldsen and Bonefeld-Jørgensen 2013).

References cited in "PFHxS"

Alkhalawi E, Kasper-Sonnenberg M, Wilhelm M, Völkel W, Wittsiepe J. 2016. Perfluoroalkyl acids (PFAAs) and anthropometric measures in the first year of life: Results from the duisburg birth cohort. J Toxicol Environ Health, Part A 79:1041-1049.

Annunziato KM, Jantzen CE, Gronske MC, Cooper KR. 2019. Subtle morphometric, behavioral and gene expression effects in larval zebrafish exposed to PFHxA, PFHxS and 6:2 FTOH. Aquat Toxicol 208:126-137.

Biomonitoring California. 2020. Available: https://biomonitoring.ca.gov/results/chemical/2183?field_chemical_name_target_id_selective%5B%5D=158&field_chemical_name_target_id_selective%5B%5D=161&field_chemical_name_target_id_selective%5B%5D=162&field_chemical_name_target_id_selective%5B%5D=1345 [accessed 28 August 2020].

Braun JM, Chen A, Romano ME, Calafat AM, Webster GM, Yolton K, et al. 2016. Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: The HOME study. Obesity (Silver Spring) 24:231-237.

Callan AC, Rotander A, Thompson K, Heyworth J, Mueller JF, Odland J, et al. 2016. Maternal exposure to perfluoroalkyl acids measured in whole blood and birth outcomes in offspring. Sci Total Environ 569-570:1107-1113.

Cao W, Liu X, Liu X, Zhou Y, Zhang X, Tian H, et al. 2018. Perfluoroalkyl substances in umbilical cord serum and gestational and postnatal growth in a chinese birth cohort. Environ Int 116:197-205.

Chang S, Butenhoff JL, Parker GA, Coder PS, Zitzow JD, Krisko RM, et al. 2018. Reproductive and developmental toxicity of potassium perfluorohexanesulfonate in CD-1 mice. Reprod Toxicol 78:150-168.

Chen Q, Huang R, Hua L, Guo Y, Huang L, Zhao Y, et al. 2018. Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and childhood atopic dermatitis: A prospective birth cohort study. Environ Health 17:8.

Costa O, Iniguez C, Manzano-Salgado CB, Amiano P, Murcia M, Casas M, et al. 2019. First-trimester maternal concentrations of polyfluoroalkyl substances and fetal growth throughout pregnancy. Environ Int 130:104830.

Dobraca D, Israel L, McNeel S, Voss R, Wang M, Gajek R, et al. 2015. Biomonitoring in California firefighters: Metals and perfluorinated chemicals. J Occup Environ Med 57:88-97.

Ernst A, Brix N, Lauridsen LLB, Olsen J, Parner ET, Liew Z, et al. 2019. Exposure to perfluoroalkyl substances during fetal life and pubertal development in boys and girls from the Danish national birth cohort. Environ Health Perspect 127:17004.

Hamm MP, Cherry NM, Chan E, Martin JW, Burstyn I. 2010. Maternal exposure to perfluorinated acids and fetal growth. J Expo Sci Environ Epidemiol 20:589-597.

Hartman TJ, Calafat AM, Holmes AK, Marcus M, Northstone K, Flanders WD, et al. 2017. Prenatal exposure to perfluoroalkyl substances and body fatness in girls. Child Obes 13:222-230.

Høyer BB, Bonde JP, Tøttenborg SS, Ramlau-Hansen CH, Lindh C, Pedersen HS, et al. 2018. Exposure to perfluoroalkyl substances during pregnancy and child behaviour at 5 to 9years of age. Horm Behav 101:105-112.

Inoue K, Ritz B, Andersen SL, Ramlau-Hansen CH, Hoyer BB, Bech BH, et al. 2019. Perfluoroalkyl substances and maternal thyroid hormones in early pregnancy; findings in the Danish national birth cohort. Environ Health Perspect 127:117002.

Jain RB. 2018. Contribution of diet and other factors to the observed levels of selected perfluoroalkyl acids in serum among US children aged 3-11 years. Environ Res 161:268-275.

Jensen RC, Glintborg D, Timmermann CAG, Nielsen F, Kyhl HB, Andersen HR, et al. 2018. Perfluoroalkyl substances and glycemic status in pregnant Danish women: The Odense child cohort. Environ Int 116:101-107.

Jorgensen KT, Specht IO, Lenters V, Bach CC, Rylander L, Jonsson BA, et al. 2014. Perfluoroalkyl substances and time to pregnancy in couples from Greenland, Poland and Ukraine. Environ Health 13:116.

Kim K, Bennett DH, Calafat AM, Hertz-Picciotto I, Shin HM. 2020. Temporal trends and determinants of serum concentrations of per- and polyfluoroalkyl substances among northern California mothers with a young child, 2009-2016. Environ Res 186:109491.

Kjeldsen LS, Bonefeld-Jørgensen EC. 2013. Perfluorinated compounds affect the function of sex hormone receptors. Environ Sci Pollut Res Int 20:8031-8044.

Lee I, Viberg H. 2013. A single neonatal exposure to perfluorohexane sulfonate (PFHxS) affects the levels of important neuroproteins in the developing mouse brain. Neurotoxicology 37:190-196.

Liew Z, Luo J, Nohr EA, Bech BH, Bossi R, Arah OA, et al. 2020. Maternal plasma perfluoroalkyl substances and miscarriage: A nested case-control study in the Danish national birth cohort. Environ Health Perspect 128:47007.

Lin HW, Feng HX, Chen L, Yuan XJ, Tan Z. 2020. Maternal exposure to environmental endocrine disruptors during pregnancy is associated with pediatric germ cell tumors. Nagoya J Med Sci 82:323-333.

Lind DV, Priskorn L, Lassen TH, Nielsen F, Kyhl HB, Kristensen DM, et al. 2017. Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort. Reprod Toxicol 68:200-206.

Manzano-Salgado CB, Casas M, Lopez-Espinosa MJ, Ballester F, Iniguez C, Martinez D, et al. 2017. Prenatal exposure to perfluoroalkyl substances and cardiometabolic risk in children from the Spanish INMA birth cohort study. Environ Health Perspect 125:097018.

Ramhoj L, Hass U, Boberg J, Scholze M, Christiansen S, Nielsen F, et al. 2018. Perfluorohexane sulfonate (PFHxS) and a mixture of endocrine disrupters reduce thyroxine levels and cause antiandrogenic effects in rats. Toxicol Sci 163:579-591.

Shi Y, Yang L, Li J, Lai J, Wang Y, Zhao Y, et al. 2017. Occurrence of perfluoroalkyl substances in cord serum and association with growth indicators in newborns from beijing. Chemosphere 169:396-402.

Skogheim TS, Villanger GD, Weyde KVF, Engel SM, Surén P, Øie MG, et al. 2020. Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children. Int J Hyg Environ Health 223:80-92.

Toft G, Jonsson BA, Lindh CH, Giwercman A, Spano M, Heederik D, et al. 2012. Exposure to perfluorinated compounds and human semen quality in arctic and european populations. Hum Reprod 27:2532-2540.

Velez MP, Arbuckle TE, Fraser WD. 2015. Maternal exposure to perfluorinated chemicals and reduced fecundity: The MIREC study. Hum Reprod 30:701-709.

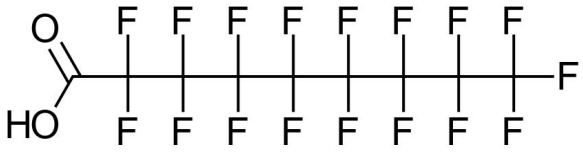
Yao Q, Shi R, Wang C, Han W, Gao Y, Zhang Y, et al. 2019. Cord blood per- and polyfluoroalkyl substances, placental steroidogenic enzyme, and cord blood reproductive hormone. Environ Int 129:573-582.

Zhang S, Tan R, Pan R, Xiong J, Tian Y, Wu J, et al. 2018. Association of perfluoroalkyl and polyfluoroalkyl substances with premature ovarian insufficiency in chinese women. J Clin Endocrinol Metab 103:2543-2551.

Zhou W, Zhang L, Tong C, Fang F, Zhao S, Tian Y, et al. 2017. Plasma perfluoroalkyl and polyfluoroalkyl substances concentration and menstrual cycle characteristics in preconception women. Environ Health Perspect 125:067012.

Perfluorononanoic acid (PFNA)

(Heptadecafluorononanoic acid, CAS No. 375-95-1)



PFNA is a perfluorinated organic compound with surfactant properties, and a member of a large group of substances collectively called per- and polyfluorinated substances (PFASs). PFASs are commonly used to make products resistant to stains, grease, soil and water, and are used in various industries. PFASs are global pollutants of air, water, soil and wildlife, and are very persistent in the environment.

Human biomonitoring studies indicate that exposure to PFNA is widespread. For example, Table 6 below summarizes data on serum concentrations of PFNA (geometric mean and 95% confidence interval [CI]) measured in studies conducted by the Biomonitoring California Program between 2010 and 2018 (Biomonitoring California 2020).

Table 6. PFNA serum concentration (ng/ml) in studies of California residents. Data from Biomonitoring California (https://biomonitoring.ca.gov/) (Biomonitoring California 2020).

Project	Sample Year	Geometric mean (ng/ml)	95% Lower CI	95% Upper CI	N	Detection Frequency
Maternal and Infant Environmental Exposure Project (MIEEP)	2010 to 2011	0.733	0.621	0.865	77	Not reported
California Teachers Study (CTS)	2011	0.92	0.89	0.95	1719	99.70%
Firefighter Occupational Exposures (FOX) Project	2010 to 2011	1.15	1.06	1.25	101	100%
Measuring Analytes in Maternal Archived Samples (MAMAS)	2012 to 2015	0.647	0.596	0.703	200	100%
Biomonitoring Exposures Study (BEST) - 1.Pilot	2011 to 2012	0.994	0.92	1.07	110	100%
Biomonitoring Exposures Study (BEST) - 2.Expanded	2013	0.787	0.726	0.853	337	99.10%
Asian/Pacific Islander Community Exposures (ACE) Project - ACE 1	2016	0.987	0.87	1.12	96	99%
Asian/Pacific Islander Community Exposures (ACE) Project - ACE 2	2017	1.1	0.988	1.22	99	99%
California Regional Exposure Study, Los Angeles County (CARE-LA)	2018	0.3	0.278	0.323	425	97.20%

Reports from other human biomonitoring studies of PFNA include:

- Serum concentration level (*μ*g/L; geometric mean and 95% CI) reported by the National Health and Nutrition Examination Survey (NHANES) in 2009 to 2010 for males aged 20 years or older was 1.40 (1.20, 1.63) (Dobraca et al. 2015).
- The unadjusted geometric mean and 95% CI for PFNA (ng/mL) in US children aged 3–11 years, from data reported by NHANES for 2013 to 2014, was 0.79 (0.68−0.93) (Jain 2018).
- PFNA was detected in the serum of 95% of mothers in the Northern California CHARGE (CHildhood Autism Risk from Genetics and Environment) case-control study (serum collected 2009 – 2016) (Kim et al. 2020).

PFNA passed the human data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Numerous human studies reporting developmental and reproductive toxicity (DART)-related effects associated with PFNA were identified in the recent literature. DART findings reported in epidemiologic studies published within the last six years are summarized here. The findings are organized by groups of outcomes.

Gestation duration

- Increased risk of preterm birth (the Danish national birth cohort study) (Meng et al. 2018).

Indicators of fetal growth

- Inverse association with birth weight (longitudinal cohort) (Gyllenhammar et al. 2018).
- No association with fetal biometry. Negative association (among smokers) with femur length and estimated fetal weight (three cohorts study) (Costa et al. 2019).
- Inverse associations with birth weight and birth length (prospective birth cohort study) (Kashino et al. 2020) and (retrospective cohort study) (Kwon et al. 2016).
- Maternal serum PFNA level was inversely associated with birth weight in girls. For height *z*-scores in boys, a significant interaction was reported with height at ages 8 and 11 years, but not with size of boys at birth (prospective study) (Wang et al. 2016).
- Associated in girls with lower birth weight for gestational age, and small for gestational age at birth (longitudinal cohort) (Wikström et al. 2020).

Neurodevelopmental effects

- PFNA was measured in maternal serum and in serum from children at ages 5 and 7 years. No associations between prenatal PFNA concentrations and strengths and difficulties questionnaire (SDQ) scores, while a two-fold increase in 5 year-old serum PFNA concentrations was associated with increases in total SDQ (birth cohort in the Faroe Islands study) (Oulhote et al. 2016).
- Maternal plasma PFNA levels measured at gestational week (GW) 15, and child behavior analysis at 5 to 9 years of age. PFNA was associated with higher SDQ-total scores (indicating more behavioral problems) in the Greenland cohort and in the combined analysis (Greenland and Ukraine cohorts), and no association with scores in the Ukraine cohort. Increased odds ratio for hyperactivity for one natural log-unit increase in prenatal PFNA (birth cohorts) (Høyer et al. 2018).
- PFNA measured in cord blood was inversely associated with inattention and oppositional defiant disorder and hyperactivity/inattention at 7 years of age (early-life cohort) (Lien et al. 2016).
- PFNA maternal plasma concentration measured once between GWs 12 to 16 was associated with increased risk of developmental problems in personal-social skills (birth cohort) (Niu et al. 2019).
- Prenatal levels of 19 PFASs were measured in maternal blood at week 17 of gestation. No associations with attention-deficit/hyperactivity disorder symptoms, language skills or intelligence quotient (IQ). Negative associations with nonverbal working memory, and positive associations with verbal working memory (prospective study) (Skogheim et al. 2020).
- PFNA was analyzed in maternal serum samples collected during the third trimester of pregnancy. Higher PFNA levels were associated with lower verbal IQ (maternal and Infant cohort study) (Wang et al. 2015).

Anogenital distance, prenatal exposure

- Associated with a decreased anogenital distance in three months-old girls (p-value for trend<0.05) after adjusting for age and weight-for-age standard deviation score (Odense child cohort) (Lind et al. 2017).

Endocrine effects

- PFNA in cord blood was associated with higher placental aromatase levels (prospective birth cohort) (Yao et al. 2019).
- PFNA levels and three thyroid hormones (THs) were measured in cord blood. Thyroid stimulating hormone (TSH) levels decreased with increasing concentrations of PFNA (cross-sectional study) (Aimuzi et al. 2019).

- PFNA was measured in the third trimester and THs levels in cord serum in 116 neonates. PFNA was associated with lower free and total thyroxine levels (prospective birth cohort study) (Wang et al. 2014).
- Associated with lower testosterone levels (cross-sectional study) (Zhou et al. 2016)

Female reproductive effects

- PFNA serum levels in pregnancy were associated with greater risk of miscarriage (nested case-control in the Odense child cohort study) (Jensen et al. 2015).
- Serum PFNA levels were associated with longer time to pregnancy in the pooled sample and specifically in women from Greenland. Increased odd ratios for infertility in the pooled sample and in women from Greenland (cohort study) (Jorgensen et al. 2014).
- Lower probability of pregnancy for women in second versus first tertile of PFNA (prospective pregnancy cohort study) (Lum et al. 2017).
- Plasma PFNA levels were associated with increased odds of self-reported history of irregular menstrual cycle, and negatively associated with self-reported history of menorrhagia (Shanghai birth cohort study) (Zhou et al. 2017).

Other DART effects

Puberty

- Prenatal exposure to PFNA was associated with a lower mean age of puberty onset in girls, and higher mean age of puberty onset in boys (Puberty cohort, nested within the Danish National Birth Cohort) (Ernst et al. 2019).

Respiratory system effects

- Maternal serum PFNA levels were associated with self-reported asthma in child at 5 years of age (child cohort) (Beck et al. 2019).
- Cord serum PFNA was positively associated with the number of lower respiratory tract infection episodes from 0 to 10 years of age (prospective birth cohort study) (Impinen et al. 2018).

Metabolic effects

- Maternal serum PFNA was associated with higher fasting insulin after adjusting for age, parity, educational level and pre-pregnancy body mass index in women with high risk for gestational diabetes (prospective cohort) (Jensen et al. 2018).
- Positive association between maternal plasma prenatal PFNA and the combined cardiometabolic-risk score of the child (birth cohort study) (Manzano-Salgado et al. 2017).

- Maternal PFNA serum levles were not associated with adiposity of the child at 8 years of age (prospective cohort) (Braun et al. 2016)
- Maternal PFNA serum levels were not associated with percent total body fat in 9 year-old girls (longitudinal study) (Hartman et al. 2017).

Animal studies

Two whole animal studies examining possible DART effects of exposure to PFNA were identified. The findings are summarized here.

Developmental and neurodevelopmental effects

- Mouse: Pups exposed *in utero* were born alive; however, 80% of pups in the high dose group died within the first 10 days of life, and surviving pups exhibited dose-dependent delays in eye opening and onset of puberty (Das et al. 2015).
- Zebrafish: Embryos were treated for the first five days post fertilization. When assessed at six months post fertilization, PFNA was not associated with any significant change in total body length or weight. In terms of behavior, PFNA was associated in males with a reduction in total distance traveled and time of immobility; increases in thigmotaxis behavior (a tendency to remain close to the tank walls, indicating anxiety) and aggressive attacks, and preference for the bright section of the tank. PFNA also decreased gene expression of two organic anion transporting polypeptides in both sexes and increased expression of growth factor genes in males (Jantzen et al. 2016).

Mechanistic, in vitro, and other relevant data

- Rat: Primary cultured Sertoli cells exposed *in vitro* developed vacuoles in the cytoplasm (Feng et al. 2010).
- Bovine: Oocytes exposed *in vitro* during maturation had larger lipid droplets during oocyte maturation and in the blastocyst stage had accumulation of very large lipid droplets and a lower proportion of small lipid droplets (Hallberg et al. 2019).
- Chinese hamster ovary cell line: Concentration-dependent androgen receptor antagonism (Kjeldsen and Bonefeld-Jørgensen 2013).

References cited in "PFNA"

Aimuzi R, Luo K, Chen Q, Wang H, Feng L, Ouyang F, et al. 2019. Perfluoroalkyl and polyfluoroalkyl substances and fetal thyroid hormone levels in umbilical cord blood among newborns by prelabor caesarean delivery. Environ Int 130:104929.

Beck IH, Timmermann CAG, Nielsen F, Schoeters G, Jøhnk C, Kyhl HB, et al. 2019. Association between prenatal exposure to perfluoroalkyl substances and asthma in 5-year-old children in the Odense child cohort. Environ Health 18:97.

Biomonitoring California. 2020. Available: https://biomonitoring.ca.gov/results/chemical/2183?field_chemical_name_target_id_selective%5B%5D=158&field_chemical_name_target_id_selective%5B%5D=161&field_chemical_name_target_id_selective%5B%5D=162&field_chemical_name_target_id_selective%5B%5D=1345 [accessed 28 August 2020].

Braun JM, Chen A, Romano ME, Calafat AM, Webster GM, Yolton K, et al. 2016. Prenatal perfluoroalkyl substance exposure and child adiposity at 8 years of age: The HOME study. Obesity (Silver Spring) 24:231-237.

Costa O, Iniguez C, Manzano-Salgado CB, Amiano P, Murcia M, Casas M, et al. 2019. First-trimester maternal concentrations of polyfluoroalkyl substances and fetal growth throughout pregnancy. Environ Int 130:104830.

Das KP, Grey BE, Rosen MB, Wood CR, Tatum-Gibbs KR, Zehr RD, et al. 2015. Developmental toxicity of perfluorononanoic acid in mice. Reprod Toxicol 51:133-144.

Dobraca D, Israel L, McNeel S, Voss R, Wang M, Gajek R, et al. 2015. Biomonitoring in California firefighters: Metals and perfluorinated chemicals. J Occup Environ Med 57:88-97.

Ernst A, Brix N, Lauridsen LLB, Olsen J, Parner ET, Liew Z, et al. 2019. Exposure to perfluoroalkyl substances during fetal life and pubertal development in boys and girls from the Danish national birth cohort. Environ Health Perspect 127:17004.

Feng Y, Fang X, Shi Z, Xu M, Dai J. 2010. Effects of PFNA exposure on expression of junction-associated molecules and secretory function in rat Sertoli cells. Reprod Toxicol 30:429-437.

Gyllenhammar I, Diderholm B, Gustafsson J, Berger U, Ridefelt P, Benskin JP, et al. 2018. Perfluoroalkyl acid levels in first-time mothers in relation to offspring weight gain and growth. Environ Int 111:191-199.

Hallberg I, Kjellgren J, Persson S, Orn S, Sjunnesson Y. 2019. Perfluorononanoic acid (PFNA) alters lipid accumulation in bovine blastocysts after oocyte exposure during *in vitro* maturation. Reprod Toxicol 84:1-8.

Hartman TJ, Calafat AM, Holmes AK, Marcus M, Northstone K, Flanders WD, et al. 2017. Prenatal exposure to perfluoroalkyl substances and body fatness in girls. Child Obes 13:222-230.

Høyer BB, Bonde JP, Tøttenborg SS, Ramlau-Hansen CH, Lindh C, Pedersen HS, et al. 2018. Exposure to perfluoroalkyl substances during pregnancy and child behaviour at 5 to 9years of age. Horm Behav 101:105-112.

Impinen A, Nygaard UC, Lodrup Carlsen KC, Mowinckel P, Carlsen KH, Haug LS, et al. 2018. Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. Environ Res 160:518-523.

Jain RB. 2018. Contribution of diet and other factors to the observed levels of selected perfluoroalkyl acids in serum among US children aged 3-11 years. Environ Res 161:268-275.

Jantzen CE, Annunziato KM, Cooper KR. 2016. Behavioral, morphometric, and gene expression effects in adult zebrafish (*Danio rerio*) embryonically exposed to PFOA, PFOS, and PFNA. Aquat Toxicol 180:123-130.

Jensen RC, Glintborg D, Timmermann CAG, Nielsen F, Kyhl HB, Andersen HR, et al. 2018. Perfluoroalkyl substances and glycemic status in pregnant Danish women: The Odense child cohort. Environ Int 116:101-107.

Jensen TK, Andersen LB, Kyhl HB, Nielsen F, Christesen HT, Grandjean P. 2015. Association between perfluorinated compound exposure and miscarriage in Danish pregnant women. PLoS One 10:e0123496.

Jorgensen KT, Specht IO, Lenters V, Bach CC, Rylander L, Jonsson BA, et al. 2014. Perfluoroalkyl substances and time to pregnancy in couples from Greenland, Poland and Ukraine. Environ Health 13:116.

Kashino I, Sasaki S, Okada E, Matsuura H, Goudarzi H, Miyashita C, et al. 2020. Prenatal exposure to 11 perfluoroalkyl substances and fetal growth: A large-scale, prospective birth cohort study. Environ Int 136:105355.

Kim K, Bennett DH, Calafat AM, Hertz-Picciotto I, Shin HM. 2020. Temporal trends and determinants of serum concentrations of per- and polyfluoroalkyl substances among northern California mothers with a young child, 2009-2016. Environ Res 186:109491.

Kjeldsen LS, Bonefeld-Jørgensen EC. 2013. Perfluorinated compounds affect the function of sex hormone receptors. Environ Sci Pollut Res Int 20:8031-8044.

Kwon EJ, Shin JS, Kim BM, Shah-Kulkarni S, Park H, Kho YL, et al. 2016. Prenatal exposure to perfluorinated compounds affects birth weight through GSTM1 polymorphism. J Occup Environ Med 58:e198-205.

Lien GW, Huang CC, Shiu JS, Chen MH, Hsieh WS, Guo YL, et al. 2016. Perfluoroalkyl substances in cord blood and attention deficit/hyperactivity disorder symptoms in seven-year-old children. Chemosphere 156:118-127.

Lind DV, Priskorn L, Lassen TH, Nielsen F, Kyhl HB, Kristensen DM, et al. 2017. Prenatal exposure to perfluoroalkyl substances and anogenital distance at 3 months of age in a Danish mother-child cohort. Reprod Toxicol 68:200-206.

Lum KJ, Sundaram R, Barr DB, Louis TA, Buck Louis GM. 2017. Perfluoroalkyl chemicals, menstrual cycle length, and fecundity: Findings from a prospective pregnancy study. Epidemiology 28:90-98.

Manzano-Salgado CB, Casas M, Lopez-Espinosa MJ, Ballester F, Iniguez C, Martinez D, et al. 2017. Prenatal exposure to perfluoroalkyl substances and cardiometabolic risk in children from the Spanish INMA birth cohort study. Environ Health Perspect 125:097018.

Meng Q, Inoue K, Ritz B, Olsen J, Liew Z. 2018. Prenatal exposure to perfluoroalkyl substances and birth outcomes; an updated analysis from the Danish national birth cohort. Int J Environ Res Public Health 15.

Niu J, Liang H, Tian Y, Yuan W, Xiao H, Hu H, et al. 2019. Prenatal plasma concentrations of perfluoroalkyl and polyfluoroalkyl substances and neuropsychological development in children at four years of age. Environ Health 18:53.

Oulhote Y, Steuerwald U, Debes F, Weihe P, Grandjean P. 2016. Behavioral difficulties in 7-year old children in relation to developmental exposure to perfluorinated alkyl substances. Environ Int 97:237-245.

Skogheim TS, Villanger GD, Weyde KVF, Engel SM, Surén P, Øie MG, et al. 2020. Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children. Int J Hyg Environ Health 223:80-92.

Wang Y, Rogan WJ, Chen PC, Lien GW, Chen HY, Tseng YC, et al. 2014. Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environ Health Perspect 122:529-534.

Wang Y, Rogan WJ, Chen HY, Chen PC, Su PH, Chen HY, et al. 2015. Prenatal exposure to perfluroalkyl substances and children's IQ: The taiwan maternal and infant cohort study. Int J Hyg Environ Health 218:639-644.

Wang Y, Adgent M, Su PH, Chen HY, Chen PC, Hsiung CA, et al. 2016. Prenatal exposure to perfluorocarboxylic acids (PFCAs) and fetal and postnatal growth in the taiwan maternal and infant cohort study. Environ Health Perspect 124:1794-1800.

Wikström S, Lin PI, Lindh CH, Shu H, Bornehag CG. 2020. Maternal serum levels of perfluoroalkyl substances in early pregnancy and offspring birth weight. Pediatr Res 87:1093-1099.

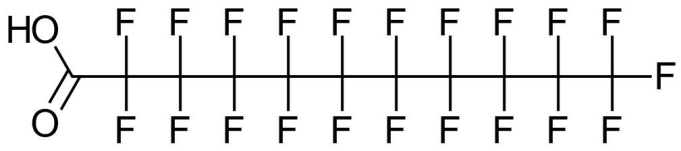
Yao Q, Shi R, Wang C, Han W, Gao Y, Zhang Y, et al. 2019. Cord blood per- and polyfluoroalkyl substances, placental steroidogenic enzyme, and cord blood reproductive hormone. Environ Int 129:573-582.

Zhou W, Zhang L, Tong C, Fang F, Zhao S, Tian Y, et al. 2017. Plasma perfluoroalkyl and polyfluoroalkyl substances concentration and menstrual cycle characteristics in preconception women. Environ Health Perspect 125:067012.

Zhou Y, Hu L-W, Qian Z, Chang J-J, King C, Paul G, et al. 2016. Association of perfluoroalkyl substances exposure with reproductive hormone levels in adolescents: By sex status. Environment international 94:189-195.

Perfluoroundecanoic acid (PFUnDA)

(Henicosafluoroundecanoic acid, CAS No. 2058-94-8)



PFUnDA is a perfluorinated organic compound with surfactant properties, and a member of a large group of substances collectively called per- and polyfluorinated substances (PFASs). PFASs are commonly used to make products resistant to stains, grease, soil and water, and are used in various industries. PFASs are global pollutants of air, water, soil and wildlife, and are very persistent in the environment.

Human biomonitoring studies indicate that exposure to PFUnDA is widespread. For example, Table 7 below summarizes data on serum concentrations of PFUnDA (geometric mean and 95% confidence interval [CI]) measured in studies conducted by the Biomonitoring California Program between 2010 and 2018 (Biomonitoring California 2020).

Table 7. PFUnDA serum concentrations (ng/ml) in studies of California residents. Data from Biomonitoring California (https://biomonitoring.ca.gov/) (Biomonitoring California 2020).

Project	Sample Year	Geometric mean (ng/ml)	95% Lower CI	95% Upper CI	N	Detection Frequency
Maternal and Infant Environmental Exposure Project (MIEEP)	2010 to 2011	0.131	0.101	0.17	77	Not reported
California Teachers Study (CTS)	2011	0.13	0.12	0.13	1759	96.80%
Firefighter Occupational Exposures (FOX) Project	2010 to 2011	0.24	0.21	0.27	101	100%
Measuring Analytes in Maternal Archived Samples (MAMAS)	2012 to 2015	0.124	0.107	0.144	200	78%
Biomonitoring Exposures Study (BEST) - 1.Pilot	2011 to 2012	0.128	0.111	0.148	110	100%
Biomonitoring Exposures Study (BEST) - 2.Expanded	2013	0.106	0.0958	0.117	337	83.40%
Asian/Pacific Islander Community Exposures (ACE) Project - ACE 1	2016	0.398	0.348	0.455	96	100%
Asian/Pacific Islander Community Exposures (ACE) Project - ACE 2	2017	0.453	0.391	0.525	99	98%
California Regional Exposure Study, Los Angeles County (CARE-LA)	2018	0.0829	0.0756	0.0909	425	82.40%

Reports from other human biomonitoring studies of PFUnDA include:

- Serum concentration level (μg/L; geometric mean and 95%CI) reported by the National Health and Nutrition Examination Survey (NHANES) in 2009 to 2010 for males aged 20 years or older was 0.18 (0.16, 0.21) (Dobraca et al. 2015).
- The percentage (with 95% CI) of observations of PFUnDA in serum samples that were above the limit of detection in children aged 3–11 years, from data reported by NHANES for 2013 to 2014, was 27.5 (21.5 − 33.5) (Jain 2018).
- PFUnDA was detected in the serum of 35% of mothers in the Northern California CHARGE (CHildhood Autism Risk from Genetics and Environment) case-control study (serum collected 2009 – 2016) (Kim et al. 2020).

PFUnDA passed the human data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

Numerous human studies reporting developmental and reproductive toxicity (DART)-related effects associated with PFUnDA were identified in the recent literature. DART findings reported in epidemiologic studies published within the last six years are summarized here. The findings are organized by groups of outcomes.

Indicators of fetal growth

- Associated with low birth weight (longitudinal cohort) (Gyllenhammar et al. 2018).
- Associated with low birth weight (retrospective cohort study) (Kwon et al. 2016).
- Associated in girls with lower birth weight for gestational age, and small for gestational age (SGA) at birth (longitudinal cohort) (Wikström et al. 2020).
- Associated in girls with low birth weight and elevated odds of SGAand lower average childhood height *z*-score (prospective study) (Wang et al. 2016).
- Non-significant increases in average birth weight (cross sectional study) (Callan et al. 2016).
- Positively associated with indications of gestational growth and postnatal growth (cross sectional study) (Cao et al. 2018).

Neurodevelopment effects

- Prenatal levels of 19 PFASs were measured in maternal blood at week 17 of gestation. Positive association between PFUnDA and verbal working memory in boys. No association with attention deficit/hyperactivity disorder symptoms, language skills or intelligence quotient (prospective study) (Skogheim et al. 2020).

Anogenital distance, prenatal exposure

- Inversely associated with anogenital distance at birth in males (prospective cohort study) (Tian et al. 2019).

Endocrine effects

- PFUnDA and three thyroid hormones (THs) were measured in cord blood. Thyroid stimulating hormone (TSH) levels decreased with increasing concentrations of PFUnDA (cross sectional study) (Aimuzi et al. 2019).

- PFUnDA and THs were measured in serum from pregnant women in their second trimester and 3 days and 6 weeks after delivery (Berg et al. 2015) and in addition, TSH concentration was analyzed in newborns in heel-prick samples at the two visits after delivery (Berg et al 2017). PFUnDA was inversely associated with mean triiodothyronine (T_3) and free T_3 (prospective cohort studies) (Berg et al. 2015; Berg et al. 2017).
- PFUnDA was measured in the third trimester and THs levels in cord serum in 116 neonates. Maternal PFUnDA levels were associated with lower cord total T_3 and total thyroxine levels (prospective birth cohort study) (Wang et al. 2014).

Other DART effects

Respiratory and allergic effects

- Cord serum PFUnDA was associated with airway infections, common colds at age two, and lower respiratory tract infections from 0 to ten years of age (prospective birth cohort study) (Impinen et al. 2018).
- Maternal plasma PFUnDA levels during pregnancy were inversed associated with ever having atopic eczema (dermatitis) in girls, wheeze and asthma (mother and child cohort study) (Impinen et al. 2019).
- Maternal plasma PFUnDA measured at 28–32 weeks of gestation was associated with eczema in age 2 female infants (longitudinal cohort study) (Okada et al. 2014).

Animal studies

One whole animal study examining possible DART effects of exposure to PFUnDA was identified. The findings are summarized here.

- Rat: In a DART guideline screening test, prenatal exposure resulted in decreased birth weight and decreased body weight gain at postnatal day 4 (Takahashi et al. 2014).

References cited in "PFUnDA"

Aimuzi R, Luo K, Chen Q, Wang H, Feng L, Ouyang F, et al. 2019. Perfluoroalkyl and polyfluoroalkyl substances and fetal thyroid hormone levels in umbilical cord blood among newborns by prelabor caesarean delivery. Environ Int 130:104929.

Berg V, Nøst TH, Hansen S, Elverland A, Veyhe AS, Jorde R, et al. 2015. Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach. Environ Int 77:63-69.

Berg V, Nøst TH, Pettersen RD, Hansen S, Veyhe AS, Jorde R, et al. 2017. Persistent organic pollutants and the association with maternal and infant thyroid homeostasis: A multipollutant assessment. Environ Health Perspect 125:127-133.

Biomonitoring California. 2020. Available: https://biomonitoring.ca.gov/results/chemical/2183?field_chemical_name_target_id_selective%5B%5D=158&field_chemical_name_target_id_selective%5B%5D=161&field_chemical_name_target_id_selective%5B%5D=162&field_chemical_name_target_id_selective%5B%5D=1345 [accessed 28 August 2020].

Callan AC, Rotander A, Thompson K, Heyworth J, Mueller JF, Odland J, et al. 2016. Maternal exposure to perfluoroalkyl acids measured in whole blood and birth outcomes in offspring. Sci Total Environ 569-570:1107-1113.

Cao W, Liu X, Liu X, Zhou Y, Zhang X, Tian H, et al. 2018. Perfluoroalkyl substances in umbilical cord serum and gestational and postnatal growth in a chinese birth cohort. Environ Int 116:197-205.

Dobraca D, Israel L, McNeel S, Voss R, Wang M, Gajek R, et al. 2015. Biomonitoring in California firefighters: Metals and perfluorinated chemicals. J Occup Environ Med 57:88-97.

Gyllenhammar I, Diderholm B, Gustafsson J, Berger U, Ridefelt P, Benskin JP, et al. 2018. Perfluoroalkyl acid levels in first-time mothers in relation to offspring weight gain and growth. Environ Int 111:191-199.

Impinen A, Nygaard UC, Lodrup Carlsen KC, Mowinckel P, Carlsen KH, Haug LS, et al. 2018. Prenatal exposure to perfluoralkyl substances (PFASs) associated with respiratory tract infections but not allergy- and asthma-related health outcomes in childhood. Environ Res 160:518-523.

Impinen A, Longnecker MP, Nygaard UC, London SJ, Ferguson KK, Haug LS, et al. 2019. Maternal levels of perfluoroalkyl substances (PFASs) during pregnancy and childhood allergy and asthma related outcomes and infections in the norwegian mother and child (MoBa) cohort. Environ Int 124:462-472.

Jain RB. 2018. Contribution of diet and other factors to the observed levels of selected perfluoroalkyl acids in serum among US children aged 3-11 years. Environ Res 161:268-275.

Kim K, Bennett DH, Calafat AM, Hertz-Picciotto I, Shin HM. 2020. Temporal trends and determinants of serum concentrations of per- and polyfluoroalkyl substances among northern California mothers with a young child, 2009-2016. Environ Res 186:109491.

Kwon EJ, Shin JS, Kim BM, Shah-Kulkarni S, Park H, Kho YL, et al. 2016. Prenatal exposure to perfluorinated compounds affects birth weight through GSTM1 polymorphism. J Occup Environ Med 58:e198-205.

Okada E, Sasaki S, Kashino I, Matsuura H, Miyashita C, Kobayashi S, et al. 2014. Prenatal exposure to perfluoroalkyl acids and allergic diseases in early childhood. Environ Int 65:127-134.

Skogheim TS, Villanger GD, Weyde KVF, Engel SM, Surén P, Øie MG, et al. 2020. Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children. Int J Hyg Environ Health 223:80-92.

Takahashi M, Ishida S, Hirata-Koizumi M, Ono A, Hirose A. 2014. Repeated dose and reproductive/developmental toxicity of perfluoroundecanoic acid in rats. J Toxicol Sci 39:97-108.

Tian Y, Liang H, Miao M, Yang F, Ji H, Cao W, et al. 2019. Maternal plasma concentrations of perfluoroalkyl and polyfluoroalkyl substances during pregnancy and anogenital distance in male infants. Hum Reprod 34:1356-1368.

Wang Y, Rogan WJ, Chen PC, Lien GW, Chen HY, Tseng YC, et al. 2014. Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environ Health Perspect 122:529-534.

Wang Y, Adgent M, Su PH, Chen HY, Chen PC, Hsiung CA, et al. 2016. Prenatal exposure to perfluorocarboxylic acids (PFCAs) and fetal and postnatal growth in the taiwan maternal and infant cohort study. Environ Health Perspect 124:1794-1800.

Wikström S, Lin PI, Lindh CH, Shu H, Bornehag CG. 2020. Maternal serum levels of perfluoroalkyl substances in early pregnancy and offspring birth weight. Pediatr Res 87:1093-1099.

Titanium dioxide nanoparticles (TiO_2 np)

Titanium dioxide nanoparticles (TiO_2 np) are ultrafine particles 1 – 100 nanometers (nm) in diameter. TiO_2 np can absorb ultraviolet (UV) radiation, act as catalysts, and confer broad antimicrobial protection, among other properties. TiO_2 np applications are highly variable, and include use as a food additive, in food packaging, and in a range of other materials and products, including cosmetics, sunscreens, protective coatings, plastics, paints, solar cells, and ceramic biomedical implants (Baranowska-Wójcik et al. 2020; Dréno et al. 2019; US FDA 2017). Approximately 48% of TiO_2 np produced today is used in paint products, 19% in plastics, 10% in medicine, food and cosmetics, 10% in resin, 8% in paper 3% in fibers, and 2% in rubber (Hong et al. 2017a).

TiO_2 np passed the animal data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant (DART) Identification Committee (DARTIC) for consultation. This is a brief overview of relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

No human studies reporting developmental and reproductive toxicity (DART)-related effects associated with TiO_2 np were identified.

Animal studies

Numerous whole animal studies examining possible DART effects of exposure to TiO_2 np were identified. Findings reported in studies published within the last five years are summarized here. .

Maternal and developmental effects

Developmental effects are associated with *in utero* exposures unless otherwise specified.

- Rat: Increased placental vascular resistance and impaired umbilical vascular reactivity (Abukabda et al. 2019).
- Rat: Perturbed the normal gestational endocrine vascular axis by increasing uterine artery vasoconstrictor responses to kisspeptin, increased placental weights, decreased placental efficiency (grams fetus/gram placenta) and pup weights (Bowdridge et al. 2019).
- Rat: No marked toxicities in dams or effects on embryo-fetal development (Lee et al. 2019).

- Rat: No evidence of maternal or developmental toxicity (Warheit et al. 2015).
- Mouse: Exposure impaired fetal/placental vascularization, inhibited placental cell proliferation, induced placental apoptosis by nuclear pyknosis, reduced the ratio of placental weight to dam body weight, increased the spongiotrophoblast area in the placenta, and decreased expression of several genes in the placenta. Exposure had no effect on the number of implanted or resorbed embryos (L Zhang et al. 2018).
- Mouse: Reduced maternal weight gain, placental and fetal weight, and number of live fetuses, and inhibited development of the fetal skeleton, with reduced or absent ossification (Hong et al. 2017b).
- Mouse: Decreased placental efficiency and impaired lung development, including decreased pulmonary expression of vascular endothelial growth factor-alpha and matrix metalloproteinase 9 at the fetal stage, and fibroblast growth factor-18 at the alveolarization stage (formation and enlargement of the gas exchange area) (Paul et al. 2017).
- Mouse: No overt fetal malformations or changes in pregnancy outcomes, or effects on postnatal growth (Notter et al. 2018).
- Mouse: Fetal cardiac changes include a 43% increase in left ventricular mass, a 25% decrease in fetal cardiac output, decreased electron transport chain complex IV activity, ten-fold higher levels of H_2O_2, increased DNA methylation, and altered gene and protein expression; sustained changes in left ventricular function observed at 11 weeks of age (e.g., 18% decrease in fractional shortening) (Kunovac et al. 2019).
- Monkey (macaque): No effects on hemoglobin regulation in the neonatal brain (Mitsunaga et al. 2016).

Neurodevelopmental effects

Effects are associated with *in utero* exposures unless otherwise specified.

- Rat: Reduced brain-derived neurotrophic factor in the hippocampus; increased hippocampal interleukin-6 concentrations; increased malondialdehyde and nitric oxide metabolites and produced other metabolic changes in hippocampal, cortical, and cerebellar tissues in the neonate brain (Asghari et al. 2019).
- Rat: Increased apoptotic cells and reduced neurogenesis in the hippocampus of offspring on postnatal days (PND) one and 21 (Ebrahimzadeh Bideskan et al. 2017).
- Rat: Increased latency to reach the visible platform (no difference on the final trial, however) and increased the number of errors in the Working Memory Correct test (repeat entries into arms that once contained a water-escape platform) when assessed at 5 months of age (Engler-Chiurazzi et al. 2016).

- Rat: Exposure of lactating rats from PND 2 – 21 impaired memory and learning in offspring on PND 21 (Mohammadipour et al. 2016).
- Mouse: Maternal exposure from GD 7 through PND 21 induced apoptosis and autophagy of hippocampal neurons and ; decreased dendritic length of hippocampal neurons in offspring on PND 21 (Zhou et al. 2017).
- Mouse: Maternal exposure on postpartum days 7-21 inhibited hippocampal development (axonal and dendritic growth) (Zhou et al. 2019b).
- Mouse: Maternal exposure during pregnancy and lactation inhibited development of the central nervous system in offspring, resulting in thinning of the cerebral and cerebellar cortex, decreased numbers of neurons per unit area of cerebrum, edema, dysplasia of neurites in hippocampal pyramidal cells, thinning of the pyramidal cell layer in the hippocampus, and decreased learning and memory, as assessed between PND 35 to 39 (Hong et al. 2018).
- Mouse: Disrupted anatomical structure of the fetal brain and liver (Naserzadeh et al. 2018).
- Mouse: Behavioral deficits with relevance to autism spectrum disorder, including dose-dependent impairments in neonatal vocal communication (PND 6) and juvenile sociability (PND 28 and 42), and dose-dependent increases in prepulse inhibition of the acoustic startle reflex (Notter et al. 2018).
- Mouse: Retarded axonal and dendritic outgrowth (Zhou et al. 2019a).

Endocrine effects

- Rat: Decreased maternal plasma estradiol on GD 20; no effect on maternal progesterone, prolactin, corticosterone or kisspeptin (Bowdridge et al. 2019).
- Rat: Exposure of adult males reduced the levels of testosterone and gonadotropins and downregulated expression of 17beta-hidroxy steroid dehydrogenase (Hussein et al. 2019).
- Rat: Exposure of adult males decreased testicular steroidogenic acute regulatory protein and serum testosterone (Shahin and Mohamed 2017).
- Rat: Exposure of adult males decreased serum testosterone (Morgan et al. 2017).
- Mouse: Exposure of 1-month old females for 30 days induced premature ovarian failure; effects included lower serum levels of estradiol, progesterone and inhibin B, and increased levels of gonadotropins and anti-Mullerian hormone (Hong and Wang 2018).
- Mouse: Exposure of adult males reduced the number of Leydig cells and testosterone concentrations in serum and the testes (Khorsandi et al. 2016).
- Mouse: Exposure of adult males decreased serum and testicular testosterone concentrations (Khorsandi et al. 2017).

- Mouse: Exposure of eight week old males impaired testicular function without changes in plasma levels of sex hormones (Miura et al. 2017).

Epigenetic effects

- Rat: Prenatal exposure resulted in significant epigenetic and transcriptomic changes in fetal cardiac tissue (Stapleton et al. 2018).
- Mouse: Increased fetal DNA methylation in cardiac tissue following prenatal exposure, along with increased hypoxia-inducible factor 1-alpha (*Hif1α*) activity and DNA (cytosine-5)-methyltransferase 1 (*Dnmt1*) protein expression (Kunovac et al. 2019).

Metabolic effects

- Rat: Exposure during pregnancy altered the dam's gut microbiota and increased fasting blood glucose (Mao et al. 2019b).

Male reproductive effects

- Rat: Increased oxidative stress in testicular tissues, reduced sperm motility, viability, and sperm cell count, and increased sperm abnormalities, in addition to damaging testicular histological architecture and upregulating proapoptotic gene (*Bax*) transcripts in testicular tissues (Hussein et al. 2019).
- Rat: Decreased sperm viability, increased incidences of sperm morphological abnormalities (e.g., deformed and detached heads, curved and coiled tails), and testicular interstitial edema and sloughing of the germinal epithelium, with apoptotic changes such as pyknosis, karyolysis and karyoschisis (Morgan et al. 2017).
- Rat: Induced prostatic and testicular injury and corresponding reproductive-related aberrations, including decreased sperm count, increased sperm malformations, and increased testicular gamma-glutamyltransferase activity (Shahin and Mohamed 2017).
- Rat: Degenerative changes in the spermatogenic epithelium, including thinning, disorganization of layers, detachment of sperm cells from the basement membrane, and reduced proliferative activity and differentiation potential of epithelial cells (Sharafutdinova et al. 2018).
- Mouse: Immunological dysfunction in mouse testes, reduction of fertility, infiltration of inflammatory cells, rarefaction, apoptosis, and/or necrosis of spermatogenic cells and Sertoli cells (Hong et al. 2016).
- Mouse: Histological changes in testicular tissues, decreased testicular weight and sperm quality, increased apoptotic index in all types of germ cells, and

increased lipid peroxidation and decreased superoxide dismutase and catalase activites in the testes (Khorsandi et al. 2017).
- Mouse: Impaired testicular function and reductions in two sperm motion parameters (motile and progressive) and in sperm numbers in cauda epididymides; no change in body weights, liver weights, or testicular-related organ weights (Miura et al. 2017).

Mechanistic, in vitro, and other relevant data

- Human: *In vitro* exposure of primary cultures of amniotic cells increased DNA fragmentation, apoptosis and DNA damage (Mottola et al. 2019).
- Human: Exposure to the trophoblastic cell line HTR8-SVneo caused proteostasis disruption and autophagy, increasing endoplasmic reticulum stress related markers, and expression of genes associated with mitophagy (Y Zhang et al. 2018).
- Human: Exposure to the trophoblastic cell line HTR8-SVneo disrupted the cytoskeleton and impaired cell invasion ability (Mao et al. 2019a).
- Rat: *In vitro* exposure of primary Leydig cells induced cellular vacuolization, and nuclear condensation, decreased cell viability, mitochondrial membrane potential, signal transduction second messengers associated with ERK1/2 PKA -PKC signaling pathways, and steroidogenic enzymes in a dose-dependent manner, and suppressed testosterone production (Li et al. 2018).
- Mouse: Retarded axonal and dendritic outgrowth was associated with increased expression of components of the extracellular signal-regulated kinase1/2 (ERK1/2) mitogen-activated protein kinase (MAPK) signaling pathway (Zhou et al. 2019a).
- Mouse: Maternal exposure on postpartum days 7-21 altered gene expression in the hippocampus of pups, including expression of genes involved in the Rho and the N-methyl-D-aspartate signaling pathways (Zhou et al. 2019b).
- Mouse: Induced cell apoptosis, altered microtubule arrangement and dynamics, and impaired cell migration ability in GC-2 and TM4 germ cell lines (derived from mouse spermatogonia and Sertoli cells, respectively) (Mao et al. 2017).
- Rainbow trout: *In vitro* exposure of sperm decreased sperm velocity and increased oxidative stress markers in sperm homogenates (Ozgur et al. 2018).

References cited in "TiO_2 np"

Abukabda AB, Bowdridge EC, McBride CR, Batchelor TP, Goldsmith WT, Garner KL, et al. 2019. Maternal titanium dioxide nanomaterial inhalation exposure compromises placental hemodynamics. Toxicol Appl Pharmacol 367:51-61.

Asghari A, Hosseini M, Beheshti F, Shafei MN, Mehri S. 2019. Inducible nitric oxide inhibitor aminoguanidine, ameliorated oxidative stress, interleukin-6 concentration and improved brain-derived neurotrophic factor in the brain tissues of neonates born from titanium dioxide nanoparticles exposed rats. J Matern Fetal Neonatal Med 32:3962-3973.

Baranowska-Wójcik E, Szwajgier D, Oleszczuk P, Winiarska-Mieczan A. 2020. Effects of titanium dioxide nanoparticles exposure on human health-a review. Biol Trace Elem Res 193:118-129.

Bowdridge EC, Abukabda AB, Engles KJ, McBride CR, Batchelor TP, Goldsmith WT, et al. 2019. Maternal engineered nanomaterial inhalation during gestation disrupts vascular kisspeptin reactivity. Toxicol Sci 169:524-533.

Dréno B, Alexis A, Chuberre B, Marinovich M. 2019. Safety of titanium dioxide nanoparticles in cosmetics. J Eur Acad Dermatol Venereol 33 Suppl 7:34-46.

Ebrahimzadeh Bideskan A, Mohammadipour A, Fazel A, Haghir H, Rafatpanah H, Hosseini M, et al. 2017. Maternal exposure to titanium dioxide nanoparticles during pregnancy and lactation alters offspring hippocampal mRNA BAX and Bcl-2 levels, induces apoptosis and decreases neurogenesis. Exp Toxicol Pathol 69:329-337.

Engler-Chiurazzi EB, Stapleton PA, Stalnaker JJ, Ren X, Hu H, Nurkiewicz TR, et al. 2016. Impacts of prenatal nanomaterial exposure on male adult Sprague-Dawley rat behavior and cognition. J Toxicol Environ Health A 79:447-452.

Hong F, Wang Y, Zhou Y, Zhang Q, Ge Y, Chen M, et al. 2016. Exposure to TiO2 nanoparticles induces immunological dysfunction in mouse testitis. J Agric Food Chem 64:346-355.

Hong F, Yu X, Wu N, Zhang Y-Q. 2017a. Progress of *in vivo* studies on the systemic toxicities induced by titanium dioxide nanoparticles. Toxicol Res (Camb) 6:115-133.

Hong F, Zhou Y, Zhao X, Sheng L, Wang L. 2017b. Maternal exposure to nanosized titanium dioxide suppresses embryonic development in mice. Int J Nanomedicine 12:6197-6204.

Hong F, Wang L. 2018. Nanosized titanium dioxide-induced premature ovarian failure is associated with abnormalities in serum parameters in female mice. Int J Nanomedicine 13:2543-2549.

Hong F, Zhou Y, Ji J, Zhuang J, Sheng L, Wang L. 2018. Nano-TiO2 inhibits development of the central nervous system and its mechanism in offspring mice. J Agric Food Chem 66:11767-11774.

Hussein MMA, Gad E, Ahmed MM, Arisha AH, Mahdy HF, Swelum AA, et al. 2019. Amelioration of titanium dioxide nanoparticle reprotoxicity by the antioxidants morin and rutin. Environ Sci Pollut Res Int 26:29074-29084.

Khorsandi L, Orazizadeh M, Mansouri E, Hemadi M, Moradi-Gharibvand N. 2016. Morphometric and stereological assessment of the effects of titanium dioxide nanoparticles on the mouse testicular tissue. Bratisl Lek Listy 117:659-664.

Khorsandi L, Orazizadeh M, Moradi-Gharibvand N, Hemadi M, Mansouri E. 2017. Beneficial effects of quercetin on titanium dioxide nanoparticles induced spermatogenesis defects in mice. Environ Sci Pollut Res Int 24:5595-5606.

Kunovac A, Hathaway QA, Pinti MV, Goldsmith WT, Durr AJ, Fink GK, et al. 2019. Ros promote epigenetic remodeling and cardiac dysfunction in offspring following maternal engineered nanomaterial (enm) exposure. Part Fibre Toxicol 16:24.

Lee J, Jeong JS, Kim SY, Park MK, Choi SD, Kim UJ, et al. 2019. Titanium dioxide nanoparticles oral exposure to pregnant rats and its distribution. Part Fibre Toxicol 16:31.

Li L, Mu X, Ye L, Ze Y, Hong F. 2018. Suppression of testosterone production by nanoparticulate TiO2 is associated with ERK1/2-PKA-PKC signaling pathways in rat primary cultured Leydig cells. Int J Nanomedicine 13:5909-5924.

Mao Z, Yao M, Xu B, Ji X, Jiang H, Han X, et al. 2017. Cytoskeletons of two reproductive germ cell lines response differently to titanium dioxide nanoparticles mediating vary reproductive toxicity. J Biomed Nanotechnol 13:409-416.

Mao Z, Guan Y, Li T, Zhang L, Liu M, Xing B, et al. 2019a. Up regulation of miR-96-5p is responsible for TiO2 NPs induced invasion dysfunction of human trophoblastic cells via disturbing Ezrin mediated cytoskeletons arrangement. Biomed Pharmacother 117:109125.

Mao Z, Li Y, Dong T, Zhang L, Zhang Y, Li S, et al. 2019b. Exposure to titanium dioxide nanoparticles during pregnancy changed maternal gut microbiota and increased blood glucose of rat. Nanoscale Res Lett 14:26.

Mitsunaga F, Umezawa M, Takeda K, Nakamura S. 2016. Maternal administration of nanomaterials elicits hemoglobin upregulation in the neonatal brain of non-human primates. J Toxicol Sci 41:265-271.

Miura N, Ohtani K, Hasegawa T, Yoshioka H, Hwang GW. 2017. High sensitivity of testicular function to titanium nanoparticles. J Toxicol Sci 42:359-366.

Mohammadipour A, Hosseini M, Fazel A, Haghir H, Rafatpanah H, Pourganji M, et al. 2016. The effects of exposure to titanium dioxide nanoparticles during lactation period on learning and memory of rat offspring. Toxicol Ind Health 32:221-228.

Morgan AM, Ibrahim MA, Noshy PA. 2017. Reproductive toxicity provoked by titanium dioxide nanoparticles and the ameliorative role of tiron in adult male rats. Biochem Biophys Res Commun 486:595-600.

Mottola F, Iovine C, Santonastaso M, Romeo ML, Pacifico S, Cobellis L, et al. 2019. NPs-TiO2 and lincomycin coexposure induces DNA damage in cultured human amniotic cells. Nanomaterials (Basel) 9.

Naserzadeh P, Ghanbary F, Ashtari P, Seydi E, Ashtari K, Akbari M. 2018. Biocompatibility assessment of titanium dioxide nanoparticles in mice fetoplacental unit. J Biomed Mater Res A 106:580-589.

Notter T, Aengenheister L, Weber-Stadlbauer U, Naegeli H, Wick P, Meyer U, et al. 2018. Prenatal exposure to TiO2 nanoparticles in mice causes behavioral deficits with relevance to autism spectrum disorder and beyond. Transl Psychiatry 8:193.

Ozgur ME, Balcioglu S, Ulu A, Ozcan I, Okumus F, Koytepe S, et al. 2018. The *in vitro* toxicity analysis of titanium dioxide (TiO2) nanoparticles on kinematics and biochemical quality of rainbow trout sperm cells. Environ Toxicol Pharmacol 62:11-19.

Paul E, Franco-Montoya ML, Paineau E, Angeletti B, Vibhushan S, Ridoux A, et al. 2017. Pulmonary exposure to metallic nanomaterials during pregnancy irreversibly impairs lung development of the offspring. Nanotoxicology 11:484-495.

Shahin NN, Mohamed MM. 2017. Nano-sized titanium dioxide toxicity in rat prostate and testis: Possible ameliorative effect of morin. Toxicol Appl Pharmacol 334:129-141.

Sharafutdinova LA, Fedorova AM, Bashkatov SA, Sinel'nikov KN, Valiullin VV. 2018. Structural and functional analysis of the spermatogenic epithelium in rats exposed to titanium dioxide nanoparticles. Bull Exp Biol Med 166:279-282.

Stapleton PA, Hathaway QA, Nichols CE, Abukabda AB, Pinti MV, Shepherd DL, et al. 2018. Maternal engineered nanomaterial inhalation during gestation alters the fetal transcriptome. Part Fibre Toxicol 15:3.

US FDA. (Food and Drug Administration). 2017. Summary of Color Additives for Use in the United States in foods, Drugs, Cosmetics, and Medical Devices. Available: https://www.fda.gov/industry/color-additive-inventories/summary-color-additives-use-united-states-foods-drugs-cosmetics-and-medical-devices. [accessed 21 August 2020].

Warheit DB, Boatman R, Brown SC. 2015. Developmental toxicity studies with 6 forms of titanium dioxide test materials (3 pigment-different grade & 3 nanoscale) demonstrate an absence of effects in orally-exposed rats. Regul Toxicol Pharmacol 73:887-896.

Zhang L, Xie X, Zhou Y, Yu D, Deng Y, Ouyang J, et al. 2018. Gestational exposure to titanium dioxide nanoparticles impairs the placentation through dysregulation of vascularization, proliferation and apoptosis in mice. Int J Nanomedicine 13:777-789.

Zhang Y, Xu B, Yao M, Dong T, Mao Z, Hang B, et al. 2018. Titanium dioxide nanoparticles induce proteostasis disruption and autophagy in human trophoblast cells. Chem Biol Interact 296:124-133.

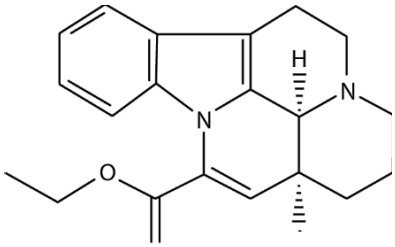
Zhou Y, Hong F, Tian Y, Zhao X, Hong J, Ze Y, et al. 2017. Nanoparticulate titanium dioxide-inhibited dendritic development is involved in apoptosis and autophagy of hippocampal neurons in offspring mice. Toxicol Res (Camb) 6:889-901.

Zhou Y, Ji J, Chen C, Hong F. 2019a. Retardation of axonal and dendritic outgrowth is associated with the MAPK signaling pathway in offspring mice following maternal exposure to nanosized titanium dioxide. J Agric Food Chem 67:2709-2715.

Zhou Y, Ji J, Hong F, Zhuang J, Wang L. 2019b. Maternal exposure to nanoparticulate titanium dioxide causes inhibition of hippocampal development involving dysfunction of the Rho/NMDAR signaling pathway in offspring. J Biomed Nanotechnol 15:839-847.

Vinpocetine

(*CAS No. 42971-09-5*)



Vinpocetine is a synthetic compound derived from a plant alkaloid that is sold as an unregulated dietary supplement (NTP 2020). Purported benefits include: enhanced brain function, rapid weight and/or fat loss, increased energy, improved visual acuity, improved memory and focus. Other claimed benefits include prevention of motion sickness, and treatment of menopausal symptoms, chronic fatigue syndrome, seizure disorders, and hearing or eye disorders. The efficacy of vinpocetine for any of these uses has not been assessed by the US Food and Drug Administration (https://www.fda.gov/food/dietary-supplement-products-ingredients/vinpocetine-dietary-supplements). Vinpocetine products sold in the US recommend daily doses of 5 to 90 mg, although actual vinpocetine levels in tested products may vary widely from what is stated on the label (Catlin 2018).

Vinpocetine passed the animal data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of the relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

No human epidemiologic studies reporting developmental and reproductive toxicity (DART)-related effects associated with vinpocetine were identified.

Animal studies

Whole animal studies examining possible DART effects of exposure to vinpocetine consist of a set of studies conducted by the National Toxicology Program (NTP), examining prenatal developmental toxicity (NTP, 2020). Dose range-finding prenatal developmental toxicity studies were performed in rats and rabbits, and a full-scale

developmental toxicity study was conducted in rats. Findings reported in these studies are summarized here.

- Rabbits: Decreased fetal survival evidenced as increased early resorptions/litter (at 300 mg/kg-day), increased percent post implantation loss, and decreased live fetuses/litter in range-finding study (NTP, 2020; Catlin 2018).
- Rats: Significant, treatment-related effect on post implantation loss for all dose groups (including lowest dose tested = 20 mg/kg-day), and total resorption of litters at doses of 80 mg/kg-day or higher in range-finding study (NTP, 2020).
- Rats: Increased post implantation loss resulting in significantly decreased live fetuses/litter (at 60 mg/kg-day) and reduced gravid uterine weight; treatment-related increased frequency of ventricular septal defect in all exposed groups (including lowest dose tested = 20 mg/kg-day); and significantly increased incidences of incompletely ossified thoracic centrum, and supernumerary ribs in the full-scale study (NTP, 2020; Catlin, 2018).

Mechanistic, in vitro, and other relevant data

- Rat: The distribution and toxicokinetics of vinpocetine and its metabolite, apovincaminic acid (AVA) was studied in pregnant rats at human-relevant doses. Vinpocetine was rapidly absorbed by dams and transferred to the fetal compartment. AVA plasma levels in dams were nearly 3-fold higher than vinpocetine; in fetuses, vinpocetine levels were higher than AVA (Waidyanatha et al. 2018).
- Xenopus/rodent model system: Vinpocetine was tested for effects on the function of excitatory amino acid receptor subtypes expressed in *Xenopus* oocytes after injection of rodent brain poly(A)+ mRNA. Vinpocetine was one of several compounds found to exert dose-dependent inhibition on the oocyte response to N-methyl-D-aspartate (NMDA) in the presence of glycine. Neither the EC50 (half maximal effective concentration) value nor the current-voltage relationship of the NMDA response below 0 millivolts were affected. Inhibition of NMDA channels by vinpocetine appeared similar to the action of Zn^{2+}, which closes the gate of the NMDA channel (Kaneko et al. 1991).

References cited in "Vinpocetin"

Catlin N, Waidyanatha S, Mylchreest E, Miller-Pinsler L, Cunny H, Foster P, et al. 2018. Embryo-fetal development studies with the dietary supplement vinpocetine in the rat and rabbit. Birth Defects Res 110:883-896.

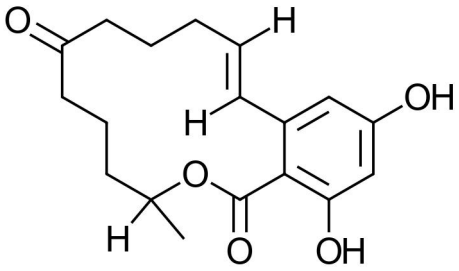
Kaneko S, Sugimura M, Inoue T, Satoh M. 1991. Effects of several cerebroprotective drugs on NMDA channel function: Evaluation using xenopus oocytes and [^{3}H]MK-801 binding. Eur J Pharmacol 207:119-128.

NTP (National Toxicology Program). 2020. NTP Developmental and Reproductive Toxicity Reports. In: NTP Developmental and Reproductive Toxicity Technical Report on the Prenatal Development Studies of Vinpocetine (CASRN 42971-09-5) in Sprague Dawley (hsd:Sprague dawley(®) sd(®)) rats and New Zealand white (hra:Nzw spf) rabbits (gavage studies): DART Report 03. Research Triangle Park (NC).

Waidyanatha S, Toy H, South N, Gibbs S, Mutlu E, Burback B, et al. 2018. Systemic exposure of vinpocetine in pregnant Sprague Dawley rats following repeated oral exposure: An investigation of fetal transfer. Toxicol Appl Pharmacol 338:83-92.

Zearalenone (ZEA)

(CAS No: 17924-92-4)



Zearalenone (ZEA) is a mycotoxin produced by certain *Fusarium* fungi (*F. graminearum* and *F. semitectum*). ZEA production is favored under conditions of high humidity and low temperature, and can be present as a contaminant of grains such as maize, wheat, rye and oats in European countries (Zinedine et al. 2007), and corn, wheat, oats and rice in the US (Zhang et al. 2018; Zinedine et al. 2007).

ZEA is stable under most cooking conditions, and only partially broken down under high temperatures. ZEA is an ingredient in many breast-enhancing dietary supplements, due to its estrogenic activity and its breast enlargement effects observed in exposed humans (Pazaiti et al. 2012). Exposure to ZEA is widespread. For example,

- ZEA is ubiquitous in human and animal feedstuff and often co-occurs with other mycotoxins. For example, in one study ZEA was detected in 12% of sampled infant/toddler foods (including multigrain-, oat-, rice-, corn-, and soy-based products), and in 10% of sampled breakfast cereals (including multigrain-, oat-, corn-, and wheat-based products) (Zhang et al. 2018).
- ZEA has been detected in milk in several countries, including the US (EFSA 2016).
- In a biomonitoring study conducted in girls in New Jersey, ZEA was detected in the urine of 55.2% of study participants (Bandera et al. 2011), with a median urinary ZEA level of 1.02 ng/ml (Rivera-Núñez et al. 2019).
- In surface waters from the central part of Illinois, ZEA was detected in 32% of the samples (limit of detection: 0.4 ng/ L); the highest level detected was 5.7 ng/L (Maragos 2012).

ZEA passed the animal data screen, underwent a preliminary toxicological evaluation, and is being brought to the Developmental and Reproductive Toxicant Identification Committee for consultation. This is a brief overview of recent relevant studies identified during the preliminary toxicological evaluation.

Human epidemiologic studies

A limited number of human studies reporting developmental and reproductive toxicity (DART)-related effects associated with ZEA were identified in the recent literature. DART findings from epidemiologic studies published within the last nine years are summarized here.

Association with puberty development

- Girls with detectable urinary ZEA levels tended to be shorter and less likely to have reached the onset of breast development (cross-sectional analysis) (Bandera et al. 2011).
- Girls with detectable urinary concentrations of total mycoestrogens (sum of ZEA and alpha-zearalanol, and their metabolites) at baseline were significantly shorter at menarche than girls with levels below detection. ZEA and total mycoestrogen concentrations were inversely associated with height- and weight-z-scores at menarche (cross-sectional analysis) (Rivera-Núñez et al. 2019).

Animal studies

Numerous whole animal studies examining possible DART effects of exposure to ZEA were identified. Findings reported in studies published within the last five years are summarized here.

Maternal and developmental effects

- Mouse: Maternal and developmental toxicity which included an increase of micronuclei formation in bone marrow, decreased maternal weight gain and litter weight; fetal growth retardation, increased number of abortions and resorbed fetuses, abnormalities of fetal bone ossification, and number of fetuses with a hematoma (Althali et al. 2019).
- Mouse: Obstruction of essential processes for establishing and maintaining pregnancy, such as embryo transport, the decidual response, and activation of luteal function. Delayed implantation and loss of conceptuses and retarded growth of the fetus after normal implantation (Kunishige et al. 2017).
- Mouse: Increased resorption of implantation sites, placental hemorrhage; decreased placental and fetal weights (Li et al. 2019).
- Rat: Decreased feed intake and body weight (bw) of pregnant rats, decreased birth weight and viability of pups and decreased feed intake and bw of F1 females at postnatal days 21 and 63 (Gao et al. 2017).

- Pig: Reduction in maternal mass gain during gestational day (GD) 35 to 70. Reduction in placenta weight at GD 70 and parturition, increase in maternal ovary weight at GD 70, and decrease in average daily feed intake during lactation. Decrease in total number and average bw of fetuses at GD 70, number of piglets born, litter birth weight, average bw of piglet at birth, number of piglets born alive, born alive litter weight, and born alive piglet bw at birth (Zhang et al. 2015).

Female reproductive effects, prenatal exposure

- Mouse: Decreased percentage of ovarian germ cells at the diplotene stage, more germ cells remained at the zygotene and pachytene stages. Reduced ovarian mRNA levels of meiosis-related genes. Decreased number of primordial follicles in newborns (Liu et al. 2017).
- Rat: Increased incidence of follicular atresia and a thinning of the uterine layer (Gao et al. 2017).

Male reproductive effects, prenatal exposure

- Mouse: Disruption of meiosis, resulting in inhibition of the spermatogenesis and diminished semen quality, as indicated by decreases in spermatozoa motility and concentration (Men et al. 2019).
- Rat: Increased weight of adult testis with atrophy of the seminiferous tubules and decreased number of spermatocytes and mature sperm (Gao et al. 2018a).
- Rat: Decreased anogenital distance (Pan et al. 2020).

Endocrine effects, prenatal exposure

- Rat: Increased serum follicle-stimulating hormone concentrations and decreased serum estradiol in F1 adult female (and also in F0 dams). Reduced levels of gonadotropin-releasing hormone receptor in fetal brain and weaned female brain. Decreased mRNA and protein levels of estrogen receptor (ER)-alpha and 3-beta-hydroxysteroid dehydrogenase in F1 adult uterus and/or ovaries. Dose-dependent increase in 3-beta-hydroxysteroid dehydrogenase in the placenta (Gao et al. 2017).
- Rat: Alteration in F1 serum hormone concentration and steroidogenic enzymes. Decreased luteinizing hormone and testosterone; increased estradiol. Decreased testicular protein levels of 3-beta-hydroxysteroid dehydrogenase and steroidogenic acute regulatory protein levels at weaning and in adulthood. Decreased gene and protein expression of gonadotropin-releasing hormone receptor and ER-alpha in the fetal brain (Gao et al. 2018a).

- Rat: Decreased serum testosterone levels, Leydig cell steroidogenic enzyme proteins, and fetal Leydig cell numbers, thought to result from a delay in commitment of stem Leydig cells to the Leydig cell lineage and proliferation (Pan et al. 2020).

Female reproductive effects, postnatal exposure

- Mouse: Neonatal exposure induced a premature vaginal opening. Disrupted estrus cycles and decreased follicular profiles (Parandin et al. 2017).
- Rat: Histological examination showed follicular atresia (Abbasian et al. 2018).
- Rat: Induced histopathologic alterations in the ovaries and uterus (Gao et al. 2018b).
- Pig: In post-weaning piglets, increased proportion of growing follicles and diameter of the largest growing follicle in ovaries (Dai et al. 2016).
- Pig: Increased size of vulva and relative weight of the reproductive organs (Fu et al. 2018; Su et al. 2018).
- Pig: Decreased proportion of primordial follicles and increased proportion of atretic primordial follicles (Yang et al. 2018).

Male reproductive effects, postnatal exposure

- Mouse: Reduction in the number and motility of spermatozoa (Boeira et al. 2015; Del Fabbro et al. 2019).
- Mouse: Decrease of epididymis and testis indicies; decrease in sperm concentration, sperm normality rate, and sperm motility parameters, including percentage of motile sperm, tropism percentage and sperm average path velocity (Long et al. 2016).
- Mouse: Reduced sperm density and sperm aberration rate (Long et al. 2017).
- Mouse: Decreased number of spermatogenic cells in seminiferous tubules. Increased DNA double stand breaks in spermatogenic cells; decreased sperm concentration, viability, motility, and hyperactive rate. Increased sperm deformity and mortality rates (Pang et al. 2017).
- Rat: Increased cellular apoptosis and DNA fragmentation in the testis (Cheraghi et al. 2015).
- Rat: Reduced mitochondrial content of germinal cells and increased germinal cell apoptosis and necrosis (Adibnia et al. 2016).
- Pig: Interstitial (Leydig) cells between the seminiferous tubules of the testes were markedly smaller and the interstitium was hyperemic, with evident blood stasis in small capillaries, and observations of degenerating seminiferous tubules. Reversible decrease in sperm motility rate, the percentage of progressively

motile sperm, and the number of sperm exhibiting rapid movement (Bielas et al. 2017).
- Rabbit: Increases in spermatozoa beat-cross frequency, in the percentages of spermatozoa with head and midpiece abnormalities, and in the percentages of spermatozoa with fragmented DNA. Histologic examination revealed no abnormal findings in the testes or epididymides (Tsouloufi et al. 2018).

Endocrine effects, postnatal exposure

- Mouse: Reduced expression of kisspeptin and neuronal density in the anteroventral periventricular and and arcuate nuclei in females. Decreased plasma levels of luteinizing hormone and increased plasma levels of estradiol (Parandin et al. 2017).
- Mouse: Reduction in plasma testosterone levels in males (Boeira et al. 2015; Del Fabbro et al. 2019).
- Rat: Decreased serum estradiol and increased serum follicle-stimulating hormone concentrations in females (Gao et al. 2018b).
- Rat: Increased plasma testosterone, progesterone and luteinizing hormone levels and reduced plasma estradiol levels in females (Abbasian et al. 2018).
- Rat: Reduced mRNA and protein levels of ER-alpha and increased mRNA and protein levels of ER-beta in the testes. Decreased mRNA levels of ER-alpha in sperm; no remarkable change in sperm ER-beta mRNA levels. Reduced Leydig cell steroidogenesis (Adibnia et al. 2016).
- Rat: Decreased serum testosterone levels and reduced Leydig cell numbers (Zhou et al. 2018).
- Pig: Reduced serum levels of luteinizing hormone, follicle-stimulating hormone, progesterone, and estradiol. Increased expression of ER-alpha in uterus and ovary and ER-beta in vagina (Fu et al. 2018).
- Pig: Decreased serum levels of estradiol, progesterone, luteinizing hormone, and follicle-stimulating hormone in females (Su et al. 2018).
- Pig: Increases in ovarian mRNA and protein expression levels of ER-alpha and ER-beta (Yang et al. 2018).

Mechanistic, in vitro, and other relevant data

- Mouse: *In vivo* mechanistic study reported increased DNA double-strand breaks at diplotene stage (Liu et al. 2017).
- Mouse: Low enzyme activity (glutathione peroxidase, glutathione reductase, glutathione-S-transferase) and non-enzymatic defenses (reduced glutathione) in testes (Del Fabbro et al. 2019).

- Mouse: Downregulation of gene and protein expression of *Bcl-2* and upregulation of gene and protein expression of *Bax* and *caspase-3* (apoptosis regulator genes) in testes. Upregulated mRNA expression of endoplasmic reticulum stress-related gene (*Xbp-1*) in testes (Long et al. 2017).
- Rat: Inhibition of androgen production and steroidogenic enzyme activities in immature Leydig cells *in vitro*, by downregulating expression levels of the cholesterol side cleavage enzyme, 3-beta-hydroxysteroid dehydrogenase, and the enzyme steroid 5alpha-reductase (Zhou et al. 2018).
- Rat: Reduced levels of the ATP binding cassette transporters b1 and c1 (ABCb1 and ABCc1) in the placenta and in fetal and weaned F1 brains (Gao et al. 2017).
- Rat: Increased ovarian expression of tumor necrosis factor-alpha and the secreted frizzled-related protein-4 (Abbasian et al. 2018).
- Pig: Increased expression of several ATP-binding cassette transporters in the vagina, uterus, and ovary (Fu et al. 2018).
- Pig: Dose-related activation of the ERs/GSK-3beta-dependent Wnt-1/beta-catenin signaling pathway in the ovaries of postweaning females (Yang et al. 2018).

References cited in "ZEA"

Abbasian N, Momtaz S, Baeeri M, Navaei-Nigjeh M, Hosseini R, Abdollahi M. 2018. Molecular and biochemical evidence on the role of zearalenone in rat polycystic ovary. Toxicon 154:7-14.

Adibnia E, Razi M, Malekinejad H. 2016. Zearalenone and 17 beta-estradiol induced damages in male rats reproduction potential; evidence for ERalpha and ERbeta receptors expression and steroidogenesis. Toxicon 120:133-146.

Althali NJ, Hassan AM, Abdel-Wahhab MA. 2019. Effect of grape seed extract on maternal toxicity and *in utero* development in mice treated with zearalenone. Environ Sci Pollut Res Int 26:5990-5999.

Bandera EV, Chandran U, Buckley B, Lin Y, Isukapalli S, Marshall I, et al. 2011. Urinary mycoestrogens, body size and breast development in New Jersey girls. Science of The Total Environment 409:5221-5227.

Bielas W, Nizanski W, Nicpon J, Nicpon JE, Partyka A, Mordak R, et al. 2017. Effect of zearalenone on circulating testosterone concentration, testicular and epididymal morphology and epididymal sperm characteristics in wild boars. Theriogenology 102:59-66.

Boeira SP, Funck VR, Borges Filho C, Del'Fabbro L, de Gomes MG, Donato F, et al. 2015. Lycopene protects against acute zearalenone-induced oxidative, endocrine, inflammatory and reproductive damages in male mice. Chem Biol Interact 230:50-57.

Cheraghi S, Razi M, Malekinejad H. 2015. Involvement of cyclin D1 and E2f1 in zearalenone-induced DNA damage in testis of rats. Toxicon 106:108-116.

Dai M, Jiang S, Yuan X, Yang W, Yang Z, Huang L. 2016. Effects of zearalenone-diet on expression of ghrelin and PCNA genes in ovaries of post-weaning piglets. Anim Reprod Sci 168:126-137.

Del Fabbro L, Jesse CR, de Gomes MG, Borges Filho C, Donato F, Souza LC, et al. 2019. The flavonoid chrysin protects against zearalenone induced reproductive toxicity in male mice. Toxicon 165:13-21.

EFSA. 2016. Appropriateness to Set a Group Health-Based Guidance Value for Zearalenone and its Modified Forms. 18314732. Available: https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2016.4425 https://efsa.onlinelibrary.wiley.com/doi/pdfdirect/10.2903/j.efsa.2016.4425?download=true. [accessed 14 August 2020].

Fu G, Wang L, Li L, Liu J, Liu S, Zhao X. 2018. Bacillus licheniformis CK1 alleviates the toxic effects of zearalenone in feed on weaned female tibetan piglets. J Anim Sci 96:4471-4480.

Gao X, Sun L, Zhang N, Li C, Zhang J, Xiao Z, et al. 2017. Gestational zearalenone exposure causes reproductive and developmental toxicity in pregnant rats and female offspring. Toxins (Basel) 9.

Gao X, Xiao Z, Li C, Zhang J, Zhu L, Sun L, et al. 2018a. Prenatal exposure to zearalenone disrupts reproductive potential and development via hormone-related genes in male rats. Food Chem Toxicol 116:11-19.

Gao X, Xiao ZH, Liu M, Zhang NY, Khalil MM, Gu CQ, et al. 2018b. Dietary silymarin supplementation alleviates zearalenone-induced hepatotoxicity and reproductive toxicity in rats. J Nutr 148:1209-1216.

Kuiper-Goodman T. 1990. Uncertainties in the risk assessment of three mycotoxins: Aflatoxin, ochratoxin, and zearalenone. Can J Physiol Pharmacol 68:1017-1024.

Kunishige K, Kawate N, Inaba T, Tamada H. 2017. Exposure to zearalenone during early pregnancy causes estrogenic multitoxic effects in mice. Reprod Sci 24:421-427.

Li R, Andersen CL, Hu L, Wang Z, Li Y, Nagy T, et al. 2019. Dietary exposure to mycotoxin zearalenone (ZEA) during post-implantation adversely affects placental development in mice. Reprod Toxicol 85:42-50.

Liu KH, Sun XF, Feng YZ, Cheng SF, Li B, Li YP, et al. 2017. The impact of zearalenone on the meiotic progression and primordial follicle assembly during early oogenesis. Toxicol Appl Pharmacol 329:9-17.

Long M, Yang S, Wang Y, Li P, Zhang Y, Dong S, et al. 2016. The protective effect of selenium on chronic zearalenone-induced reproductive system damage in male mice. Molecules 21.

Long M, Yang S, Zhang Y, Li P, Han J, Dong S, et al. 2017. Proanthocyanidin protects against acute zearalenone-induced testicular oxidative damage in male mice. Environ Sci Pollut Res Int 24:938-946.

Maragos CM. 2012. Zearalenone occurrence in surface waters in central illinois, USA. Food Addit Contam Part B Surveill 5:55-64.

Massart F, Saggese G. 2010. Oestrogenic mycotoxin exposures and precocious pubertal development. International Journal of Andrology 33:369-376.

Men Y, Zhao Y, Zhang P, Zhang H, Gao Y, Liu J, et al. 2019. Gestational exposure to low-dose zearalenone disrupting offspring spermatogenesis might be through epigenetic modifications. Basic Clin Pharmacol Toxicol 125:382-393.

Pan P, Ma F, Wu K, Yu Y, Li Y, Li Z, et al. 2020. Maternal exposure to zearalenone in masculinization window affects the fetal Leydig cell development in rat male fetus. Environ Pollut 263:114357.

Pang J, Zhou Q, Sun X, Li L, Zhou B, Zeng F, et al. 2017. Effect of low-dose zearalenone exposure on reproductive capacity of male mice. Toxicol Appl Pharmacol 333:60-67.

Parandin R, Behnam-Rassouli M, Mahdavi-Shahri N. 2017. Effects of neonatal exposure to zearalenone on puberty timing, hypothalamic nuclei of AVPV and ARC, and reproductive functions in female mice. Reprod Sci 24:1293-1303.

Pazaiti A, Kontos M, Fentiman IS. 2012. Zen and the art of breast health maintenance. Int J Clin Pract 66:28-36.

Rivera-Núñez Z, Barrett ES, Szamreta EA, Shapses SA, Qin B, Lin Y, et al. 2019. Urinary mycoestrogens and age and height at menarche in New Jersey girls. Environ Health 18:24.

Su Y, Sun Y, Ju D, Chang S, Shi B, Shan A. 2018. The detoxification effect of vitamin C on zearalenone toxicity in piglets. Ecotoxicol Environ Saf 158:284-292.

Tsouloufi TK, Tsakmakidis IA, Tsousis G, Papaioannou N, Tzika E, Kritsepi-Konstantinou M. 2018. Effect of subchronic oral exposure to zearalenone on the reproductive system of rabbit bucks. Am J Vet Res 79:674-681.

Yang LJ, Zhou M, Huang LB, Yang WR, Yang ZB, Jiang SZ, et al. 2018. Zearalenone-promoted follicle growth through modulation of Wnt-1/beta-catenin signaling pathway and expression of estrogen receptor genes in ovaries of postweaning piglets. J Agric Food Chem 66:7899-7906.

Zhang K, Flannery BM, Oles CJ, Adeuya A. 2018. Mycotoxins in infant/toddler foods and breakfast cereals in the US retail market. Food Addit Contam Part B Surveill 11:183-190.

Zhang Y, Gao R, Liu M, Shi B, Shan A, Cheng B. 2015. Use of modified halloysite nanotubes in the feed reduces the toxic effects of zearalenone on sow reproduction and piglet development. Theriogenology 83:932-941.

Zhou S, Wang Y, Ma L, Chen X, Lu Y, Ge F, et al. 2018. Zearalenone delays rat Leydig cell regeneration. Toxicol Sci 164:60-71.

Zinedine A, Soriano JM, Moltó JC, Mañes J. 2007. Review on the toxicity, occurrence, metabolism, detoxification, regulations and intake of zearalenone: An oestrogenic mycotoxin. Food Chem Toxicol 45:1-18.