EVIDENCE ON THE DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF

2,4-DP

DRAFT

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Reproductive and Cancer Hazard Assessment Section
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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq.*) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that one of the mechanisms by which "a chemical is known to the state to cause cancer or reproductive toxicity [is] if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity" (Health and Safety Code Section 25249.8(b)). The "state's qualified experts" regarding findings of reproductive toxicity are identified as members of the Developmental and Reproductive Toxicant (DART) Identification Committee of the Office of Environmental Health Hazard Assessment's Science Advisory Board (Title 22, California Code of Regulations, Section 12301 (22 CCR 12301)). The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency.

Another mechanism by which a chemical may be put on the Proposition 65 list is if the chemical has been formally identified as causing cancer or reproductive toxicity by an organization that has been designated by the State's qualified experts as "authoritative" for purposes of Proposition 65. One such "authoritative body" is the U.S. Environmental Protection Agency (22 CCR 12306). If the lead agency finds that there is no substantial evidence that the criteria specified in regulation (22 CCR 12306(g)(1) and (2)) have been met, the listing under the Proposition can be reconsidered (22 CCR 12306(j)(1)). Chemicals listed as causing reproductive toxicity which are under reconsideration and which have been placed on the list by the authoritative bodies mechanism are referred to the (DART) Identification Committee. The Committee then makes a recommendation as to whether the chemical should remain on the list.

2,4-DP was added to the Proposition 65 list of chemicals known to the state to cause reproductive toxicity under the authoritative bodies provision of Proposition 65, based on its formal identification as causing developmental toxicity by the U.S. Environmental Protection Agency. Subsequent to that listing, a Court of Appeal decision restricted the evidence that could be reviewed by OEHHA for potential authoritative bodies listings [Third District Court of Appeal, Western Crop Protection et al. vs. Gray Davis et al. (Case No. CO29727, May 9, 2000 as modified on denial of rehearing, June 8, 2000)]. In conducting the more restrictive review of the evidence, per that decision, OEHHA has found that there is no substantial evidence that the scientific criteria for listing 2,4-DP were met (22 CCR 12306(g)). As required by regulation (22 CCR 12306(j)), 2,4-DP is being referred to the DART Identification Committee for a recommendation as to whether it should remain on the Proposition 65 list.

This draft document provides the DART Identification Committee with information relevant to the reproductive toxicity of this chemical. While this hazard identification

document does not provide dose-response evaluation, exposure assessment, or determination of allowable or safe exposure levels, the document does provide information which may be useful in such appraisals.

A public meeting of the Committee will be held on December 17, 2001, in Sacramento, California. Following discussion and Committee deliberation, the Committee will determine whether 2,4-DP "has been clearly shown through scientifically valid testing according to generally accepted principles" to cause reproductive toxicity.

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A. Abstract

2,4-DP is a chlorophenoxy herbicide used in landscaping, forestry, and agriculture. It has minimal use in the production of food crops. In 1999, 1,110 lbs of 2,4-DP dimethylamine salt were applied in California for agricultural use. 2,4-DP is a 50/50 racemic mixture of optical isomers. The (+) isomer is biologically active as an herbicide. Both the mixture (2,4-DP) and the dextrorotatory isomer (2,4-DP(+)) are discussed in this review. 2,4-DP is readily absorbed orally and excreted largely unchanged.

Data on developmental and reproductive toxicity are available mainly from pesticide registration studies. 2,4-DP was found to produce lower fetal weights and skeletal effects in rats and mice in developmental toxicity studies with administration during organogenesis and examination at term. At high doses of 2,4-DP(+) in the mouse, statistically significant increases in malformations were found. No effects on fertility were identified in two rat multigeneration studies. Perinatal toxicity (mortality, growth retardation and developmental delay) was seen at the highest dose in the multigeneration studies. These offspring effects were accompanied by prolonged gestation and impaired maternal behavior. The perinatal toxicity could reflect developmental and/or female reproductive toxicity. No effects on male reproduction were indicated in two multigeneration studies and a dominant lethal study.

B. Introduction

B.1. Chemical structure and main physical characteristics

2,4-DP (2,4-dichlorophenoxypropionic acid, CAS # 120-36-5) is an odorless, colorless crystalline solid which is minimally soluble in water and is not volatile. The molecular weight is 235. 2,4-DP has two optical isomers; the (+) isomer is biologically active as an herbicide. Both isomers are contained as a racemic mixture in commercial preparations and the (+) isomer is also available commercially. 2,4-DP is used as an emulsion or, more commonly, made into soluble esters (isooctyl/ ethylhexyl) or salts (potassium, dimethylamine) for application.

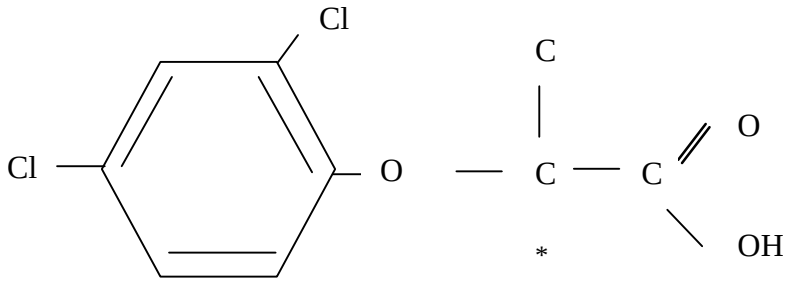


Figure 1. Structure of 2,4-DP. * asymmetric carbon.

2,4–DP, also called dichlorprop and dichloroprop, belongs to the family of phenoxy acid herbicides that includes:
2,4-D (2,4 dichlorophenoxyacetic acid),
2,4-DB (2-(2,4 dichlorophenoxy) butyric acid),
dichlofop (2-(4-(2,4-dichlorophenoxy) phenoxy)propionic acid),
MCPP [2-(2-methyl-4-chlorophenoxy) propionic acid],
2,4,5-T (2,4,5-trichlorophenoxyacetic acid),
MCAP(2 methyl-4 chlorophenoxyacetic acid).
The herbicide Agent Orange, which has been investigated epidemiologically for its teratogenic effects in humans (Sterling and Arundel 1986; IOM 2001), contained 2,4,5,-T and 2,4-D, and was also contaminated with dioxin, but did not contain 2,4-DP.

B.2. Regulatory history

2,4-DP was added to the Proposition 65 list of chemicals known to the state to cause reproductive toxicity effective April 27, 1999. This listing took place under the authoritative bodies provision of Proposition 65, based on a formal identification of 2,4-DP as causing developmental toxicity by the U.S. Environmental Protection Agency (U.S. EPA), an identified authoritative body (22 CCR 12306). Minimal regulatory action has been taken on this chemical. 2,4-DP is not separately recognized as a carcinogen by Proposition 65, U.S. EPA or the International Agency for Research on Cancer (IARC). However, IARC has identified the class of chlorophenoxy acid herbicides as possibly carcinogenic (Group 2B) (see section B.5.1). No non-cancer reference dose has been

developed by USEPA, and no threshold limit value has been established by the American College of Governmental Industrial Hygienists. It is not on California's Toxic Air Contaminant list and no Public Health Goal has been established for drinking water in California.

B.3. Use and exposure information

The chemical 2,4-DP itself is not currently registered in California for use as a pesticide but there are over 50 registered products that contain 2,4-DP salts and esters.

2,4-DP and its salts and esters are used alone or in combination with other herbicides for broad leaf weed control. 2,4-DP is used in the agricultural, landscaping and forestry industries, and for weed control next to highways.

The most recent data available shows that 1,110 lbs of 2,4-DP dimethylamine salt were applied in California for agricultural use in 1999 (DPR 2000). The largest single uses within this category were landscape maintenance (639 lbs) and ornamental turf (241 lbs). 2,4-DP dimethylamine salt was not used on food crops. No agricultural uses were reported for other 2,4-DP salts and esters.

2,4-DP dimethylamine is also registered for use in California in several "Weed and Feed" lawn care products. Phenoxyherbicides are broad-leaf herbicides that do not affect grasses and are therefore valuable in lawn maintenance. There is no available quantitative information on home use.

Little information is available on environmental fate and media contamination. 2,4-DP does not adsorb to soil. The United States Department of Agriculture has estimated a half-life in soil of 10 days (HSDB 2000). Half-lives in ground water are very long, estimated at 196 to over 1200 days (HSDB 2000). The estimated half-life in air is 12 days. Degradation occurs via side-chain breakdown (2-dichlorophenol), ring hydroxylation, and ring opening. 2,4-DP does not degrade to 2,4-D. Bioconcentration does not appear to be a factor in aquatic species; a bioconcentration factor of 23 has been estimated (HSDB 2000).

No estimates of exposure in human populations were located. In a study of workers applying herbicides with sprayers (Libich et al. 1984), the range of daily excretion of 2,4-DP in urine was 1.8-5.1 mg/day, corresponding to a fraction of a mg per kg. In this small sample (n=7) exposure was assumed to occur via inhalation and dermally.

B.4. Pharmacokinetics

The pharmacokinetics of several phenoxy acid herbicides (not including 2,4-DP) were reviewed as a group by Arnold and Beasley (1989). The authors concluded that phenoxyherbicides are completely absorbed from the GI tract but minimally absorbed from skin. They are highly protein bound, have a high volume of distribution and are

largely excreted unchanged by the kidney organic acid transport mechanism. A small amount may be hydrolysed and/or conjugated prior to excretion. At high doses, enterohepatic circulation occurs via biliary excretion. Most of the information reviewed by these authors came from rat studies.

In a study of 2,4-DP (Beitz et al. 1985), 30 or 300 mg/kg/day was administered orally to Wistar rats. Concentrations of 2,4-DP were twice as high in bile as in serum 8 h after gavage administration, indicating biliary excretion at these doses. The serum half-life was 9.5 h for the 30 mg/kg dose and 10.5 h for the 300 mg/kg dose. Conjugated 2,4-DP (leucine conjugate) had higher biliary concentrations and a shorter half-life.

The study by Beitz et al. also produced evidence of hepatic enzyme induction by 2,4-DP, even though 2,4-DP is not metabolized by these enzymes. After two weeks of treatment with 80 mg/kg by gavage, relative liver size was greater, CYP activity (particularly CYPIVA1) was more than doubled, and hexobarbital sleeping time was decreased. After four weeks, additional enzymes (aniline hydroxylase, morphine N–demethylase, cytochrome b_s) were induced. Ascorbic acid excretion was more than doubled after both exposure periods indicating upregulation of kidney organic acid transport mechanisms. A very similar study using 80 mg/kg 2,4-DP for 21 days in Wistar rats confirmed the findings on morphine N-demethylase and hexobarbital sleep time (Clausing et al. 1991). Also, *in vitro* studies showed that 2,4-DP, along with other phenoxy acid herbicides, binds to and inhibits hepatic glutathione (Dierickx 1983).

Pharmacokinetic studies with 2,4-DP (+) have been done in connection with pesticide registration (Lappin 1996). As was the case for 2,4-DP, 2,4-DP (+) was excreted in urine largely unchanged (>70% in male rats, >81% in female rats). One to 3 h after administration the highest concentrations were found in lung, liver, kidney, thyroid and adrenals. Half-lives ranged from 4.4 to 7.3 h in individual rats. *In vitro* studies with various metabolic systems indicated that the dimethylamine salt and the ethylhexyl ester of 2,4-DP(+) degrade to 2,4-DP (+) which undergoes little further metabolism (John and Noctor 1995; John 1995).

Pharmacokinetic studies in animals with inhalation or dermal exposure were not located. In a study of workers applying herbicides along roads and right-of-ways, 2,4-DP concentrations were measured in air and in urine (Libich et al. 1984). Exposure under these circumstances would likely be dermal or by inhalation. From the air and urine measures, it was calculated that 2-3% of the 2,4-DP excreted was from inhalation exposure.

B.5. Non-DART toxicities

B.5.1. Mutagenicity and cancer

The pesticide registration database maintained by the California Department of Pesticide Regulation (DPR 2000) contains a number of studies of mutagenicity (Ames test, Chinese

hamster ovary HPGRT locus assay, reverse mutation assay), chromosome abnormalities (metaphase analysis of human lymphocytes, chromosome aberration assay in human lymphocytes, sister chromatid exchange, bone marrow chromosome aberration test in Chinese hamster cells), and DNA damage (micronucleus test) conducted for 2,4-DP, 2,4-DP(+), and their salts and esters. No mutagenic effects were recorded but possible effects on chromosomes were seen in sister chromatid exchange, hamster bone marrow, and human lymphocyte studies. The *in vivo* Chinese hamster bone marrow assay, indicated effects at single doses of 1780 mg/kg but not at doses of 939 mg/kg or below.

As a class, chlorophenoxy acid herbicides have been recognized as possibly carcinogenic to humans (Group 2B) by IARC on the basis of epidemiological studies showing associations of exposure with soft tissue sarcoma and non-Hodgkin's lymphoma (IARC 1987). 2,4-DP is among the phenoxy herbicide exposures in these studies (Bond and Rossbacher 1993).

Two animal carcinogenicity studies have been performed with 2,4-DP. A rat carcinogenicity study (CDC Research Laboratories 1980) failed to identify increased tumor incidence. The doses used were 25, 50 and 200 mg/kg/day. The 200 mg/kg/day dose was reduced to 150 mg/kg/day after 15 months. However this study was judged unacceptable by DPR due to protocol and performance deficiencies (DPR 2000). A mouse carcinogenicity study of 2,4-DP (CDC Research Laboratories 1979) was essentially negative. However, in its review of this study, DPR noted that high dose animals who were sacrificed for morbidity had a high incidence of palpable abdominal masses which the authors interpreted as enlarged livers but did not examine (DPR 2000). For this reason, the study was not considered definitive.

B.5.2. Experimental animal non-DART toxicities

The toxicity profile of 2,4-DP can be derived from subchronic and chronic studies conducted in support of pesticide registration. The primary toxic effects are hepatoxicity, kidney toxicity, altered lipid metabolism and anemia. A report in the pesticide registration database described seven studies in mice ranging from 3 weeks to 3 months that were conducted as pilot work for a carcinogenicity study with 2,4-DP(+) (BASF 1992). At a diet concentration of 2700 ppm for 3 weeks, 2,4-DP(+) increased liver weight, circulating alkaline phosphatase and alanine aminotransferase. Central lobular hypertrophy in the liver was reported along with retarded body weight gain and eosinophilia. At 3600 ppm for 3 months additional findings were disrupted lipid metabolism (decreased circulating triglycerides and increased cholesterol) along with signs of anemia (decreased hemoglobin and mean corpuscular volume (MCV)). In the 3 week studies no effects were seen at 100, 300 or 900 ppm. In the 3 month study effects were seen at doses as low as 600 ppm, or about 60 mg/kg/day based on a food intake of 10% body weight.

A syndrome of body weight loss, increased liver weight, and urinary obstruction was reported in a study in rats at a dose of 0.04 mmol/kg (94 mg/kg) (Ohta et al. 1987). The

authors also described leg paralysis, which is seen in poisoning with other phenoxy acid herbicides. This toxicity profile was confirmed in rats in the 2-generation study described below (Hellwig 1992). Decreased cholesterol and triglycerides, increased alkaline phosphatase, increased urinary crystals, and decreased hematocrit were seen in the parental Wistar rats at 2000 ppm 2,4-DP in diet.

The altered lipid metabolism associated with 2,4-DP toxicity has been studied in the context of peroxisomal enzyme induction. Many of the steps in cholesterol biosynthesis occur in peroxisomes (Krisans 1996). Induction of rat liver peroxisomal enzymes has also been demonstrated for both isomers of 2,4-DP at a dose (150 mg/kg body weight for 2 weeks) that also led to hepatomegaly and decreased serum cholesterol and triglycerides (Koibuchi et al. 1993). Ohta et al. (1987) found that systemic cholesterol concentrations were reduced by almost 50% after five months of treatment of rats with 2,4-DP at a dose of 94 mg/kg body weight. They further determined that interference with cholesterol synthesis, rather than absorption or distribution, was responsible for this reduction. Additionally, Ohta et al. found that 2,4 DP at a dose of 94 mg/kg for 8 days had a greater cholesterol lowering effect than an equimolar dose of clofibrate. Clofibrate is a peroxisome proliferating agent that acts via the peroxisome proliferator activated receptors (PPARs) to lower cholesterol, and has been used therapeutically as a hypolipedimic drug . The authors compared 2,4-DP to clofibrate because of the similarities between the two agents in their structure (see Figure 2) and their hypolipedimic actions.

C. Developmental Toxicity

C.1. Developmental toxicity studies

C.1.1. Overview

No human developmental toxicity studies were identified. Available animal studies using a developmental toxicity design (administration during organogenesis, examination at term) are shown in Table 1. All studies were by the oral route. One study of developmental toxicity in mice comparing the racemic mixture with the (+) isomer has been published in the open literature (Roll and Matthiaschk 1983). Animal studies performed with 2,4-DP for registration include rat and rabbit developmental toxicity studies performed in 1979/80 and an earlier rat developmental toxicity study from 1973. Also relevant are more recent (1993) rat and rabbit developmental toxicity studies of the isolated (+) isomer (2,4-DP-P, CAS # 15165-67-0). Individual studies are described below.

Table 1. Developmental toxicity studies with oral 2,4-DP and 2,4-DP(+). All dosing was by gavage except for the Litton Bionetics (1973) study which used administration in diet.

Agent	Species	Doses (mg/kg/day)	Reference
2,4-DP	Mouse	0, 100, 200, 300, 400, 500	Roll and Matthiaschk, 1983
2,4-DP (+)	Mouse	0, 200, 300, 400, 500	Roll and Matthiaschk, 1983
2,4-DP	Rat	0, 10, 30, 100	Litton Bionetics, 1973
2,4-DP	Rat	0, 8, 20, 50, 125	Hazleton, 1980
2,4-DP	Rabbit	0, 12, 30, 75	Hazleton, 1979
2,4-DP(+)	Rabbit	0, 20, 50, 100	Hellwig, 1993a
2,4-DP(+)	Rat	0, 20, 80, 160	Hellwig, 1993b

C.1.2. Individual studies

A teratology study conducted with a number of phenoxy acid herbicides included 2,4-DP and 2,4-DP(+) (Roll and Matthiaschk 1983). NMRI mice 8-10 weeks old were treated by oral gavage on gestation day (GD) 6-15 with 0, 100, 200, 300, 400 or 500 mg/kg 2,4-DP or 2,4-DP(+) (excluding the 100 mg/kg dose). The 2,4-DP was suspended in arachis (peanut) oil and administered at a volume of 0.1 ml/10 g body weight. Controls received vehicle only.

Effects determined at the fetal exam (GD18) included growth retardation (fetal weight), embryotoxicity, (resorption) and malformations (cleft palate, fused ribs, exencephaly, and deformed vertebrae) (Table 2). Fetal weight was the most sensitive index and was statistically different from the controls at the 300, 400 and 500 mg/kg doses for both 2,4-DP and 2,4-DP(+). 2,4-DP(+) was somewhat more effective in producing resorption with significant increases seen at both 400 and 500 mg/kg for 2,4-DP(+), and 500 mg/kg for 2,4-DP. 2,4-DP(+) was also the more effective teratogen, demonstrating a significantly increased incidence of exencephaly and malformed vertebrae, neither of which was recorded in the 2,4-DP groups. Fused ribs and cleft palate occurred in both groups with the lowest effective dose being 400 mg/kg in both groups for fused ribs. Soft tissue examinations were performed but no malformations were reported.

Two aspects of the statistical analysis need to be taken into account in interpreting the data from the Roll and Mattiaschk study. First, the sensitivity of the statistical analysis for 2,4-DP(+) was enhanced by a larger control group than was used for 2,4-DP. Second, only comparisons significant at p- values of .009 and .0027 were reported. However, the group mean data (Table 2) did not suggest that lower doses would have been found effective with a larger control group for the 2,4-DP groups or a different criterion for statistical significance.

As regards maternal toxicity, maternal weight gain was lower than control at the 500 mg/kg dose, with no dose-related trends at the intermediate doses (Table 2). The reduction in weight gain at 500 mg/kg was 18% for 2,4-DP and 27% for 2,4-DP(+). The

Table 2. Data from Roll and Matthiaschk (1984) mouse developmental toxicity study. Mean values are presented; the report did not provide variability measures.

doses	dams	pregnancy weight gain	fetuses /dam	resorptions early		resorptions late		dead fetuses		post-implant loss	fetal weight	cleft palate		fused ribs		exencephaly		malformed vertebrae	
mg/kg	*n*	g	*n*	*n*	%	*n*	%	*n*	%	%	*g*	*n*	%	*n*	%	*n*	%	*n*	%
2,4-DP																			
0	24	20.6	11.0	19	7.1	2	0.8	4	1.5	9.4	1.18	4	1.7	1	0.4	0	0	0	0
100	38	22.4	11.8	54	12.1	4	0.9	1	0.2	13.2	1.16	12	3.1	1	0.3	0	0	0	0
200	21	21.3	10.8	17	7.5	2	0.9	0	0	8.4	1.20	3	1.4	0	0	0	0	0	0
300	26	21.3	10.9	26	9.2	5	1.8	1	0.4	11.4	1.08**	8	3.2	0	0	0	0	0	0
400	30	21.9	12.1	30	8.2	3	0.8	0	0	9.0	1.08**	7	2.1	10	3.0*	0	0	0	0
500	12	16.8*	10.5	31	24.6**	3	2.4*	1	0.8	27.8**	0.94**	14	15.4**	5	5.5*	0	0	0	0
2,4-DP(+)																			
0	59	24.2	12.7	63	8.4	14	1.9	2	0.3	10.6	1.17	11	1.6	0	0	2	0.3	2	0.3
200	27	26.8	13.6	42	11.4	5	1.4	0	0	12.8	1.14	4	1.3	3	0.9	0	0	2	0.6
300	33	25.8	12.8	38	9.0	10	2.4	2	0.5	11.9	1.09**	6	1.6	1	0.3	0	0	8	2.2*
400	34	23.2	13.0	90	20.3**	4	0.9	2	0.5	21.7**	0.93**	15	4.3*	25	7.2**	0	0	42	12.1**
500	10	17.0*	13.6	19	14.0*	4	2.9	0	0	16.9*	0.87**	15	13.3**	2	1.8	5	4.4**	14	12.4**

*p<.009, **p<.0027

number of dams treated is not stated but would be judged to be between 20 and 35 from the information on dams examined at term. No other indices of maternal toxicity were reported.

Cleft palate and fused ribs were also reported at multiple doses and in a dose dependent pattern for the other phenoxy acid herbicides tested by Roll and Matthiaschk (MCPP, MCPP(+) and MCPA), and deformed vertebrae were reported only for MCPP(+). In their discussion the authors state that only skeletal effects were found (with the exception of cleft palate) and there were no soft tissue findings.

Standard design rat teratology studies intended to support pesticide registration were conducted for 2,4-DP in 1973, 1979 and 1980. The doses were lower than those used in the mouse study of Roll and Matthiaschk and both fetal and maternal toxicity were limited.

The first, minimally reported, study (Litton Bionetics 1973) administered 0, 10, 30 or 100 mg/kg/day 2,4-DP to Sprague Dawley rats in diet (0, 150, 450, 1500 ppm) on GD 6-15 (n=16-21/group). A tabular presentation of group means was provided without apparent statistical analysis (Table 3). The authors concluded that there were no effects on malformations, resorption, live pups/litter, maternal food consumption (measured during treatment), or pregnancy weight gain. Fetal weight and litter size were not mentioned.

Table 3. Data from Litton Bionetics (1973) rat developmental toxicology study. No statistical analysis was presented in the report.

	2,4-DP (mg/kg/day)			
Endpoint	0	10	30	100
Litters (#)	16	21	19	19
Maternal food consumption (during dosing) (g/day)	19.2±0.8[1]	19.5±0.5	21.0±0.6	19.4±0.6
Pregnancy weight gain (g)	112±6	110±4	116±5	98±4[2]
Resorptions/implants	6/168	3/221	1/206	7/212
Live fetuses/litter	10.5±0.2	10.5±0.1	10.8±0.1	11.1±0.1
Abnormal fetuses (litters)	6(3)	4(1)	1(1)	2(1)

[1] mean ± s.e.m.
[2]Stated to *not* be statistically different from controls in the report.

A later 2,4-DP rat teratology study with statistical analysis (Hazleton 1980) also found no fetal or maternal effects (Table 4). Sprague Dawley rats were treated by gavage with 8, 20, 50, or 125 mg/kg/day on GD 6-15 (n=21-22/group). The vehicle was methyl cellulose and aspirin was used as a positive control. No treatment effects were found on maternal food intake, body weight or weight gain. At the fetal examination (GD 21) no effects were found on implantation, litter size, early or late resorptions, fetal weight, fetal length or external, visceral or skeletal defects or skeletal variants. The high dose for this

study was based on a pilot study indicating reduced fetal weight and length in four dams treated with 100 mg/kg/day vs 10 controls. However, these effects were not seen in the full study.

Table 4. Data from Hazleton (1980) rat developmental toxicology study. Litter means are shown. The report did not provide variability indices.

	2,4-DP (mg/kg/day)				
Endpoint	0	8	20	50	125
Litters (#)	18	15	14	11	16
Early resorptions (# per dam)	0.0	0.13	0.14	0.00	0.19
Late resorptions (# per dam)	0.06	0.07	0.07	0.00	0.06
Fetuses/litter	11.39	14.87	13.43	13.82	12.44
Fetal weight (g)	5.56	5.22*	5.30	5.46	5.33
Fetal crown-rump length (mm)	45.07	43.87*	44.36	44.71	44.29
Major external & visceral defects (% of fetuses)	0.0	0.0	0.0	0.0	0.5
Minor external & visceral defects (% of fetuses)	3.9	4.5	5.9	3.3	6.0
Minor skeletal defects (% of fetuses)	9.5	7.8	2.1	9.0	5.9
Skeletal variants (% of fetuses)	56.2	67.2	47.4	52.6	64.7

*significantly different from control, $p<.05$, Wilcoxon's test

A rabbit teratology study with 2,4-DP (Hazleton 1979) showed much more severe toxicity to both the dams and fetuses in a lower dose range than the rat studies (Table 5). In a pilot study with Dutch-belted rabbits using 0, 25 and 100 mg/kg/day 2,4-DP by gavage, three of five does at the high dose were sacrificed moribund with weight loss and ataxia. The litters of these three dams were found dead in utero at necropsy. Only one of the five rabbits delivered offspring, which were judged to be small. Based on this pilot study, a full study using 0, 12, 30 or 75 mg/kg by gavage on GD 6-18 was conducted (Hazleton 1979). Four of 15 does died at the high dose (75 mg/kg/day); however, mortality rates of 2/15 and 4/15 and 0/15 were seen in the other groups (0, 12 and 30 mg/kg/day). The authors stated that "the deaths observed were considered not to be related to the administration of 2,4-DP". Necropsy data indicated that all the deaths were associated with lung pathology—congestion, "consolidation", pleurisy, and/or pleural effusion. No diseases of infectious origin were proposed by the authors, suggesting that gavage aspiration may have been involved. One of the dams died acutely after gavage. Maternal food intake and pregnancy weight gain were not affected by treatment.

Fetuses in all the treated dose groups had lower mean litter weight and lengths than controls, and this difference was significant for the 75 mg/kg dose (Table 5). However, the authors attributed the lower fetal weights to the larger litter size. Additional statistical analysis prepared for the present document indicated that fetal weight and litter size were highly correlated in the sample as a whole and that the effect of 2,4-DP on fetal weight was not significant when litter size was included in the analysis of variance. The litter

size in the control group was said to be smaller than for historical controls and was associated with a high preimplantation loss, which was also higher than in historical controls. These problems with the concurrent control complicate interpretation of these data sets.

Table 5. Data from Dutch-belted rabbit developmental toxicity study (Hazleton 1979). Means are presented. Variability measures were not given in the report.

	2,4-DP (mg/kg/day)			
Endpoint	0	12	30	75
Dams pregnant (#)	15	15	15	15
Dam mortality (#)	2	4	0	4
Pregnancy weight gain (%)	11.2	11.5	9.2	13.0
Pre-implant loss (%)	23.0	16.2	7.0	3.7
Litter size (fetuses/dam)	4.9	5.6	6.3	7.2
Fetal weight litter mean (g)	37.5	32.6	33.2	33.7*
% minor skeletal variation	37.5	52.2	48.7	56.8

*p<.05.

There were 3 fetuses in the 75 mg/kg/day group with multiple major malformations:
Fetus 1: cleft palate, arthrogryposis (limb flexure), hydrocephalus, midbrain not fused
Fetus 2: cleft palate, arthrogryposis , exencephaly, cerebral aqueduct enlarged, external pinna absent, ectrodactyly, adactyly, microopthalmia, omphalocele, macroglossia
Fetus 3: truncus arteriosus, ventricular septal defect, abnormal ribs, abnormal vertebrae

The more recent studies performed with 2,4-DP(+) are summarized briefly.

In the rabbit study (Hellwig 1993a), Himalayan rabbits were given 0, 20, 50 or 100 mg/kg 2,4-DP(+) in methyl cellulose by gavage on GD 7-19 (n=13-15/group). Maternal and fetal toxicity were identified in Himalayan rabbits only at the highest dose (100 mg/kg/day) and consisted of somewhat decreased food consumption and increased weight loss in the dams early in dosing (GD 7-9) and increased incidence of fetuses with accessory 13^{th} ribs (Table 6). Dam weight gain during pregnancy (GD 7-29) was very similar across groups. Thus the results were much less striking than in the earlier study conducted with Dutch-belted rabbits, in which fetuses with multiple malformations were found in the 75 mg/kg group.

Table 6. Data from the Hellwig (1993a) rabbit developmental toxicology study of 2,4-DP(+).

	2,4-DP(+) (mg/kg/day)			
Endpoint	0	20	50	100
Litters (#)	14	15	13	12
Dam food intake GD 7-19 (g/day)	90	83	95	78*
Dam weight gain GD 7-9 (g/day)	0.2±3[1]	-4.9±20.1	-13.8±32.1	-26.8±28.3*
Pregnancy weight gain GD 7-29 (g)	239±87	230±101	235±106	200±80
Accessory ribs (#)	1(1)[2]	4(4)	2(2)	9*(6)*
Skeletal retardations (#)	50(14)	45(15)	30(11)	27*(8)*

[1] mean ± s.d. [2] fetuses(litters) * p<.05.

The rat (Wistar) study (Hellwig 1993b) also reported decreased food intake and weight gain in the dams during treatment. This occurred at both the 80 and 160 mg/kg dose, with the pregnancy weight gain at term being significantly (13%) lower in the dams of the 160 mg/kg dose than in controls (Table 7). In the fetus, skeletal effects were seen at 160 mg/kg/day (increased skeletal variants and retardations, including rudimentary cervical ribs). Decreased fetal weights were also reported at 160 mg/kg/day. No effects on intrauterine death or gross or soft tissue malformations were reported.

Table 7. Data from the Hellwig (1993b) rat developmental toxicology study of 2,4 DP(+).

	2,4-DP (mg/kg/day)			
Endpoint	0	20	80	160
Litters (#)	20	21	21	21
Dam food intake GD 6-15 (g/day)	26	25.3	23.8	21.6*
Dam weight gain GD 6-15 (g/day)	50	47	42*	31*
Pregnancy weight gain GD 0-20 (g)	156±22[1]	154±21	145±22	136±31*
Fetal weight: litter mean (g)	4.0±.22	3.9±.22	4.0±.20	3.5±.30*
Skeletal variations (#)	70(20)[2]	62(21)	71(21)	94*(19)
Skeletal retardations (#)	60(19)	65(19)	57(19)	125*(21)
Rudimentary cervical ribs (#)	9(5)	5(4)	13(6)	57*(8)*

[1] mean ± s.d. [2] fetuses(litters) * p<.05.

Thus, unlike the previous studies with 2,4-DP (Litton Bionetics 1973; Hazleton 1980) , this rat teratology study with 2,4-DP(+) identified developmental toxicity. Differences between the studies besides the agent included strain of rat (Sprague Dawley in the earlier studies) and dose range (lower in the earlier studies). The rabbit teratology study with 2,4-DP(+) (Hellwig 1993a) identified developmental toxicity but not the malformations reported in the earlier rabbit study with 2,4-DP (Hazleton 1979).

C.2. Developmental toxicity data from multigeneration studies.

Two multigeneration studies in rats have been conducted for 2,4-DP. Neither study reported effects on fertility parameters (see Section D below), but both studies reported effects on offspring growth and viability at a 2000 ppm dietary concentration. The design of the first study (Huntingdon Research Center 1978) was based on diet concentrations of 0, 125, 500 and 2000 ppm 2,4-DP for three generations. However the high dose was reduced to 1000 ppm at the time of breeding for the second generation due to high postnatal mortality in the F1A and B litters. The second study (Hellwig 1992) used dietary concentrations of 0, 80, 400 and 2000 ppm 2,4-DP for two generations. Unlike the previous study (Huntingdon Research Center 1978), the investigators maintained the 2000 ppm dietary concentration throughout the second generation in spite of the high pup mortality.

In the Huntingdon 1978 study, the authors concluded that there were effects on litter weight (sum of all pups born) and postnatal mortality in the F1A and F1B generations at the high dose. Only the F0 dams received this dose, while dams in the later generations were fed 1000 ppm. No statistical analysis or variability measures were given in the report of this study. Thus the size of the effects described by the authors is difficult to determine.

In the Hellwig 1992 study, there was also a striking effect on offspring viability in both litters of both generations at the high dose (2000 ppm). Litters were small at birth and, as a result of high pup mortality prior to postnatal day (PND) 4, no pups were culled on PND 4. Pup mortality was more comparable to controls after PND 4, and did not differ statistically. Nonetheless only a small number of pups survived to weaning in each generation: F1A 2 per litter, 47 total; F1B, 4 per litter, 101 total; F2A, 1.1 per litter, 19 total; F2B, 1.5 per litter, 26 total. The data on offspring survival are shown in Table 8.

Additionally findings of increased incidence of dilated renal pelves were seen in offspring of all litters (except F1B). Little indication of dose related findings were seen at the 80 and 400 ppm doses.

In addition to high early pup mortality, surviving offspring in all four litters in the 2000 ppm group showed a distinctive pattern of developmental retardation and delay. All four litters from the 2000 ppm group were significantly (10%) growth retarded as reflected in birthweight, and even more severely growth restricted (25%) as reflected in weight at weaning (PND 21). Also, testing showed developmental delay in terms of pinna detachment on PND 4, auditory canal opening on PND 13 in all litters, and eye opening on PND 15 in the F2 litters only. Behavioral testing did not find effects on grip reflex at PND13, startle response on PND21, or pupillary response on PND 20, with the exception that the grip reflex was found in a smaller percent of the 2000 ppm F1B pups (74%) than in controls (99%).

Table 8. Offspring viability in the four litters of the Hellwig (1992) two generation rat study.

	2,4-DP (mg/kg/day)[1]			
Endpoint	0	8	42	226
Litter size at birth				
F1A	15.0±2.1[2]	14.5±3.2	14.5±2.2	11.8±4.1**
F1B	14.8±2.9	15.1±3.0	15.8±2.9	10.9±4.1**
F2A	12.6±4.0	13.1±3.6	12.6±3.2	9.2±4.7**
F2B	13.9±3.8	13.9±4.1	14.4±2.8	11.9±3.4
Born live (%)				
F1A	97	97	96	45**
F1B	97	97	95	65**
F2A	97	97	95	70**
F2B	95	95	97	65**
Viability index[3] (%), PND 0-4				
F1A	95	93	94	40**
F1B	98	90	95	64**
F2A	94	91	96	18**
F2B	95	94	93	25**
Viability index (%), PND 4-21				
F1A	99	97	100	92*
F1B	99	98	96	97
F2A	99	99	98	90
F2B	99	100	98	87**

[1] doses were estimated by the authors, apparently based on premating food intake of males and females in both generation.
[2]mean ±s.d.
[3] pups alive at the end of the period/pups alive at the beginning of the period.
* p<.05, **p<.01 Dunnett's test or Fisher's exact test.

Parental toxicity in the Hellwig multigeneration study was examined in some detail, including body weight, food and water intake, clinical signs, organ weights and pathology, complete blood counts and clinical chemistry panels. As was the case for developmental effects, parental effects were almost entirely confined to the 2000 ppm dose group.

In agreement with chronic and subchronic studies, parents in the 2000 ppm group demonstrated disruption of lipid metabolism and signs of kidney toxicity at necropsy following weaning of their litters. Decreased cholesterol and triglycerides were reported in the F1 males and females, decreased cholesterol in the F0 males and decreased triglycerides in the F0 males and females. Increased alkaline phosphatase was reported in the F0 females and the F1 males and urinary crystals in the F0 and F1 males. Decreased

hematocrits were reported in the F0 males and females and the F1 females. It should be noted that these effects were found after weaning of the second litter in each generation and it is not known whether these conditions existed during pregnancy.

Food intake was slightly decreased during the early weeks of the premating period in the F0 parents of the 2000 ppm group. The F1 parents were growth-retarded from birth and weighed less and ate less in the premating period. There was a consistent increase in water intake during the premating period in all parents (F0 males, 25%, F0 females 16%, F1males 18%, F1 females, 27%). Pregnancy weight gain was lower in the 2000 ppm group than controls (F1A 7%, F1B 14%, F2A 19%, F2B 9%, all relative to controls). Body weights and body weight gains were dramatically lower in the 2000 ppm group than in controls during lactation. For example, control dams nursing the F1A litter gained an average of 24 g, while the 2000 ppm dams lost 10 g during lactation. However, the average litter size for the 2000 ppm dams was 1-2 pups compared to 8 pups for controls. Thus the 2000 ppm dams who had small litters due to postnatal mortality ate less and gained less weight during lactation.

C.3. Relationship between developmental and maternal toxicity

Table 5 presents a summary of developmental effects by dose in the combined developmental toxicity and multigeneration studies for 2,4-DP and 2,4-DP(+). Effects in this table *exclude* those detected in pups one or more days after birth in multigeneration studies due to the current interpretation of Proposition 65 which excludes consideration of effects resulting from exposures occurring after birth. However, effects in mothers detected after weaning of their 2nd litter are included.

Table 9. Summary of developmental toxicity findings detected at or before birth.
Effects were statistically significant or identified as treatment-related by authors.

Study	Dose (mg/kg/day)[1]	Developmental Effects	Maternal Effects
RATS			
Litton Bionetics 1973 (Sprague Dawley) 2,4-DP	8, 20, 50, 125	None	None
Hazleton 1980 (Sprague Dawley) 2,4-DP	10, 30, 100	None	None
Huntingdon 1978 (Sprague Dawley) (multigeneration) 2,4-DP	19, 75	None	None
	300[2]	↓ litter weight	↓ food intake, weight gain
Hellwig 1992 (Wistar) (multigeneration) 2,4-DP	8, 42	None	None
	226	↓ birthweight ↓ littersize ↑ stillbirth ↑ dilated renal pelves	↓ pregnancy food intake (6-8%) & weight gain (7-19%) ↓ cholesterol, triglycerides, hematocrit ↑ alkaline phosphatase, urine crystals
Hellwig 1993b (Wistar) 2,4-DP(+)	20, 80	None	None
	160	↑ extra ribs ↓ fetal weight ↓ ossification ↑ hydroureter	↓ food intake (17%), weight gain (37%) during dosing ↓ pregnancy weight gain (28%) ↓ corrected pregnancy weight gain
RABBITS			
Hazleton, 1979 (Dutch-belted) 2,4-DP	12	None	Mortality 4/15; control 2/15
	30	None	Mortality 0/15; control 2/15
	75	↓ fetal weight 3 malformed fetuses	Mortality 4/15; control 2/15
Hellwig 1993a (Himalayan) 2,4-DP(+)	20, 50	None	None
	100	↓ fetal weight ↑ extra ribs	↓ food intake during dosing (13%)

[1] all studies by gavage except Litton Bionetics study and multigeneration studies, which used dietary administration.
[2] diet administration; mg/kg dose estimated by RCHAS based on food intake 5% of body weight.

Table 9 (cont).

Study	Dose (mg/kg/day)[1]	Developmental Effects	Maternal Effects
MICE			
Roll and Matthiaschk, 1983 (NMRI) 2,4-DP	100, 200	None	None
	300	↓ fetal weight	None
	400	↓ fetal weight ↑ fused ribs	None
	500	↓ fetal weight ↑ fused ribs ↑ resorptions ↑ postimplant loss ↑ cleft palate	↓ pregnancy weight gain (18%)
Roll and Matthiaschk, 1983 (NMRI) 2,4-DP(+)	200	None	None
	300	↓ fetal weight ↑ malformed vertebrae	None
	400	↓ fetal weight ↑ malformed vertebrae ↑ early resorp ↑ post implant loss ↑ cleft palate ↑ fused ribs ↓ fetal weight ↑ malformed vertebrae	None
	500	↑ early resorp ↑ post implant loss ↑ cleft palate ↑ exencephaly	↓ pregnancy weight gain (30%)

[1] all studies by gavage except Litton Bionetics study and multigeneration studies, which used dietary administration.

In providing information relevant to consideration of the relationship between developmental and maternal toxicity, three issues were considered:

- Whether maternal toxicity was greater than minimal and made the developmental effects difficult to interpret.
- Whether fetal toxicity occurred in the absence of maternal toxicity
- Whether fetal toxicity occurring in the presence of maternal toxicity was likely to be secondary to the maternal toxicity.

The U.S. EPA Guidelines for Developmental Toxicity Risk Assessment (USEPA 1991) provide guidance in this regard which has been exhaustively reviewed and which therefore may be considered to represent generally accepted principles. The following discussion is in the context of the guidance provided in the U.S. EPA document.

Greater than minimal maternal toxicity as defined by U.S. EPA (1991) (mortality greater than 10%) was seen in the Dutch-belted rabbit teratology study (Hazleton 1979). Mortality was also greater than 10% in the control group, and thus mortality in the treated groups was not attributed by the authors to 2,4-DP. However, examination of the study indicated that most dams that died had lung pathology. Clinical observations and dam pathology at sacrifice also indicated symptoms of pneumonia in other animals. In addition, the controls had lower litter size than any of the treated groups, and litter size was highly negatively correlated with fetal weight. Taken together, this information makes the developmental toxicity findings difficult to interpret. This difficulty is also recognized in the DPR review of this study which concluded that it was "unacceptable" for regulatory purposes. No other studies demonstrated greater than minimal maternal toxicity.

Table 9 indicates that fetal toxicity occurred in the absence of reported maternal toxicity (pregnancy weight gain) at the mid-doses (300 and 400 mg/kg/day) in the mouse teratology study (Roll and Matthiaschk 1983). This study had the largest number and greatest range of doses and showed clear dose response patterns for the fetal effects. In addition the doses were higher than for the rat and rabbit studies. However, only one index of maternal toxicity (pregnancy weight gain) was reported.

Other studies outlined in Table 9, showed a co-occurrence of developmental and maternal toxicity. The studies in which maternal and fetal toxicity co-occurred were considered individually for the likelihood that the fetal toxicity was secondary to maternal toxicity. To aid in this assessment, reports were obtained from the literature that analyzed the relationship between maternal food restriction and fetal outcome.

In the rabbit study of Hellwig (1993), fetal toxicity (reduced fetal weight, increased extra ribs) and maternal toxicity (reduced food intake during dosing) co-occurred at the 100 mg/kg dose. The results of a study of food restriction during organogenesis in rabbits (Petrere et al. 1993) suggests that food intake reduction as small as those seen in the Hellwig study (13%) should not cause adverse fetal effects. Petrere et al. (1993) fed New Zealand white rabbits 32% less food than controls from GD 6 to 18. No effects on fetal

weight or skeletal variations were found. In groups that were 93% food restriction during organogenesis, effects on fetal weight and external/visceral variations were seen.

2,4-DP influenced maternal food intake and weight gain in the multigeneration studies (Huntingdon Research Center 1978; Hellwig 1992). Two studies in the literature restricted food intake of rats before, during and after birth (Young and Rasmussen 1985; Rasmussen and Fischbeck 1987). In the first study (Young and Rasmussen 1985) food was restricted 25, 40 or 50% from control *ad libitum* levels for four weeks before breeding, continuing through gestation to lactation day 14. Sprague Dawley rats used in the study were 6-7 weeks of age at the beginning of food restriction. Live litter size was lower than control at each of the food restriction levels. Birth weight was reduced at the 40 and 50% levels, but not with 25% food restriction. In the second study (Rasmussen and Fischbeck 1987) food restriction of 25 or 40% was maintained through a second pregnancy. Again, live litter size was influenced at both food restriction levels, but birth weight was only affected at the 40% level. Studies with a lower level of food restriction (<20% as in the Hellwig study) were not located. However, the studies by Rasmussen and colleagues using 25% restriction suggest that an effect on birthweight would not be anticipated as a result of reduced food intake alone in the Hellwig study.

Effects on lipid metabolism as reported in the dams of the Hellwig multigeneration study could be suggested to influence steroid hormone metabolism and, secondarily, pregnancy outcome. In this case, the 2,4-DP perinatal toxicity might be more appropriately considered as female reproductive than developmental toxicity. No information was found that would help resolve this issue.

Both maternal and fetal toxicity were reported at 80 and 160 mg/kg/day doses of 2,4-DP(+) in the Hellwig (1993) rat developmental toxicity study. The maternal toxicity was in the form of lower body weight gain during dosing at both doses, and lower pregnancy weight gain and body weight gain during dosing, and lower pregnancy weight gain at the higher dose. Several studies in the literature have used food restriction to reduce food intake and weight gain during organogenesis in rats. For example, when food was restricted to 50% of control from GD 5 to 13 of gestation in Sprague Dawley rats (Ahokas et al. 1981), litter size and fetal weight were not affected, although total and corrected maternal weight gain during pregnancy were significantly lower. This finding suggests that the 17% lower food intake during organogenesis comparable to that seen in the Hellwig study does not in itself lead to adverse fetal outcomes.

C.4. Developmental toxicity: Other relevant data

C.4.1. Distribution and metabolism in pregnant females and conceptuses

No information is available on distribution of maternally administered 2,4-DP or 2,4-DP(+) to fetuses. The large volume of distribution of phenoxy acid herbicides (Arnold and Beasely 1989) suggests that 2,4-DP would be distributed to the fetus. Since 2,4-DP is not metabolized in adult rats, it is probable that 2,4-DP is not metabolized by fetuses.

C.4.2. Mechanism(s) of 2,4-DP developmental toxicity.

C.4.2.1. Active agent

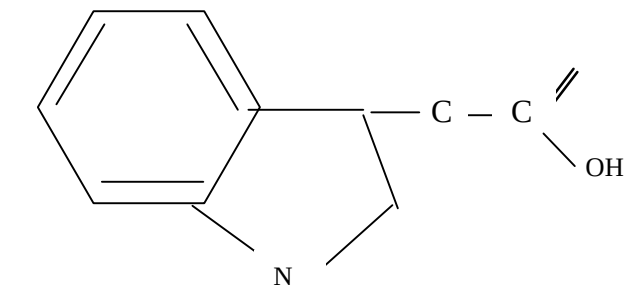
2,4-DP, the subject of this document, is a 50/50 mixture of the (+) and (-) optical isomers. The (+) isomer is thought to be responsible for 2,4-DP herbicidal activity. In general the available data on developmental activity do not suggest that 2,4-DP(+) is exclusively responsible for 2,4-DP developmental toxicity. 2,4-DP (+) appears to have somewhat greater potency and to be a stronger teratogen in a study in mice (Roll and Matthiaschk 1983) that directly compared the two agents. Evaluations of 2,4-DP and 2,4-DP(+) in rats and rabbits did not occur in the same study and there are differences between the studies in areas besides agent used, so that comparisons cannot readily be made. Optical isomers of 2,4-DP also differ only slightly in their effects on plasma cholesterol, with the (+) isomer somewhat more active (Ohta et al. 1987). Similarly, the optical isomers of MCPA, a related phenoxy acid herbicide, do not differ in hepatotoxicity and peroxisome enzyme inducing properties (Koibuchi et al. 1993).

C.4.2.2. Biological mechanisms of action

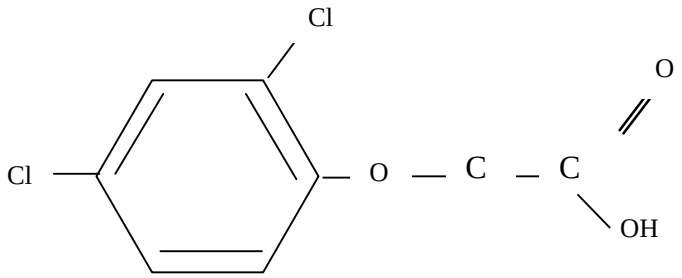
Phenoxy acid herbicides are structural analogues of the auxin plant hormones (see Figure 2) and interfere with their function. In animals, phenoxy acid herbicides are rough structural analogues of fatty acids. They are known to be peroxisome proliferators and to influence circulating cholesterol and triglycerides in chronic animal studies (see Section B.4). 2,4-DP structurally resembles the classic peroxisome proliferator and hypolipidemic agent clofibrate (Figure 2).

No studies have been conducted of the mechanism of 2,4-DP developmental toxicity. However, the knowledge that 2,4-DP is a peroxisome proliferator may have some implications for its mechanism of developmental toxicity. PPARs, particularly PPAR-delta and PPAR-gamma are expressed during embryonic and fetal development (Keller et al. 2000). PPARs form heterodimers with retinoic acid, an important embryonic growth factor (Takase et al. 2000). PPAR-alpha ligands are also implicated in altered estradiol metabolism (Corton et al. 1997).

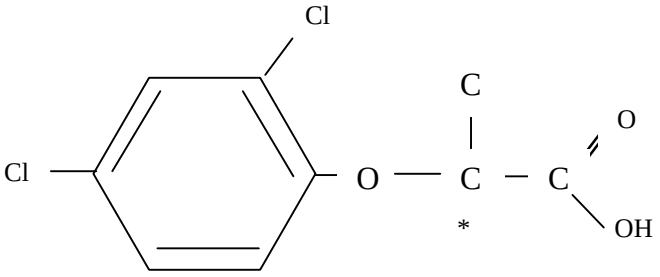
As a carboxylic acid and peroxisome proliferator, 2,4-DP resembles the teratogenic agents, valproic acid and ethylhexanoic acid, which are structural isomers (Figure 2). Recent evidence demonstrates that valproic acid binds to the PPAR-delta receptor, which is critical to differentiation of teratocarcinoma cells (Lampen et al. 1999), (Werling et al. 2001). This pathway is thus a possible mechanism for 2,4-DP induced teratology. 2,4-DP has not been studied for PPAR binding.



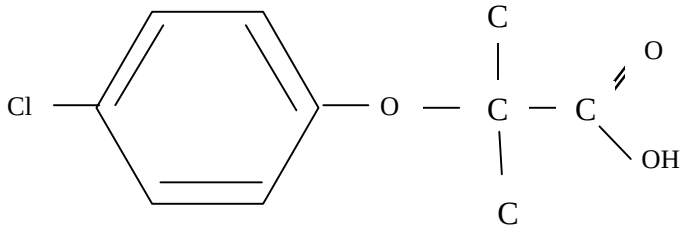
Auxin (Indole-3-acetic acid)



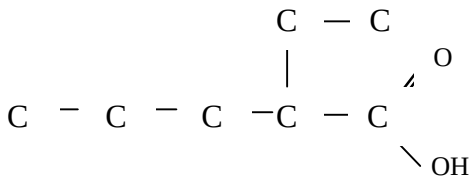
2,4-D



2,4-DP



Clofibric Acid



Valproic acid

Figure 2. Compounds structurally related to 2,4-DP

C.5. Integrative evaluation

Developmental toxicity has been observed at 2,4-DP doses of 226 mg/kg/day and greater and 2,4-DP(+) doses of 80 mg/kg/day and greater in rodent studies using oral administration. Developmental toxicity was reflected in retarded growth and skeletal variations at low doses and intrauterine death and malformations at higher doses in teratology studies with fetal examination at term. In rabbits, one study in the Himalayan strain demonstrated intrauterine growth retardation and skeletal variations at 100 mg/kg/day. Developmental toxicity occurred at doses below those producing maternal toxicity in a mouse study, and at the same doses as those producing developmental toxicity in rat and rabbit studies. The maternal toxicity in rodent and rabbit studies consisted of reduced food intake and pregnancy weight gain. The reduction in food intake was less than 20% of controls.

A very striking perinatal mortality was seen when rats fed 2000 mg 2,4-DP/kg diet were allowed to deliver in multigeneration studies. Surviving offspring demonstrate marked growth retardation and developmental delay. Parents in the multigeneration treated at this dose exhibited signs of 2,4-DP toxicity including altered steroid metabolism and kidney toxicity when examined after the weaning of the second litter in each generation.

D. Female Reproductive Toxicity

D.1. Female reproductive toxicity studies

No studies in humans with information on 2,4-DP were located. Information on female fertility in animals is limited to one fully reported two-generation study using rats and an older three generation study with no statistical analysis. No effects on fertility were found in either study. Observations related to maternal behavior suggested perinatal effects in dams of the two-generation study. In addition, reproductive organ toxicity data are available from chronic and subchronic toxicity studies. No consistent pattern of effects on reproductive ovarian weights were reported.

D.1.1. Fertility

An early three-generation study was conducted using administration of 2,4-DP in diet at 0, 125, 500 and 2000 ppm for the first litter of the first generation (F1A) (Huntingdon Research Center 1978). Subsequently the high dose was reduced from 2000 to 1000 ppm because of the small number of surviving offspring from the F1A litter. The breeders were mated 1 male: 2 females continuously for 20 days. No statistics or variability measures were given in the study report. The authors stated that there were no effects on parental mortality, estrus cycle, rate of conception or gestation length.

According to the authors, pup mortality was affected in the F1A and F1B litters. Some elevation in postnatal mortality was indicated in the F2A litter, but there was limited postnatal mortality in the F2B litter (after reduction of the feed concentration from 2000 to 1000 ppm). The developmental toxicity data are described more fully in Section C.2.

A fully reported two-generation study was conducted using administration of 2,4-DP in diet at doses of 0, 80, 400 and 2000 ppm (Hellwig 1992). The doses were estimated by the authors as 0, 8.3, 42, and 226 mg/kg/day. The breeders were mated overnight at a 1 male: 1 female ratio for three weeks. Due to low survival in the F1 parents at the 2000 dose, they were mated 1 male: 2 females. No effects on fertility were reported in the study at any dose. Developmental toxicity identified at the highest dose in this study is reported more fully in Section C.2.

The high postnatal pup mortality recorded shortly after birth (PND 1-4) was associated with a variety of endpoints, including prolonged gestation, indicating disrupted female reproductive function in the perinatal period, as shown in Table 10. In addition to reports

Table 10. Endpoints possibly reflecting female reproductive toxicity in the 2-generation rat study (Hellwig 1992). No statistics were reported for endpoints other than gestation length. All endpoints were assessed at delivery.

		2,4-DP (mg/kg/day)[1]			
Endpoint	generation	0	8	42	226
Gestation length (days)	F1A	22.0	21.8	22.0	22.7#
	F1B	21.8	21.9	21.8	22.6#
	F2A	22.0	22.0	22.0	22.4#
	F2B	21.9	21.8	21.9	22.4#
Dams with all pups dead at birth	F1A	0/24	0/24	0/25	4/24
	F1B	0/24	0/24	0/25	5/23
	F2A	0/22	0/24	1/20	3/18
	F2B	0/23	0/24	0/25	0/17
Insufficient maternal care	F1A	0/24	0/24	0/25	8/24
	F1B	0/24	0/24	0/25	0/23
	F2A	0/22	0/24	0/20	4/18
	F2B	0/23	0/24	0/25	5/17
Umbilical cord not cut	F1A	0/24	1/24	1/25	6/24
	F1B	1/24	1/24	1/25	3/23
	F2A	1/22	1/24	5/20	4/18
	F2B	3/23	2/24	1/25	5/17
Placenta not consumed	F1A	0/24	0/24	0/25	6/24
	F1B	1/24	1/24	1/25	3/23
	F2A	1/22	1/24	5/20	4/18
	F2B	3/23	2/24	1/25	5/17

[1] doses were estimated by the authors, apparently based on premating food intake of males and females in both generation.
#p<.01, test not stated

of maternal abnormalities at birth, two F0 dams in the 2000 ppm group died while attempting to deliver. The pathology report stated that dystocia was the probable cause of death. Also, another dam in the 2000 ppm group had undelivered pups which were palpated in her abdomen during lactation.

D.1.2. Reproductive organ toxicity

Although a number of chronic and subchronic studies of 2,4-DP and 2,4-DP(+) have been conducted in rats and mice, not all these studies recorded weights of female reproductive organs, and the only female reproductive organ weighed was the ovary (see Table 13).

After 13 weeks oral administration of 0, 100, 300, 1000 and 3000 ppm 2,4-DP to rats, no effects were found on ovary weights (Institute of Environmental Toxicology 1984). In a chronic rat study, which used the same diet concentrations (Institute of Environmental Toxicology 1985), ovarian weights were obtained at 26, 52, 78 and 104 weeks after initiation of treatment (see Table 11). After 26 and 78 weeks reduced absolute ovarian weights were reported in the 300 ppm group, and the relative ovarian weights were also lower at 78 weeks. This effect was not seen at 52 or 104 weeks. In fact, after 104 weeks, increased absolute and relative ovarian weights were reported at the 100 ppm group. One rat in the 1000 ppm group and one rat in the 3000 ppm group developed ovarian tumors. The ovarian weights for these rats are excluded from the means.

Subchronic and chronic studies in beagle dogs using 2,4-DP and 2,4-DP(+) reported ovarian weight and pathology (Reuzel et al. 1980; Hellwig 1994; Bachmann et al. 1997). No 2,4-DP effects were reported in this study. A chronic study using 2,4-DP(+) in mice at 0, 40, 400, 2000 and 3500 ppm in diet found no effects on ovarian weights or histopathology at 40, 400 or 2000 ppm (Mellert et al. 1996). Females in the highest dose group (3500 ppm) were sacrificed after nine months due to excess weight loss and mortality.

D.2. Integrative evaluation

No effects on the female's ability to mate and conceive were suggested in available animal studies. Resorptions seen in developmental toxicity studies could be due to either developmental or female reproductive toxicity. Perinatal toxicity including lengthened gestation, death during labor, and failure to deliver live pups in a multigeneration rat study could be due to female reproductive toxicity. Postnatal toxicity, including pup mortality, growth retardation and developmental delay, seen in this study could also be attributable to female reproductive toxicity. No mechanism studies are available that bear on the biological plausibility of these effects being mediated by female reproductive toxicity. Effects on ovarian weights at high doses in chronic studies are difficult to interpret in the context of reproductive toxicity.

Table 11. Absolute and relative ovarian weights from the 24-month chronic oral toxicity study in rats (Institute of Environmental Toxicology 1985).

Dose (ppm in diet)	Absolute weights (mg)	Relative Weights (%)
	26 Weeks	
0	60.2 ± 6.2[1]	0.028 ± 0.003
100	57.2 ± 10.9	0.026 ± 0.005
300	53.4 ± 5.1*	0.025 ± 0.003
1000	61.5 ± 8.2	0.028 ± 0.003
3000	57.0 ± 6.1	0.029 ± 0.003
	52 Weeks	
0	59.5 ± 7.7	0.023 ± 0.001
100	63.9 ± 12.6	0.025 ± 0.005
300	62.5 ± 5.1	0.025 ± 0.002
1000	65.1 ± 9.0	0.026 ± 0.003
3000	55.8 ± 9.7	0.025 ± 0.004
	78 Weeks	
0	73.6 ± 10.9	0.024 ± 0.003
100	65.0 ± 12.8	0.022 ± 0.004
300	60.8 ± 7.3*	0.019 ± 0.002
1000	64.4 ± 7.7	0.022 ± 0.004
3000	75.9 ± 17.2	0.029 ± 0.005
	104 Weeks (Terminal)	
0	66.0 ± 12.2	0.020 ± 0.004
100	74.1 ± 15.9*	0.022 ± 0.005*
300	68.0 ± 16.7	0.021 ± 0.005
1000	70.7 ± 16.4[2]	0.021 ± 0.005[2]
3000	69.3 ± 11.00[2]	0.021 ± 0.006[2]

[1]mean ± s.d.

[2]values calculated by OEHHA (weights of ovaries with cysts omitted)

* $p<.05$

E. Male Reproductive Toxicity

E.1. Male reproductive toxicity studies

E.1.1. Multigeneration studies

No relevant studies in humans were located. No effects on fertility were found in rat multigeneration studies (Huntingdon Research Center 1978; Hellwig 1992) described above under developmental and female reproductive toxicity (Sections C2 and D).

E.1.2. Dominant lethal and sperm effects

A dominant lethal study was conducted in rats using doses of 0, 10, 25 and 50 mg/kg for five days prior to mating (Pharmakon Laboratories 1979). No effects were seen on fertility rate, or pre- or postimplantation loss in mating conducted over the next eight weeks. DPR considered this study unacceptable due to the small group size (n=10/group).

Another study from the open literature (Seiler 1979) tested a number of phenoxy acid herbicides by examining tritiated thymidine uptake into DNA of testes after oral administration of each agent to mice. At a concentration of 200 mg/kg a number of phenoxy herbicides were found to inhibit testicular DNA synthesis, but 2,4-DP was ineffective at this dose.

E.1.3. Reproductive organ toxicity

Effects on testicular weight were reported in the two generation reproduction study (Hellwig 1992) as shown in Table 12. No treatment-related pathological findings were reported in testes.

Table 12. Absolute and relative testes weights in males of the rat two-generation study (Hellwig 1993).

		2,4-DP (mg/kg/day)[1]			
	generation	0	8	42	226
N/group	F0 F1	24 25	25 25	24 25	25 20
Testes weight (g)	F0 F1	3.8±0.3 4.0±0.3	3.7±0.3 4.0±0.3	3.7±0.2 4.0±0.3	3.6±0.2* 3.6±0.3*
Testes/body (%)	F0 F1	.737±.100 .730±.087	.686±.082 .668±.081	.697±.076 .683±.073*	.694±.087 .706±.080

[1] doses estimated by authors *p<.05, Dunnett's test

Testicular weights and pathology were examined in a number of chronic and subchronic studies (Institute of Environmental Toxicology 1984) (CDC Research Laboratories 1980;

Bachmann et al. 1997; Mellert et al. 1996; Institute of Environmental Toxicology 1985; CDC Research Laboratories 1979; Mellert et al. 1993; Reuzel et al. 1980; Hellwig 1994; Kirsch et al. 1986; Kuhborth et al. 1988) (see Table 13).

No adverse effects on reproductive organs were reported in most of these studies. However, a 13 week study using the highest doses in rats found decreased absolute and relative testes weight at the 3000 ppm diet concentration and decreased relative testes weight at 1000 ppm (Institute of Environmental Toxicology 1985). This effect was not seen with longer exposures in older rats (Institute of Environmental Toxicology 1984). In the longer (24 month) study, increased relative testes weights were seen at the highest diet concentration (3000 ppm) after 26 and 52 weeks but not after 78 and 104 weeks. The increased relative testes weight could be attributed to reduced body weight at the 3000 ppm diet concentration. An increased incidence of prostatitis was found in the two highest dose groups in comparison to controls (1000 ppm group 18/80, 3000 ppm group 18/80, controls 9/80).

No effects on testis or epididymis weights were found in subchronic (3 month) studies (Reuzel et al. 1980; Hellwig 1994) or in a chronic (12 month) study (Bachmann et al. 1997) in beagle dogs. Instances of testicular pathology were noted at the highest dose (48 mg/kg/day) used in the 3 month study of 2,4-DP (Reuzel et al. 1980), but all dogs in this group died or were sacrificed moribund.

E.1.4. Human data

In male foresters using 2,4-D pesticides, luteinizing hormone (LH) was found to be increased during the spraying season (Garry et al. 2001). No information was available for 2,4-DP specifically.

Table 13. Effects of 2,4-DP and 2,4-DP(+) on reproductive organ weights in subchronic and chronic toxicity studies.

CITATION	SPECIES STRAIN	AGENT DURATION (ROUTE)	DOSE mg/kg/day	REPRO. ORGANS WEIGHED	ORGAN WEIGHT FINDINGS[2]
Mellert et al. 1993	Mice B6C3F1	2,4-DP (+) 3-Month Oral (diet)	27, 302, 863	Testes	No effects reported
Kirsch et al. 1986	Rats Wistar	2,4-DP 2,4-DP (+) 4-Week Oral (diet)	11, 53 11, 53	Testes	↑abs testes weights, 11 & 53 mg/kg No effects reported for 2,4-DP (+)
Reuzel et al. 1980	Dogs Beagles	2,4-DP 13-Week Oral (diet)	0, 3, 12, 48	Testes, Ovaries	Testicular effects with high mortality, 48 mg/kg
Inst Environ Toxicology 1985	Rats F344	2,4-DP 13-Week Oral (diet)	0, 5, 15, 50, 150[1]	Testes, Ovaries	↓ abs and rel testes weight, 15 mg/kg ↓ rel testes weight, 50 mg/kg
Kuhborth 1988	Rats Wistar	2,4-DP 3-Month Oral (diet)	8, 39, 201	Testes	No effects reported
Hellwig 1994	Dogs Beagles	2,4-DP(+) 3-Month Oral (diet)	0, 1, 5, 17	Testes, Epididymides	No effects reported
Inst Environ Toxicology 1984	Rats F344	2,4-DP 24-Month Oral (diet) Necropsy at 26, 52, 78 and 104 weeks	0, 4, 11, 36, 116	Testes, Ovaries	↓ abs ovary weight 26 wks, 11 mg/kg ↓abs & rel ovary weight 78 wks, 11 mg/kg ↑abs & rel ovary weight 104 wks, 4 mg/kg ↑rel testes weight 26 & 52 wks, 116 mg/kg ↓ abs and rel testes weight; 78 weeks
Bachmann 1997	Dogs Beagles	2,4-DP(+) 12-Month Oral (diet)	0, 4, 7, 24	Testes, Ovaries Epididymides	↑rel testes weight, 4 mg/kg Testicular pathology with high mortality, 24 mg/kg
CDC Research, Inc. 1980	Rats Sprague-Dawley	2,4-DP 24-Month Oral (diet)	1.25, 2.5, 7.5[1]	Testes	No effects reported
CDC Research, Inc. 1979	Mice CD1	2,4-DP 18-Month Oral (diet)	36, 141, 440 [1]	Testes	No effects reported
Mellert et al. 1996	Mice B6C3F1	2,4-DP(+) 18-Month Oral (diet)	0, 7, 69	Testes, Ovaries	No effects reported

[1] doses calculated from diet concentrations by RCHAS. [2] "abs"= absolute; "rel" =relative.

E.2. Integrative evaluation

No effects on male reproductive capacity were found in two multigeneration studies, one dominant lethal study in the pesticide registration data base, or one study of testicular DNA synthesis in the open literature. No consistent, interpretable pattern of adverse effects on testes weight or histopathology were seen in subchronic and chronic studies.

F. Summary

No human studies of relevance to 2,4-DP developmental and reproductive toxicity were located. Most of the available animal studies of developmental and reproductive toxicity were performed in connection with pesticide registration. No toxicokinetic or mechanism studies directly relevant to 2,4-DP developmental or reproductive toxicity have been performed.

F.1. Developmental Toxicity

A number of developmental toxicity studies are available for 2,4-DP and its (+) optical isomer. Mouse studies using a wide range and number of doses report growth retardation and skeletal variations at the lower doses and intrauterine death and malformations at the higher doses. Effects at the lower doses occur in the absence of maternal toxicity. Developmental toxicity studies in rats and rabbits reported lower fetal weight and skeletal variations. These effects co-occurred with reduced maternal food intake and/or pregnancy weight gain. Increased stillbirth, and decreased litter size and birth weight were recorded in a two-generation study in rats. Parents in the multigeneration study demonstrated the same signs of 2,4-DP toxicity as were seen in chronic and subchronic toxicity studies.

F.2. Female Reproductive Toxicity

No effects on fertility were identified in two rat multigeneration studies. Female reproductive toxicity may have been reflected in prolonged gestation length, delivery complications, deficiencies in maternal care and perinatal pup death reported in the multigeneration studies in rats. Effects on ovarian weight in subchronic and chronic animal studies performed for pesticide registration are difficult to interpret in the context of reproductive toxicity.

F.3. Male Reproductive Toxicity

There was no indication of male reproductive toxicity in multigeneration studies or a dominant lethal study. Effects on testes weight in subchronic and chronic animal studies

performed for pesticide registration are difficult to interpret in the context of reproductive toxicity. Absolute testes weights of F0 and F1 2,4-DP breeder males were lower than control males in a rat multigeneration study.

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