EVIDENCE ON THE DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF

Diuron

DRAFT

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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 "a chemical is known to the state to cause … reproductive toxicity 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 … reproductive toxicity" (Health and Safety Code Section 25249.8(b)). The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of reproductive toxicity are identified as the members of the Developmental and Reproductive Toxicant (DART) Identification Committee of OEHHA's Science Advisory Board (Title 22, California Code of Regulations, Section 12301) (22 CCR 12301).

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 4, 2002, in Sacramento, California. Following discussion and Committee deliberation, the Committee will determine whether diuron "has been clearly shown through scientifically valid testing according to generally accepted principles" to cause reproductive toxicity.

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A. ABSTRACT

Diuron is a substituted urea compound registered for use as a herbicide to control a wide variety of annual and perennial broadleaf and grassy weeds on both crop and non-crop sites. It is used on non-crop areas and many agricultural crops such as fruit, cotton, sugar cane, alfalfa, and wheat. Diuron works by inhibiting photosynthesis. It may be found in formulations as wettable powders and suspension concentrates and is readily absorbed through the root system of plants and less readily through the leaves and stems.

Diuron has low acute toxicity, and its uses are not expected to affect avian wildlife. Diuron is slightly toxic to mammals with the oral LD_{50} in rats being 3400 mg/kg. The dermal LD_{50} in rabbits is greater than 2000 mg/kg. Some signs of central nervous system depression have been noted at high levels of diuron exposure. Male rats given extremely high doses of diuron over a 2-week period showed changes in their spleen and bone marrow. Other chronic effects attributed to moderate to high doses of the pesticide over time included changes in blood chemistry, increased mortality, growth retardation, abnormal blood pigment, and anemia. Increased incidences of urinary bladder transitional epithelial cell carcinomas (all at $p < 0.01$) were observed in both sexes of Wistar rats exposed to 2500 ppm (high dose) in the diet. An increased incidence of adenocarcinomas in the uterus was also observed at the high dose level. These effects were observed along with marked hyperplasia in bladder and renal epithelium. Increased incidence of mammary adenocarcinomas in female mice (NMRI) along with hematological, liver effects and bladder hyperplasia in females were also reported. Effects on erythocyte counts and hypochromic anemia were noted in dogs. Diuron has not produced mutations in animal cells or in bacterial cells in the majority of tests undertaken.

Data on developmental and reproductive toxicity come primarily from developmental studies in rat and rabbits and reproduction studies in rats that are conducted in accordance with standards and protocols established pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Findings from these studies indicate that at maternal dose levels above 80 mg/kg/day some amount of developmental toxicity (delayed ossification of the calvarium and wavy ribs at doses of 125 mg/kg/day and above; lower birth weights at 144 mg/kg/day; delayed ossification at 400 mg/kg/day) were observed in offspring of rats. However, the effects observed do not suggest a pattern of malformations or a specific system being affected at these levels. Known mechanisms of toxicity for diuron and related compounds suggest that the appropriate system that needs examination is the developing reproductive system, but such studies have not been conducted.

Diuron is structurally related to another herbicide, linuron, studies of which have demonstrated testicular adenomas in rats, liver cell adenomas in female mice and adverse effects on the developing male reproductive system subsequent to late gestational and early postnatal exposure. The findings from studies on linuron demonstrate that teratology studies (with traditional dosing regimes and assessment periods) and multigeneration reproduction studies fail to clearly identify the hazard of chemicals with antiandrogenic potential. Although there are no data gaps for diuron under current federal regulations, the effects of diuron on the developing reproductive system have not

been evaluated. Despite the structural similarity, based on publicly available information, some of the toxicological activities of diuron and linuron do differ. Nonetheless, the protocols for related developmental studies of diuron should reflect the concerns raised by the linuron data.

B. INTRODUCTION

B.1 Background, Chemical Structure and Physical Properties

Diuron (CAS No. 330-5401) was introduced under the trademark "Karmex" in 1954 by E.I. Du Pont de Nemours & Co. It is currently registered for use as an herbicide in the United States. Through the pesticide reregistration and tolerance reassessment programs, U.S. EPA plans to make risk management decisions for diuron soon; however, no reregistration eligibility document is available at this time. Diuron is also registered for use in California by the California Department of Pesticide Regulation and there are no data gaps under SB 950 (the Birth Defects Prevention Act of 1984). Diuron is listed under Proposition 65 as a chemical known to the State to cause cancer, based on a formal identification by an authoritative body (U.S. EPA). Diuron was formally identified by the U.S. Environmental Protection Agency (U.S. EPA) as causing reproductive toxicity (developmental) in the course of the implementation of the Toxic Release Inventory (TRI) program (i.e. Section 313 of the Emergency Planning and Community Right-to-Know Act of 1986) (U.S. EPA 1994a, 1994b). Diuron was considered for listing under Proposition 65 because of this formal identification by an authoritative body. Subsequent to publication of a Notice of Intent to List diuron, OEHHA determined that scientific criteria for "as causing reproductive toxicity" specified in regulation (Title 22, California Code of Regulations, Section 12306(g) (22CCR 12306 (g))) were not satisfied for diuron. Consequently, diuron has been referred to the Developmental and Reproductive Toxicant (DART) Identification Committee as required by regulation (22 CCR 12306(i)) so that the State's Qualified Experts can render an opinion as to whether the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause reproductive toxicity.

This document reviews all the available data on the reproductive and developmental toxicity of diuron, including studies not cited by U.S. EPA in the TRI process. The scope of the review here is broader than that previously reviewed by OEHHA during the data call-in and notice of intent to list stages for diuron under that authoritative bodies mechanism. This is due to the fact that 22 CCR 12306(i) specifies a different inquiry for the DART Identification Committee than that undertaken by OEHHA during its review of the chemical to determine if it met the "sufficient evidence" standard specified in 22 CCR 12306(g). The determination by the Committee is distinct from OEHHA's determination whether U.S. EPA had sufficient data for its formal identification of diuron as causing reproductive (developmental) toxicity and calls for a review of all relevant information regarding diuron, not just that cited by U.S. EPA.

Diuron is registered for use on numerous crops such as citrus fruit, cotton, asparagus, sugar cane, alfalfa, wheat and grapes (Farm Chemicals Handbook, 2000). In non-crop applications, diuron is used as a pre-emergence herbicide for general weed control and is also used as a soil sterilant. About 57% of the total usage is reported to be on industrial

sites, on rights-of-way, around farm buildings, and on irrigation and drainage ditches (Hayes and Laws, 1991; U.S. EPA, 1983a,b). Diuron is applied to contained ponds used in commercial catfish production during a 2 to 4-month period in the summer and fall and the fish are harvested from the ponds the year round (U.S. EPA, 1999). Diuron is available as wettable powder, granular, flowable, pelleted/tableted, liquid suspension, and soluble concentrate formulations. Technical diuron is a white, crystalline, odorless solid. It is stable towards oxidation and moisture under conventional conditions with a melting point of 158-159ºC and a boiling point of 180-190ºC. The chemical does not exhibit any unusual handling hazards. It has a water solubility of about 42 ppm(mg/l) at 25ºC (Extoxnet,1996). The structure is shown in Figure 1.

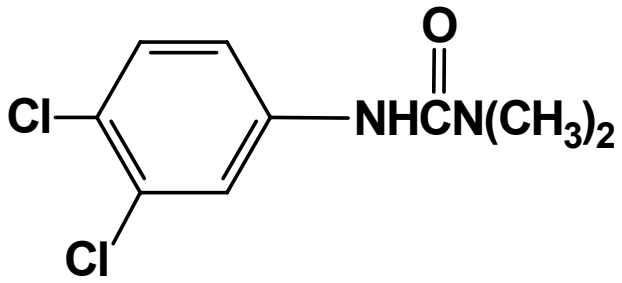


Figure 1

Structure of diuron

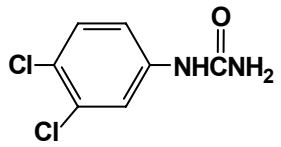
B.2. California Use and Exposure Information

The estimated usage in the United States as of 1995 was about 2-4 million pounds (Aspelin, 1997) and the reported average use in California over the five years between 1994 and 1998 was 1,257,345 lbs/year. (CDPR, 2001). The herbicide is applied by either ground or air equipment at rates ranging from 0.6 to 8.0 pounds active ingredient per acre on crop sites and 15 to 48 pounds active ingredient per acre on non-crop sites (U.S. EPA, 1983b). The main exposures are likely to be to agricultural workers, with some exposure to the general population from consumption of vegetables, and possibly through contact with ornamental plants and landscaping. Breakdown of the compound is similar in animals, plants, and soil. Cows fed very low doses of diuron in their diets had small amounts of residues in whole milk. Cattle fed small amounts accumulated low levels of diuron in fat and muscle, liver, and kidney (Extoxnet, 1996). A voluntary occupational exposure limit based on an eight-hour time-weighted average for diuron in workplace air was established at 10 mg/m^3 by the American Conference of Governmental Industrial Hygienists in the United States (International Labor Office, 1980). Diuron is moderately to highly persistent in soils. Residue half-lives are from one month to one year (Wauchope et al., 1992) but some fields contained residues three years after the last application. Mobility in the soil is related to organic matter and to the type of the residue. The metabolites are less mobile than the parent compound (Howard, 1991). In California, diuron has been found in groundwater in the 2 to 3 ppb range. It has also been

found in Ontario groundwater where it has been linked with land applications (Howard, 1991). Other reports indicate the concentrations of diuron in California well water have ranged from 0.05 to approximately 2.8 ppb (CDPR, 1998; Troiano and Segawa, 1987; Zhang et al., 1997). In the most recent update of the California well water inventory, diuron was detected in 50 of 522 wells sampled with concentrations ranging from 0.6 to 1.5 ppb (CDPR, 1998). Diuron is relatively stable in neutral water. Microbes are the primary agents in the degradation of diuron in aquatic environments (Howard, 1991). Irrigation practices appear to have a greater influence over movement of diuron from soil into groundwater than rain events (Pickett et al., 1990) with diuron residues in surface water occurring as a result of agricultural runoff. Also, concentrations of the chemical in runoff water are a function of the amount of the herbicide applied and time since the last application.

B.3. Pharmacokinetics and Biochemical Effects

No studies have been located of pharmocokinetics of diuron in humans. Diuron is readily absorbed through the gastrointestinal tract in rats and dogs and is excreted in the feces and urine. Tissue levels of diuron were positively correlated with dosage (Liu, 2001). In mammals diuron is metabolized by dealkylation of the urea methyl groups. Hydrolysis of diuron to 3,4-dichloroaniline and oxidation to 3,4-dichlorophenol, as well as hydroxylation at carbon 2 and/or 6 of the benzene ring, has also been reported. The predominant metabolite of diuron in urine was N-(3,4-dichlorophenyl)-urea (Figure. 2). Diuron is also partially excreted unchanged in feces and urine (Boehme and Ernst, 1965; Hodge et al., 1967). Metabolites found in mammals were qualitatively similar to those found in soil and plants wherein dealkylation was also the major metabolic pathway (Dalton et al., 1966; Geissbuhler et al., 1963). As a dihalogenated substituted urea herbicide diuron is reported to be a more potent inducer of hepatic metabolizing enzymes (e.g., benzo (a) pyrene mono-oxygenase (BP-MOO), 7-ethoxy coumarin *O*-deethylase (EROD)) compared to phenyl urea herbicides with one or no halogen substitutions such as isoproturon or chlorotoluron (Schoket and Vincze, 1985 1986, 1990). Schoket and coworkers (1987) found that repeated diuron exposures (1/6 LD50 for 3 days) decreased the plasma half-life of antipyrine significantly indicating hepatic cytochrome P_{450} isozymes were induced. Hepatic enzymes such as cytochrome P_{450}, BP-MOO, microsomal epoxide hydrolase, glutathione-S-transferase were all induced by diuron in a dose-related manner (oral dosing 1/20 to 1/4 LD_{50}) in rats (Schoket and Vincze, 1990). Dose-related induction of hepatic microsomal enzymes was also seen in rats fed a diuron-containing diet (100, 250, 1000 and 2000 ppm) for 13 weeks with maximum induction within the first 3 weeks of feeding (Kinoshita and DuBois, 1970). Also, a sex difference was noted in the response of the animals, where male rats were more sensitive than females to the enzyme-inducing activity of diuron (Liu, 2001).



3,4 dichlorophenyl urea

Figure 2

B.4. Non-DART Toxicities

No studies have been located of human effects of diuron exposure. There have been numerous studies in animals conducted for pesticide registration purposes, and a small number of studies published in the peer-reviewed literature.

B.4.1. Acute Toxicity

The rat acute oral single dose LD50 is 3.4 g/kg with 95% confidence limits of 2.9-4.0 g/kg (Hodge et al., 1967). It has been reported that the protein content of the diet can influence the acute toxicity of diuron in weanling rats (Boyd and Krupa, 1970). The rat acute inhalation LD50 is reported to be less than 2.5 mg per liter and the rabbit acute dermal single dose LD50 to be greater than 2 g/kg of bodyweight. A primary eye irritation study in the rabbit shows that diuron is moderately irritating to the unwashed eye when instilled undiluted and primary dermal irritation showed that diuron is not a skin irritant when applied undiluted (Drexel Chemical Company, 1997). A skin sensitization study (Buehler method) in the guinea pig shows that diuron is not a skin sensitizer when applied undiluted (Drexel Chemical Company, 1997). Signs of acute toxicity following near-lethal dosages of diuron in rats included drowsiness, ataxia, decrease and subsequent increase in reflexes, irritability and bradypnea. Diarrhea, diuresis, shedding of bloody tears and nosebleed were also noted. Hypothermia, glycosuria, proteinuria and aciduria were detected at 24 hours after exposure with respiratory failure being the immediate cause of death (Boyd and Krupa, 1970). For humans, the only reported case of acute, oral exposure to the herbicide produced no significant symptoms or toxicity (Extoxnet, 1996).

B.4.2. Subchronic and chronic toxicity

Subchronic toxicity: Wistar rats (10/group) administered diuron (98.8%) in the diet at 0, 4, 10 and 25 ppm for six months (Bayer AG, 1986), demonstrated minor hematological changes in females and increased iron pigment deposition was noted in both males and females at 25 ppm suggesting a NOEL = 10 ppm. The scope of the study was primarily restricted to parameters affecting the erythrocytes. The authors reported that in two previous studies (details not available), rats showed growth retardation and anemia after

being fed a diet containing diuron at 4000 and 8000 ppm for 30 days. Red blood cell counts and hemoglobin levels were also reduced.

Chronic toxicity: Groups of 60 Wistar rats per sex were administered diuron (98.7% purity) at 0, 25, 250, or 2,500 ppm in their diet for a period of up to 24 months (Bayer, 1985a). Ten animals per sex per group were sacrificed at 12 months. The corresponding administered doses were 1, 10 and 111 or 1.7, 17 and 203 mg/kg-day for males and females, respectively. The testes and other organs collected were fixed in a 10% buffered formaldehyde solution and specimens for histopathology were embedded in Paraplast, microtomed (approximately 5 µm) and stained with Hemalum Eosin. Decreased erythrocyte counts, reduced hematocrit, increased cellular hemoglobin content and mean corpuscular volume were observed in all treatment groups. Hemosiderin deposition in the spleen and splenic enlargement was also observed in all treatment groups. The dose of 25 ppm was identified as a LOAEL based on increased erythrocyte count, increased hemosiderin in the spleen, increased spleen weight, bone marrow activation, increased hematopoietic marrow, decreased fat marrow, at the two higher doses and thickened urinary bladder wall (transistional epithelial cell carcinomas at the high dose only). Data on ophthalmology and histology of the spinal cord were not presented. No significant effects in the reproductive system were noted in the animals at the interim sacrifice (12 months). The tumor incidences in the rat bioassay are shown below in Table 1.

An early study (Hodge et al., 1967) administered technical diuron (80% a.i.) to beagle dogs (two males and three females per dose) in the diet at levels of 0, 25, 125, 250 or 1,250 ppm for two years. The approximate corresponding doses were 0, 0.625, 3.12, 6.25 and 31.25 mg/kg/day. Body and organ weights, clinical chemistry, hematology, gross pathology and histopathology were evaluated. No adverse effects were reported at 25 ppm (0.625 mg/kg/day). Abnormal blood pigment was observed in males at 125 ppm and in both sexes at greater dose levels. Depression of red blood cell number, reduced hematocrit and hemoglobin were observed at 250 ppm and greater. Weight loss, increased erythrogenic activity in bone marrow and hemosiderosis of the spleen were observed at 1,250 ppm supporting a NOAEL of 25 ppm (0.625 mg/kg/day). In another study, diuron (98.2% purity) was administered in the diet for 12 months at 0, 50, 300, or 1,800 ppm to six beagle dogs/sex/group (Bayer, 1985b). Approximate corresponding doses were 0, 1.9, 11, and 64 mg/kg-day. Reduced erythrocyte counts, hemoglobin, hematocrit, MCHC and alpha-1 and alpha-2 globulin values were reported at 300 ppm and higher in both sexes. Increases in mean corpuscular values, Heinz bodies in erythrocytes, reticulocytes, leukocytes, beta globulins, platelets, alanine amiotranferase and alkaline phosphatase activities, and relative spleen weights were noted at 300 ppm in males and in both sexes at 1,800 ppm. Increased relative spleen weights were reported in both sexes at 1,800 ppm and in male dogs at 300 ppm. Increased deposition of iron pigment was found in liver, kidney and spleen in both sexes at 300 and 1,800 ppm. A NOAEL of 50 ppm (1.9 mg/kg-day) was identified based on blood effects and on iron pigment deposition in the two highest doses.

B.4.3. Genotoxicity and Carcinogenicity

Genotoxicity: Diuron was not found to be mutagenic to TA97, TA98, TA100 and TA1535 strains of Salmonella typhimurium (Ames Salmonella plate assay) either with or

without metabolic activation at the concentrations tested (-S9: 0.5, 1, 2.5, 5 and 10 µg/plate; +S9: 10, 25, 100 and 250 µg/plate). In the CHO/HGBRT assay, the results for diuron are negative up to cytotoxic levels in the presence of S9 activation (0.75 mm) and in the absence of S9 metabolic activation (1.25 mm). For the *in vivo* cytogenic study in rats, diuron is clastogenic at 5,000 mg/kg, the highest dose level tested. For the *in vitro* unscheduled DNA synthesis assay in primary rat hepatocytes, diuron is negative up to 20 mm, the highest concentration tested (Liu, 2001).

Carcinogenicity: In the rat chronic toxicity study (details outlined previously in B.4.2.) submitted for regulatory purposes (Bayer, 1985a) statistically significant increased incidences (increasing trends and pair-wise comparisons in the high dose groups) of urinary bladder transitional epithelial cell carcinomas (all at $p < 0.01$) were observed in both sexes. The incidence of bladder epithelial cell carcinomas in the control, low, mid and high-dose groups in the males and females are shown in Table 1. Marked hyperplasia was found in bladder and renal epithelium at the high dose of 2500 ppm in males and females and to a lesser extent in females at the mid-dose of 250 ppm. Male rats also displayed a significant increasing trend ($p < 0.05$) in renal pelvis epithelial papillomas or carcinomas. Information on carcinogenicity in the reproductive tract is presented below (Tables 2 and 3). In the uterus there was a slight increase in the incidence of adenocarcinoma and endometrial sarcoma at the high dose level, and squamous cell carcinoma was observed in one mid-dose female and one high-dose female. Overall, in the chronic studies, diuron appears to be more carcinogenic than uniquely toxic to the reproductive system since the tumors were not restricted to the reproductive system.

Table 1. Tumor Incidence in Male and Female Rats Administered Diuron in the Diet (Bayer, 1985a).

Dose, ppm (mg/kg-day: males, females)	Urinary bladder epithelial carcinomas (%)[1]		Kidney renal pelvis epithelial carcinomas or papillomas in males (%)[3]
	Males	Females	
0 (0, 0)	1/49 (2)	1/47 (2)	0/49 (0)
25 (1, 1.7)	0/50 (0)	0/49 (0)	0/50 (0)
250 (10, 17)	1/49 (2)	1/50 (2)	0/50 (0)
2500 (111, 203)	35/48 (73)[2]	13/49 (27)[2]	3/48 (6)

[1] Statistically significant increasing trends for both males and females; $p < 0.01$ (Exact trend test).
[2] Statistically significant increased incidence versus controls; $p < 0.01$ (Fisher's Exact test).
[3] Statistically significant increasing trend; $p < 0.05$ (Exact trend test).

Table 2. Tumor Incidence in Female Reproductive Organs from the Two-year Dietary Study in Rats (Bayer 1985a)

Tumor Site and Type	Dose Levels ppm (mg/kg/day)			
	0	25 (1.7)	250 (17)	2500 (203)
Tumor incidence at terminal sacrifice (%)				
Animals examined	48	50	50	50
Uterus :				
Polyp	7 (15)	7 (15)	6(12)	3 (6)
Adenocarcinoma	5 (10)	5 (10)	5 (10)	10[a] (20)
Squamous cell carcinoma	0	0	1 (2)	1 (2)
Endometrial sarcoma	0	0	0	2 (4)
Ovary :				
Adenocarcinoma	0	3 (6)	1 (2)	0
Thecal cell tumor	0	1 (2)	0	0
Granulosa cell tumor	0	0	0	2 (2)
Mammary gland:				
Fibroadenoma	-	4 (8)	-	-
Adenocarcinoma	-	1 (2)	-	-

[a]includes one slightly differentiated carcinoma
- not examined

Table 3. Tumor Incidence in Male Reproductive Organs from the Two-year Dietary Study in Rats (Bayer 1985a)

Tumor Site and Type	Dose Levels ppm (mg/kg/day)			
	0	25 (1)	250 (10)	2500 (111)
Tumor incidence at terminal sacrifice (%)				
Animals examined	50	50	50	49
Testes:				
Mesothelioma (malignant)	1(2)	0	0	0
Benign Leydig cell tumor (unilateral)	4(8)	1(2)	2 (4)	5(10)
Benign Leydig cell tumor (bilateral)	0	2(4)	0	1 (2)
Prostate (Sarcoma Infiltration)	0	0	2 (4)	0
(squamous cell carcinoma of bladder origin)	0	0	0	1 (2)
Seminal Vesicle (Sarcoma Infiltration)	0	0	2 (4)	0
Epididymis (tumor infiltration)	0	0	0	1(2)

The oncogenicity phase of this combined chronic toxicity/oncogenicity study in rats was considered to be supplementary in satisfying the oncogenicity data requirement under

FIFRA. The deficiency in the study was that several organs were not examined, such as the mammary glands.

In a two-year feeding (oncogenicity) study in NMRI mice, 60 animals/dose/sex were administered 0, 25, 250, or 2,500 ppm diuron in their diet (Bayer, 1983). Approximate corresponding doses were 5, 51 or 640 for males and 8, 78 or 867 mg/kg/day for females. Ten animals of each sex/group were sacrificed at 12 months. No effects were noted in either sex at 25 or 250 ppm. At 2,500 ppm, body weights were reduced in both sexes and relative liver and spleen weights and hemosiderin deposits in the liver were increased at both 12 and 24 months in males. Increased leukocyte and reticulocyte counts, mean corpuscular volume and hemoglobin and bilirubin values were also observed in both sexes at the highest dose. An increased incidence of liver single cell necrosis and increased mitosis was observed in the livers of both sexes. No significant effects in the reproductive system were noted in the animals at the interim sacrifice (12 months). An increased incidence of urinary bladder edema and epithelial cell hyperplasia, thickened mucosa and enlarged uterine horn was observed in the high dose females at 24 months. A NOAEL of 250 ppm (51 mg/kg-day in males) was identified for non-cancer effects. No statistically increased incidence of tumors was observed in the males. In the females, a statistically significant trend of increased incidence of mammary gland adenocarcinomas was observed. Mammary gland tumors (adenocarcinoma type A and B) in the 2,500 ppm group were statistically significantly higher than the concurrent control (12%, $p \leq 0.05$ vs. 4% in the concurrent control) and higher than the historical control incidence of 3.3%. The incidence of mammary adenocarcinomas observed in the mouse bioassay are shown in Table 4. In the ovary, a higher incidence of unilateral granulosa/theca cell tumors was noted at the 250 ppm dose level. Both these tumors are derived from the same cell of origin i.e., the granulosa cell and luteomas are derived predominantly from granulosa-cell and theca-cell tumors (Lemon and Gubareva, 1979). Although the total number of ovarian tumors was not influenced by treatment, the variation was in tumor type and not in number. Also there was increased trend of ovarian luteomas (Table 5). The study authors and U.S. EPA in its analysis (U.S. EPA, 1997), combined sex cord-stromal tumors, which included luteomas, thecomas, Sertoli cell tumors, Leydig cell tumors, androblastomas, and arrhenoblastomas for further statistical analysis of the tumor data. It is thought that these tumors are morphological variants of the same tumor type (Bayer, 1983; U.S. EPA, 1997). The incidence of combined sex cord-stromal tumors was not significantly increased compared to controls, nor was there an increasing trend. Also, the incidence of these tumors in all dose groups was within the range reported by Bayer (1983), as was the incidence of spontaneous occurrence (0 to 35.5%).

Table 4. Tumor Incidence in Female Mice Administered Diuron in the Diet (Bayer, 1983)

Dose, ppm (mg/kg-day)	Mammary gland adenocarcinomas (%)[1]
0 (0)	2/50 (4)
25 (7.5)	1/47 (2)
250 (77.5)	1/49 (2)
2500 (867.0)	6/50 (12)

[1]Statistically significant increasing trend; $p < 0.05$ (Exact trend test).

Table 5. Tumor Incidence in Reproductive Organs (Female) of Mice Administered Diuron in the Diet (Bayer, 1983)

Two-year only:

TUMOR SITE AND TYPE	TREATMENT LEVEL ppm (mg/kg/day)			
	0	25 (7.5)	250 (77.5)	2500 (869)
Ovaries – Number of animals examined:	34	32	46	41
Unilateral Luteoma (benign)	3	1	2	7**
Granulosa/theca cell tumor Unilateral (benign)	7	4	11	5
Bilateral (benign)	1	1	2	2
Unilateral (malignant)	0	1	0	0
Tubular cystadenoma -Unilateral (benign)	2	1	1	0
Leiomyoma – Unilateral (benign)	0	0	0	1
Teratoma – Unilateral (benign)	0	1	0	0

* Significantly different from control by Trend test, p=0.0034
** Significantly different from control by Trend test, p=0.158

B.5. Summary of non-DART toxicity

The data indicate that exposure to diuron can result in increased reticulocyte count, suggestive of hemolytic anemia in female Wistar rats at 25 ppm in the diet. Hemosiderin deposition in spleen and splenic enlargement in males and females were also noted at 25 ppm. Additionally, increased erythropoiesis in bone marrow and related signs at higher doses were noted. Epithelial cell carcinomas in the urinary bladder were observed in both sexes in Wistar rats exposed to 2500 ppm (high dose) in the diet. An increased incidence of adenocarcinomas in the uterus was also observed at the high dose level. These effects were observed along with marked hyperplasia in bladder and renal epithelium. The LOEL was reported to be 25 ppm (1.02 and 1.69 mg/kg/day for males and females, respectively), the lowest dose level tested in this study, based on increased erythrocyte count in females, increased hemosiderin in the spleen, increased spleen weight, bone marrow activation, increased hematopoietic marrow, decreased fat marrow,

and thickened urinary bladder wall in males. Increased incidence of mammary adenocarcinomas in female mice (NMRI) along with hematological, liver effects and bladder hyperplasia in females were also reported. Effects on erythocyte counts and hypochromic anemia were noted in dogs.

C DEVELOPMENTAL TOXICITY

C.1 Overview

Four studies in experimental animals of developmental toxicity of diuron were located, three in rat and one in rabbit (Khera et al., 1979; Talakin et al., 1984; Argus Laboratories 1986a, 1986b). Also, two studies on reproduction and fertility effects in the rat contain information relevant to developmental endpoints (Haskell Laboratory, 1990; Hodge, et al., 1967). No studies of human exposure and developmental effects were located.

C.2 Animal developmental toxicity studies

C.2.1 Developmental toxicity studies in rats

Khera et al. (1979)

In this study the formulation Karmex® (containing 80% diuron) was given in corn oil by oral gavage to 20 mated (as determined by positive vaginal smear on day 1 of gestation) female Wistar rats per group, on days 6 to 15 of gestation, at 0, 125, 250 or 500 mg/kg/day. Early resorptions or implantation sites and fetuses dying at a late developmental stage were recorded as dead fetuses. The incidence of 'non-pregnant' females appeared to increase with dose, although the exposure began during the peri-implantation period, on day 6 of gestation. The authors reported that the pregnancy rate was variable in all groups and not related to treatment. Only the highest dosage level demonstrated both maternal and fetal reduced body weights that were statistically significant (individual data not provided). Average maternal weight for the 250 mg/kg dose level was reduced on the 15th day of pregnancy and later at necropsy (without uteri and contents), but the decreases were not statistically significant. Wavy ribs were noted in the mid- and high-dose groups and delayed ossification of the calvarium was noted in fetuses from dams that received 125 mg/kg/day of diuron. The number of fetuses examined for skeletal findings (with Alizarin red staining) was two-thirds of the total number alive from each litter but the actual figure is not given. The remaining fetuses were fixed in Bouin's fluid, sectioned and examined for visceral anomalies. The total number of all fetuses were 199, 189, 164 and 147 in the control, low, mid-and high-dose groups, respectively. No individual data were included for evaluation. The litter was considered the basic unit and the proportion of a litter having a particular attribute was calculated. Analyses of inter-group comparisons were performed using Student's t-test. Differences of $p<0.05$ were considered significant. From these results it appears that the maternal NOEL is 250 mg/kg (reduced body weight) and the developmental NOEL is less than 125 mg/kg. Data from the study are summarized in Table 6.

Table 6. Selected Results of Rat Developmental Study with Karmex (Diuron 80%) (Khera et al, 1979)

Parameter	Dose levels (mg/kg/day)			
	0	125	250	500
Number of dams pregnant/Number of females initiated	19/20[a]	18/20	15/20	14/20
Number "not pregnant" at necropsy	0	2	5	6
Number of corpora lutea per pregnancy Mean±SE	12.9±0.3	13.0±0.4	13.9±0.6	13.1±0.3
Number of live fetuses per pregnancy Mean±SE	10.5±0.9	10.5±0.9	10.9±0.6	10.5±0.5
Dead or resorbed fetuses[b] (%)	7	8	12	9
Fetal Weight Mean±SE	5.2±0.1	5.2±0.1	5.0±0.1	4.6±0.1*
Number of anomalous fetuses	10	15	16*	12
Number of fetuses examined	199	189	164	147
Number of litters with anomalous fetus (%)	8 (40)	9 (50)	9* (60)	7 (50)
Number of litters examined	19	18	15	14
Anomalies (number of fetuses affected)				
Wavy ribs	3	7	7*	7*
Extra ribs	6	7	5	0
13th rib, rudimentary	0	2	1	0
Sternoschisis	0	0	1	2
Calvarium delayed ossification	1	4*	3	4
Runted fetus	0	1	0	0
Hydroureter	0	0	0	1

[a] one dam died of causes unrelated to the treatment; * $p<0.05$.

[b] $\frac{\text{Number of resorption sites + dead fetuses}}{\text{Total implants}} \times 100$

Talakin et al. (1984)

In this study (published in Russian with limited technical details), five groups of 15 (animals/group) of female white rats were exposed by gavage to diuron (in 2% starch) at oral doses of 0, 9, 18, 36, or 720 mg/kg on days 6 to 15 of gestation. A dose of 720 mg/kg resulted in maternal weight loss and decreased embryo weight and size on the 20th day. At this dose, there was a 1.3-fold increase in embryo death and 3.4-fold increase in postimplantational loss. The most common anomalies were related to the circulatory system (30%), liver (9%) and skeleton (5%). At 36 mg/kg, the toxic effects were less severe, with implantation and survival comparable to the control group. The only effects at 18 mg/kg were an increase in the average weight of placenta and in the placental implant coefficient (however, these were not dose-related). The authors determined the NOAEL to be 9 mg/kg.

Argus Research Laboratories, Inc. (1986a)

In this developmental toxicity study, diuron (99.0%) in aqueous 0.5% hydroxypropyl methylcellulose was administered by gavage at 0, 16, 80, or 400 mg/kg/day, to 25 mated females/group [CD (SD) rats], on days 6 through 15 of gestation (day 0 = sperm and/or plug). Reduced feed consumption, reduced maternal body weight and body weight gain at 80 and 400 mg/kg/day was noted. Reductions in fetal body weight (9%) and delayed ossification of the vertebrae and sternal centers of fetuses were observed at 400 mg/kg/day (See Table 7 below). The authors concluded that this study demonstrated no adverse developmental effects, with a maternal NOEL of 16 mg/kg/day and a developmental NOEL of 80 mg/kg/day.

Table 7. Selected Results of Rat Developmental Study with Diuron (99.0% purity), (Argus Research Laboratories, Inc., 1986a)

Parameter	Dosage mg/kg/day			
	0	16	80	400
Number females mated	25	25	25	25
Number found dead/killed *in extremis*	0	0	0	0
Feed consumption period at end of dosing period[a] (on day 16)		nd	-22%	-46%
Difference in body weight[a] (on day 16)		nd	-8%	-16%
Body weight gain between days 6-16[a]		nd	-63%	-131%
Number that had abortions	0	0	0	0
Number with total litter resorptions	0	0	0	2
Number of litters at Cesarean section	22	23	22	20
Mean litter size (live)	13.1	13.3	13.5	14
Mean fetal weight (g)	3.36	3.42	3.35	3.07
Delayed ossification in fetuses (vertebrae & sternum)	-	-	-	+

– not present
\+ present
[a] compared to control;
nd: not different from control

The California Department of Pesticide Regulation (CDPR) found this study acceptable according to FIFRA guidelines and found no adverse effects with a developmental NOEL of 80 mg/kg/day and a maternal NOEL of 16 mg/kg/day (CDPR, 1987). The Integrated Risk Information System summary (US. EPA, 1988) does not include the review of this study and identified data gaps for the Rat Teratology Study endpoint. However, the U.S. EPA Office of Pesticide Programs/Health Effects Division (OPP/HED) reviewed this study and concluded the maternal (systemic) NOAEL to be 16 mg/kg/day, based on reduction in body weight and food consumption at the LOAEL of 80 mg/kg/day. The developmental (fetal) NOAEL was determined to be 80 mg/kg/day, based on increases in delayed ossification of vertebrae and sternebrae as well as decreased fetal weights at the LOAEL of 400 mg/kg/day (U.S. EPA, 1999, 2001).

C.2.2. Developmental toxicity study in rabbits

Argus Research Laboratories, Inc. (1986b)

In a developmental toxicity study, diuron (99.0%) in aqueous 0.5% hydroxypropyl methylcellulose was administered by gavage on days 7 through 19 of gestation to New Zealand White Rabbits (artificially-inseminated females), 23, 24 or 25/group, at 0, 2, 10, or 50 mg/kg/day. Maternal toxicity as demonstrated by decreased feed consumption and

weight gains was noted at 50 mg/kg/day. Also one abortion was noted at this dose level. No adverse developmental effects were observed resulting in a maternal NOEL of 10 mg/kg/day and a developmental NOEL greater than 50 mg/kg/day. Data from this study are summarized in Table 8.

Table 8. Selected Results of Rabbit Developmental Study with Diuron (99.0% purity) (Argus Research Laboratories, Inc., 1986b)

Parameter	Dosage mg/kg/day			
	0	3	10	50
Number of females mated	23	24	25	25
Number found dead/killed *in extremis*	0	0	0	0
Feed consumption at end of dosing period[a] (on day 20)	-	nd	nd	-22%
Difference in body weight at end of dosing period[a]	-	nd	nd	-5%
Number that had abortions	0	0	0	1
Number with total litter resorptions	0	0	0	0
Number of litters at Cesarean section	21	22	23	22
Mean litter size (live)	6.7	6.8	7	7.5
Mean fetal weight (g)	46.11	45.89	46.13	45.17

[a]compared to controls
nd: not different from controls

The CDPR found this study acceptable according to FIFRA guidelines and found no adverse effects with a developmental NOEL ≥ 50mg/kg/day and a maternal NOEL of 10 mg/kg/day (CDPR, 1987). The Integrated Risk Information System summary (U.S. EPA, 1988) does not include the review of this study and identified data gaps for the Rabbit Teratology Study endpoint. However, the U.S. EPA, OPP/HED reviewed this study and concluded that the maternal (systemic) NOAEL was 10 mg/kg/day, based on decreased body weight and food consumption at the LOAEL of 50 mg/kg/day. The developmental (pup) NOAEL was 50 mg/kg/day, the highest dose tested, and there were no developmental effects (U.S. EPA, 1999, 2001).

C.2.3. Reproductive toxicity studies in rats.

Hodge et al. (1967); University of Rochester School of Medicine and Dentistry (1964)

In this study, diuron was supplied in the diet at either 0 or 125 ppm (approximate dose of 6.25 mg/kg-day) to rats in a three-generation reproduction study (8 males and 16 females per group). Fertility rate, body weight, hematology and histopathology were monitored. No effect was observed except for body weights, which were significantly decreased in the F_{2b} and F_{3a} litters. A LOAEL of 125 ppm (6.25 mg/kg-day) was identified. The entire study was repeated with a different lot of diuron and no adverse effects on body weights were observed. The two replicates in the study were performed with the same numbers of animals. No adverse reproductive effects were reported in the replicate

component of the study but postweaning growth retardation was noted in the first component. The reason for the difference was not explained. The same set of studies was submitted to the California Department of Food and Agriculture (CDFA) to meet the FIFRA guidelines for reproduction and fertility effects in rats. A NOEL could not be determined since the report had several deficiencies such as single dose, no analysis of diet, no data on food consumption, inadequate number of pregnant animals (per FIFRA guidelines), and parental animals were not necropsied (CDFA, 1985).

Haskell Laboratory for Toxicology and Industrial Medicine (1990)

Diuron technical grade (97.1% purity) was fed in the diet to 30 Crl:CD*BR rats/dose/sex for two generations at 0 (control), 10, 250, and 1750 ppm (Male: 0, 0.68, 16.9 and 120 mg/kg/day; Female: 0, 0.80, 20.3 and 144 mg/kg/day). F0 animals were fed treated diet 73 days prior to mating and F1 animals received treatment diet for at least 105 days after weaning and prior to mating. According to the authors, these dose levels were selected based on the observations from previous sub-chronic and chronic feeding studies. At 1750 ppm, decreased F0 and F1 body weights (5% to 20% less than controls; see Table 9) and food consumption were noted. During gestation, a reduction in weight gain was noted in the high dose groups for both F0 and F1 matings; this was statistically significant only for the F1 generation. During lactation however, a reduction in weight gain was noted in the F0 dams. This reduction in weight gain was lower in the high dose group (statistically significant). For the F1 generation, a reduction in weight gain (dams) was also noted in the control animals and 10 ppm group during lactation, but a weight gain was noted in the 250 ppm group and at the high dose group (Table 9). Data on other reproductive parameters from the study are summarized in Table 10. The pup weights at the high dose level were significantly lower than control pups in both generations on postnatal day 7, 14 and 21, as summarized in Table 11. No adverse effects on the reproductive system were observed. Testicular weights in this study did not exhibit a clear dose-response relationship. The authors concluded that the parental NOEL was 250 ppm based on reduced F0 and F1 body weights and food consumption at 1750 ppm and the reproductive NOEL was 250 ppm based on reduced F1 and F2 pup weights at 1750 ppm. Histopathological examination of reproductive tissues (testes, epididymides, prostate, seminal vesicles, ovaries, uterus, cervix and vagina) was conducted only for the control and high dose groups and for gross lesions noted in other doses. No significant effects were reported from these examinations.

Table 9. Mean Body Weight Gains (g) of Dams During Gestation and Lactation

Duration	Treatment Levels (ppm)			
	0	10	250	1750
F0 Gestation day 0-21	153.3±23.5	148.9±38.1	154.2±17.8	141.0±15.2
F0 Lactation day 0-21	-25.1±20.4	-21.5±22.4	-8.9±19.7	-0.8±27.5*
F1 Gestation day 0-21	150.6±37.8	152.7±23.6	154.5±28.4	122.3±26.9*
F1 Lactation day 0-21	-11.5±30.3	-11.7±24.2	2.6±25.2	34.1±20.2*

*significantly different from control by one-way ANOVA and Dunnett's test ($p<0.05$)

Table 10. Selected Results from the Multigeneration Reproduction Study in Rats with Diuron Technical (97.1 % purity) (Haskell Laboratory, 1990)

Parameter	Treatment Levels (ppm)			
	0	10	250	1750
F0 Generation				
Number of F0 females on study	30	30	30	30
Number of F0 females copulated/cohoused	28/30	30/30	29/30	30/30
Number of F0 males on study	30	30	30	30
Number of F0 males found Dead/KE	0	0	0	0
Number of F0 females found Dead/KE	1	1	0	0
Number of F0 live litters	21	24	23	29
Number of still-born litters	1	1	0	0
Mean live litter size (day 0)	14.3	14.3	14.9	14.4
Mean live litter size (day 4):				
Preculling	14.1	13.9	14.7	14.2
Postculling	8.0	7.9	7.9	8.0
Mean live litter size (day 21)	8.0	7.9	7.9	7.9
F1 generation				
Number of F1 females on study	30	30	30	30
Number of F1 females copulated/cohoused	29/30	26/30	28/30	28/30
Number of F1 males on study	30	30	30	30
Number of F1 males found Dead/KE	1	0	1	1
Number of F1 females found Dead/KE	0	0	0	0
Number of F1 live litters	26	20	23	23
Number of still-born litters	0	0	0	0
Mean live litter size (day 0)	13.1	14.0	13.4	12.5
Mean live litter size (day 4):				
Preculling	12.5	13.8	13.3	12.6
Postculling	7.3	7.9	7.7	7.9
Mean live litter size (day 21)	7.2	7.8	7.7	7.9

KE: Killed *in extremis*

Table 11. Selected Mean Pup Weights, from the Multigeneration Reproduction Study in Rats with Diuron Technical (97.1 % purity) (Haskell Laboratory, 1990)

Parameter	Treatment Levels (ppm)			
	0	10	250	1750
F0				
Mean pup weight (g) (day 0)	7.0	7.0	7.0	6.6*
Mean pup weight (g) (day 4) Preculling	11.8	12.2	11.4	10.1*
Mean pup weight (g) (day 4) Postculling	11.9	12.4	11.4	10.1*
Mean pup weight (g) (day 7)	19.1	19.7	18.3	16.2*
Mean pup weight (g) (day 14)	38.7	39.6	38.1	32.9*
Mean pup weight (g) (day 21)	58.9	61.3	57.9	48.4*
Male:Mean pup weight (g) (day 0)	7.3	7.3	7.2	6.8*
Mean pup weight (g) (day 4) Preculling	12.1	12.5	11.8	10.4*
Mean pup weight (g) (day 4) Postculling	12.2	12.7	11.7	10.4*
Mean pup weight (g) (day 7)	19.6	20.1	18.9	16.7*
Mean pup weight (g) (day 14)	39.6	40.3	39.0	33.6*
Mean pup weight (g) (day 21)	61.0	63.1	59.0	49.6*
Female: Mean pup weight (g) (day 0)	6.9	6.8	6.8	6.4*
Mean pup weight (g) (day 4) Preculling	11.5	11.9	11.1	9.8*
Mean pup weight (g) (day 4) Postculling	11.5	12.1	11.0	9.8*
Mean pup weight (g) (day 7)	18.6	19.3	17.7	15.7*
Mean pup weight (g) (day 14)	37.9	38.9	37.1	32.2*
Mean pup weight (g) (day 21)	56.9	59.3	56.6	47.2*
F1				
Mean pup weight (day 0)	6.7	7.0	6.9	6.5
Mean pup weight (g) (day 4) Preculling	11.0	11.9	11.4	10.3
Mean pup weight (g) (day 4) Postculling	11.1	11.9	11.4	10.3
Mean pup weight (g) (day 7)	18.7	19.6	18.8	16.6*
Mean pup weight (g) (day 14)	39.1	39.4	37.7	32.7*
Mean pup weight (g) (day 21)	62.7	64.7	61.1	51.1*
Male: Mean pup weight (g) (day 0)	6.9	7.2	7.1	6.7
Mean pup weight (g) (day 4) Preculling	11.4	12.1	11.6	10.5
Mean pup weight (g) (day 4) Postculling	11.4	12.1	11.7	10.6
Mean pup weight (g) (day 7)	19.2	19.8	19.3	17.0*
Mean pup weight (g) (day 14)	39.7	39.8	38.3	33.1*
Mean pup weight (g) (day 21)	63.9	66.2	62.0	52.5*
Female: Mean pup weight (g) (day 0)	6.5	6.9*	6.7	6.4
Mean pup weight (g) (day 4) Preculling	10.5	11.7*	11.2	10.1
Mean pup weight (g) (day 4) Postculling	10.7	11.7	11.2	10.0
Mean pup weight (g) (day 7)	17.9	19.3	18.5	16.2*
Mean pup weight (g) (day 14)	38.1	38.9	37.6	32.3*
Mean pup weight (g) (day 21)	60.8	63.1	60.2	49.5*

*Statistically significant difference from control at $\alpha = 0.05$ (Mann-Whitney U test performed on litter means)

This study was found to meet with FIFRA guidelines and was acceptable to both CDPR and U.S. EPA. The NOEL and LOEL for reproductive (developmental) toxicity were 250 ppm (20.3 mg/kg/day) and 1750 ppm (144 mg/kg/day) respectively.

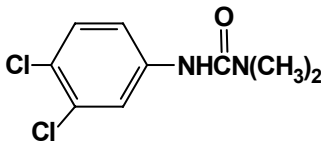
C.2.4. Developmental endpoints from studies on reproduction and fertility effects

In the multi-generation reproduction study in rats (Haskell Laboratory, 1990) a statistically significant reduction in mean pup body weight (both sexes) on postnatal day 0, day 4, day 7, day 14 and day 21 was observed in the first generation (F1 pups) at the highest dose level (1750 ppm). A statistically significant reduction in mean pup body weight (both sexes) was observed on postnatal day 7, day 14 and day 21 in both generations (F1 and F2 pups) with some evidence of a dose-response pattern (trend test not conducted). Examining the mean data for each sex separately, a similar dose-response was found as shown in Table 11. Lactation weights of F1 high-dose litters were less than control from day 0 through 21, while those of F2 litters were reduced from lactation day 7 through 21. Compound-related developmental toxicity was observed at the high dose of 1750 ppm (144 mg/kg/day in females) as evidenced by significantly decreased pup body weight at birth in the F1 generation and during the lactation period in both generations. The lack of a strong dose-response relationship with statistically significant effects only at the high dose serves to support a NOEL of 250 ppm (20.3 mg/kg/day in females; i.e., the animals receiving the compound).

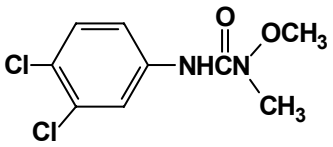
C.3 DEVELOPMENTAL TOXICITY: Other Relevant Information

C.3.1. Metabolism, Compounds of Similar Structure and Mechanism of Action.

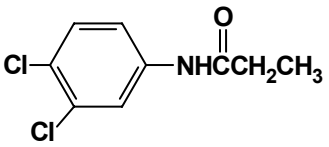
Diuron, the archetypical urea, exerts its herbicidal action by inhibiting the Hill reaction (Hill and Whittingham 1955), thereby inhibiting photosynthesis in plants. The Hill reaction is a light-initiated reaction that splits water (photolysis), resulting in the production of free oxygen (O_2) by plants. (Chlorophyll, the green pigment of plants, is an essential ingredient in the reaction, since it catalyzes the product). Diuron shares a common metabolite, 3,4-dichloroaniline (DCA) with herbicides linuron and propanil (U.S. EPA, 1999). The non-cancer dietary risk assessments take into consideration the residues of concern for diuron, namely the parent compound and all metabolites convertible to DCA (Figure. 3).



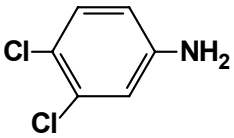
Diuron



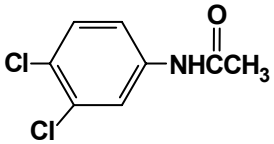
Linuron



Propanil



3,4 dichloroaniline



3,4-dichloroacetanilide

Figure 3

3,4-DCA is not readily biodegradable, or hydrolisable. In anaerobic conditions, slow degradation occurs to more resistant monochloroanilines. The major degradation route is photodegradation. Therefore, 3,4-DCA is relatively persistent in water, sediments and soil, while it is rapidly degraded in the atmosphere. Due to its physico-chemical properties and emission patterns, the main compartments exposed are water and soil. This metabolite, 3,4-DCA, is rapidly taken up by fish and metabolized to 3,4-dichloroacetanilide (DCAc). It is also structurally similar to the therapeutically used antiandrogen, flutamide.

Chemicals which act like androgens or which block the androgen receptor interfere with physiological androgen functions and can lead to impairments in sexual development and reproduction. Available data demonstrate that phenyl urea herbicides such as linuron are able to displace testosterone bound to the androgen receptor (Cook et al., 1993). The potency of different substances for [3H]dihydrotestosterone ([3H]DHT) displacement from the bovine androgen receptor was tested in an *in vitro* system and the relative binding affinities (RBA) were determined and compared with that of DHT (Bauer et al., 1998). The RBA of the various compounds were about $1/10^4$ to $1/10^5$ of that of DHT with 3,4-DCAc demonstrating the highest (1.31×10^{-4}), followed by linuron, 3,4-dichlorophenylurea, flutamide, 3,4-DCA and diuron, which had the lowest RBA (2.4×10^{-5}). The RBA of diuron was 25% that of linuron. 3,4-DCA, the metabolite of both linuron and diuron, has been reported to have a 1.7-fold lower androgen RBA than linuron. However, 3,4-dichloroacetanilide (DCAc), the metabolite of DCA, is structurally similar to the therapeutically used antiandrogen, flutamide, and DCAc has a two-fold higher RBA than DCA. These findings suggest that diuron could have effects *in vivo* similar to that of other known antiandrogens (linuron and flutamide).

Many pesticides are able to block or activate the steroid hormone receptors and/or affect the levels of sex hormones, thereby potentially affecting the development or expression

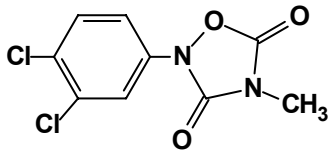
of the male and female reproductive system (Vingaard et al., 2000). *In utero* exposure to linuron appears to affect androgen-dependent development of the male reproductive system. Linuron clearly demonstrates effects on the male reproductive system of the offspring after maternal exposure during mid and late gestation (McIntyre et al., 2000, 2002). Data on the effects of diuron during late gestation are not available at this time. An *in utero* study of diuron exposing animals during the latter period of gestation is being planned and results from such a study will help elucidate the effects of diuron on the developing reproductive system (Gray, 2002).

An important enzyme in the steroid synthesis pathway is CYP19 aromatase, which catalyses the conversion of androgens to estrogens, thereby being responsible for the homeostatic balance between the male and female sex hormones. In 1999 the American Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) recommended the screening of potential endocrine disruptors for aromatase inhibition using human placental microsomes. In a study testing twenty-two pesticides for their ability to affect CYP19 aromatase activity in human placental microsomes using the classical $^{3}[H]_2O$ method, diuron and linuron did not affect the aromatase activity suggesting that these compounds do not exert their effects by affecting the aromatase pathway (Vingaard et al., 2000).

Another approach in evaluating the mechanism of action of these structurally similar compounds is to compare their carcinogenicity. Diuron induces urinary bladder carcinomas in rats (both sexes) and mammary gland carcinomas in female mice. In addition, an increase in the incidence of a rare kidney tumor was observed in male rats. Linuron, however, induces testicular interstitial cell adenomas in rats and hepatocellular adenomas in mice and propanil may induce malignant lymphomas of the spleen in female mice, testicular interstitial cell tumors in male rats and hepatocellular adenomas in female rats (at a dose level which exceeds the maximum tolerated dose). Available mechanistic and metabolism data indicate that linuron and diuron may be inducing tumors through different mechanisms of action. Hence the target organs for carcinogenicity may be similar for linuron and propanil, but not for diuron. Whether a similar pattern for the developmental toxicity of these two compounds exists needs to be examined.

Another herbicide, methazole (Figure 4.) acts as a precursor for a urea that inhibits the Hill reaction and the first metabolite of methazole is closely related to diuron (Corbett et al., 1984). This metabolite is approximately as herbicidal as methazole itself and was shown to cause 50% inhibition of the Hill reaction at 0.1 μM. Methazole itself is reported to be about 100 times less active than diuron as a Hill reaction inhibitor.

Examining other effects noted for methazole, preliminary results from a rat reproduction study indicated that a high percentage of the dosed generations' offspring developed cataracts. Some studies with linuron also documented increased incidence of cataracts in offspring of rodents exposed to the compound (CDPR, 1993). Such effects were not observed in any of the studies with diuron.



Methazole

Figure 4

C.3.2. Determination of Safety for Infants and Children

The Federal Food Drug and Cosmetic Act (FFDCA) section 408 provides that the U.S. EPA shall apply an additional tenfold margin of safety for infants and children in the case of threshold effects. This is to account for pre- and postnatal toxicity and the completeness of the database unless a different margin of safety will be safe for infants and children. Based on the developmental and reproductive toxicity studies discussed above for diuron and available data that reasonably account for potential exposures, U.S. EPA determined there did not appear to be an extra sensitivity for pre- or postnatal effects and that an additional safety factor was not required in assessing the risk posed by this chemical for a tolerance action (U.S. EPA, 1999).

C.4. Integrative evaluation.

Four studies in experimental animals of developmental toxicity of diuron were located, three in rat and one in rabbit (Khera et al., 1979; Talakin et al., 1984; Argus Laboratories 1987a, 1987b). Also, two studies on reproduction and fertility effects in the rat contain information relevant to developmental endpoints (Hodge et al., 1967; Haskell Laboratory, 1990). No studies in humans have been located. Findings from these studies indicate that at maternal dose levels above 80 mg/kg/day some amount of developmental toxicity (delayed ossification of the calvarium and wavy ribs at 125 mg/kg/day and above; lower birthweights at 144 mg/kg/day; delayed ossification at 400 mg/kg/day) may be observed in offspring of rats. However, the effects observed do not suggest a specific system being affected or contribute to a pattern of malformations or specific syndrome, although reduced birth weights and delayed ossification are typical indications of growth retardation. The developmental toxicity studies are designed to evaluate adverse effects on the developing organism resulting from maternal pesticide exposure during gestation and the reproduction studies provide information relating to effects from exposure to the pesticide on the reproductive capability of mating animals along with data on systemic toxicity. Effects on the development of the reproductive system and other organ systems developing during late gestation and early postnatal period may be detected in the reproduction studies. However, these studies are not designed to specifically determine such effects. Consequently, the lack of reported effects these systems does not conclusively demonstrate that they are not affected. Testing regimes that include dosing

during the androgen-dependent period of sexual differentiation and examination of neonates would allow detection of reproductive tract abnormalities that would not be evident in the standard teratology study conducted (dosing from gestation day 6-15, with examination of only fetal animals required). The mechanism of action of diuron and related compounds suggests that the developing reproductive system may be particularly susceptible; however, no studies designed to specifically examine such potential effects have been conducted. Data on the effect of diuron during late gestation are not available at this time. Studies evaluating the potency of diuron to displace testosterone bound to the androgen receptor *in vitro* have been reviewed, and the findings suggest that diuron could have effects *in vivo* similar to that of other known antiandrogens (linuron and flutamide).

D. TOXICITY TO THE FEMALE REPRODUCTIVE SYSTEM

D.1. Human

No human studies are available at this time.

D.2 . Animal Studies

D.2.1. Studies of reproductive toxicity

Hodge et al. (1967); University of Rochester School of Medicine and Dentistry

In this study (previously discussed in Section C.2.3), diuron was supplied in the diet at either 0 or 125 ppm (approximate dose of 6.25 mg/kg-day) to rats in a three-generation reproduction study (8 males and 16 females per group). Fertility rate, body weight, hematology and histopathology were monitored. No effect was observed except for body weights, which were significantly decreased in the F_{2b} and F_{3a} litters. A LOAEL of 125 ppm (6.25 mg/kg-day) was identified. The entire study was repeated with a different lot of diuron and no adverse effects on body weights were observed. The two replicates in the study were performed with the same numbers of animals. No adverse reproductive effects were reported in the replicate component of the study but postweaning growth retardation was noted in the first component. The reason for the difference was not explained. The same study was submitted to the California Department of Food and Agriculture (CDFA) to meet the FIFRA guidelines for reproduction and fertility effects in rats. A NOEL could not be determined since the report had several deficiencies such as single dose, no analysis of diet, no data on food consumption, inadequate number of pregnant animals (per FIFRA guidelines), and parental animals were not necropsied (CDFA, 1985).

Haskell Laboratory for Toxicology and Industrial Medicine (1990)

As discussed previously in Section C.2.3, diuron technical (97.1% purity) was fed in the diet to 30 Crl:CD*BR rats/dose/sex for two generations at 0 (control), 10, 250, and 1750 ppm (Male: 0, 0.68, 16.9 and 120 mg/kg/day; Female: 0, 0.80, 20.3 and 144 mg/kg/day).

F0 animals were fed treated diet 73 days prior to mating and F1 animals received treatment diet for at least 105 days after weaning and prior to mating. The dose levels appear to have been selected based on the observations from previous sub-chronic and chronic feeding studies. At 1750 ppm, decreased F0 and F1 body weights (5% to 20% less than controls: see Table 9) and food consumption were noted. No adverse effects on the reproductive system were observed. The authors concluded that the parental NOEL was 250 ppm based on reduced F0 and F1 body weights and food consumption at 1750 ppm and the reproductive NOEL was 250 ppm based on reduced F1 and F2 pup weights at 1750 ppm. Histopathological examination of female reproductive tissues (ovaries, uterus, cervix and vagina) was conducted only for the control and high dose groups and for gross lesions noted in other doses. The detailed findings on the female reproductive system in this study are provided below (Tables 12 and 13).

This study was determined to meet with the FIFRA guidelines and found to be acceptable to CDPR with no adverse effects on female reproduction. The parental and reproductive NOELs were both 250 ppm with LOELs of 1750 ppm based on reduction in parental and pup body weights. In the U.S. EPA Toxicology One-liner database for Diuron, this study was reviewed and the parental (systemic) NOAEL was 16.9 (males) and 20.3 (females) mg/kg/day, based on decreased body weight, body weight gain and food consumption in both sexes at the LOAEL of 120 (males) and 144 (females) mg/kg/day and the developmental (pup) NOAEL was 20.3mg/kg/day, based on decreased pup body weight during the lactation period for both sexes and generations at the LOAEL of 144 mg/kg/day. The female reproductive NOAEL was 144 mg/kg/day, the highest dose tested (U.S. EPA, 2001). The toxicological data base evaluating pre- and postnatal toxicity for diuron was considered complete with respect to current data requirements and it was determined that there were no reproductive effects (U.S. EPA, 1999).

Table 12. Selected Results of Multigeneration Reproduction Study in Rats with Diuron Technical (97.1 % purity) (Haskell Laboratory, 1990)

Parameter	Treatment Levels (ppm)			
	0	10	250	1750
Number of F0 females on study	30	30	30	30
Number of F0 females copulated/cohoused	28/30	30/30	29/30	30/30
Number of F0 males on study	30	30	30	30
Number of F0 males found dead/KE	0	0	0	0
Number of F0 females found dead/KE	1	1	0	0
Number of F0 live litters	21	24	23	29
Number of still-born litters	1	1	0	0
Mean live litter size (day 0)	14.3	14.3	14.9	14.4
Mean pup weight (g) (day 0)	7.0	7.0	7.0	6.6*
Mean live litter size (day 4)				
Preculling	14.1	13.9	14.7	14.2
Postculling	8.0	7.9	7.9	8.0
Mean live litter size (day 21)	8.0	7.9	7.9	7.9
Mean pup weight (g) (day 21)	58.9	61.3	57.9	48.4*
Number of F1 females on study	30	30	30	30
Number of F1 females copulated/cohoused	29/30	26/30	28/30	28/30
Number of F1 males on study	30	30	30	30
Number of F1 males found dead/KE	1	0	1	1
Number of F1 females found dead/KE	0	0	0	0
Number of F1 live litters	26	20	23	23
Number of still-born litters	0	0	0	0
Mean live litter size (day 0)	13.1	14.0	13.4	12.5
Mean pup weight (day 0)	6.7	7.0	6.9	6.5
Mean live litter size (day 4)				
Preculling	12.5	13.8	13.3	12.6
Postculling	7.3	7.9	7.7	7.9
Mean live litter size (day 21)	7.2	7.8	7.7	7.9
Mean pup weight (g) (day 21)	62.7	64.7	61.1	51.1*

KE : killed *in extremis*
*Statistically significant difference from control at $\alpha = 0.05$ (Mann-Whitney U test)

Table 13. Selected Results from Histopathology of Female Reproductive System from the Multigeneration Reproduction Study in Rats with Diuron (Haskell Laboratory, 1990)

Parameter	Dosage (ppm)			
	0	10	250	1750
Microscopic observations (F0)				
Ovaries (cysts)	1/30	-	-	0/30
Uterus :				
Deciduoma	0/30	2/4	-	0/30
Dilatation (body and horns)	1/30	1/4	-	0/30
Dilatation (glands)	1/30	1/4	-	0/30
Inflammation (Purulent)	1/30	1/4	-	0/30
Cervix (Inflammation)	1/30	-	-	0/30
Microscopic observations (F1)				
Ovaries	0/30	-	0/1	0/30
Uterus :				
Dilatation (body and horns)	0/30	-	-	1/30

D.2.2. Rat Chronic Study

Bayer (1985a)

The design of this chronic study in Wistar rats is discussed above in section B.4.2. While this study was conducted primarily in accordance with protocols determining the chronic toxicity potential of compounds regulated under FIFRA, the following findings in female animals were noted. In the histopathological evaluation of sections of the uterus at the high dose level it appears that there was an increase in adenocarcinomas in the animals examined at the end of the study i.e., terminal sacrifice (Table 1). Additional data are presented below in Table 14. One neoplastic alteration, a benign tumor of the ovary (granulosa cell tumor), was found in one animal in the 2500 ppm dose group in the animals examined at 12 months.

Table 14. Selected Results from the Two-year Dietary Study in Rats (Bayer 1985a)

Organ	Dosage ppm			
	0	25	250	2500
Histopathological findings of animals that died or were killed in extremis				
Uterus:				
Glandular-cystic hyperplasia	0/9	0/9	0/3	1/11
Dilated	0/9	1/9	0/3	0/11
Hyperemia	1/9	0/9	0/3	0/11
Hemometra	0/9	1/9	0/3	0/11
Hemorrhage	0/9	1/9	0/3	0/11
Ovaries:				
Cysts	1/9	1/9	0/2	3/11
Hyperemia	2/9	0/9	0/2	1/11
Atrophy	1/9	0/9	0/2	0/11
Tumor infiltration	0/9	2/9	1/2	1/11

D.3. Integrative evaluation

The two-generation reproduction study in rats (Haskell, 1990) did not demonstrate any specific effects on the female reproductive system or on fertility. Compound-related parental toxicity was observed at the high dose of 1750 ppm (144 mg/kg/day in females) as evidenced by decreased body weight gain and food consumption in both sexes and both generations. The lack of a dose-response with effects only at the high dose serves to support a systemic NOEL of 250 ppm (20.3 mg/kg/day in females). In the chronic toxicity study in mice no significant effects on the female reproductive system were noted in the animals examined at 12 months; at terminal sacrifice (Bayer, 1983 in Tables 4 and 5) an increased incidence of both ovarian tumors and mammary tumors was observed at the high dose (869 mg/kg/day). In the chronic study in rats (Bayer, 1985a) no significant effects of the female reproductive system were noted in the animals examined at 12 months. In these studies submitted to meet regulatory requirements for pesticide use, an increased incidence in adenocarcinomas of the uterus at the high dose (203 mg/kg/day) in rats was observed. However, the tumors in rats were not restricted to the reproductive system.

E. TOXICITY TO THE MALE REPRODUCTIVE SYSTEM

E.1. Human

No human studies are available at this time.

E. 2 . Animal Studies

E. 2.1 Studies of reproductive toxicity

Hodge et al. (1967); University of Rochester School of Medicine and Dentistry, 1964

In this study (previously discussed in Sections C.2.3 and D.2), diuron was supplied in the diet at either 0 or 125 ppm (approximate dose of 6.25 mg/kg-day) to rats in a three-generation reproduction study (8 males and 16 females per group). Fertility rate, body weight, hematology and histopathology were monitored. No effect was observed except for body weights, which were significantly decreased in the F_{2b} and F_{3a} litters. A LOAEL of 125 ppm (6.25 mg/kg-day) was identified. The entire study was repeated with a different lot of diuron and no adverse effects on body weights were observed. The two replicates in the study were performed with the same numbers of animals. No adverse reproductive effects were reported in the replicate component of the study but postweaning growth retardation was noted in the first component. The reason for the difference was not explained. The same study was submitted to the California Department of Food and Agriculture (CDFA) to meet the FIFRA guidelines for reproduction and fertility effects in rats. A NOEL could not be determined since the report had several deficiencies such as single dose, no analysis of diet, no data on food consumption, inadequate number of pregnant animals (per FIFRA guidelines), and parental animals were not necropsied (CDFA, 1985).

Haskell Laboratory for Toxicology and Industrial Medicine (1990)

As discussed previously in Sections C.2.3 and D.2, diuron technical (97.1% purity) was fed in the diet to 30 Crl:CD*BR rats/dose/sex for two generations at 0 (control), 10, 250, and 1750 ppm (Male: 0, 0.68, 16.9 and 120 mg/kg/day; Female: 0, 0.80, 20.3 and 144 mg/kg/day). F0 animals were fed treated diet 73 days prior to mating and F1 animals received treatment diet for at least 105 days after weaning and prior to mating. The dose levels appear to have been selected based on the observations from previous sub chronic and chronic feeding studies. At 1750 ppm, decreased F0 and F1 body weights (5% to 20% less than controls) and food consumption were noted. No adverse effects on the reproductive system were observed. Testicular weights in this study did not exhibit a clear dose-response. The authors concluded that the parental NOEL was 250 ppm based on reduced F0 and F1 body weights and food consumption at 1750 ppm and the reproductive NOEL was 250 ppm based on reduced F1 and F2 pup weights at 1750 ppm. Histopathological examination of male reproductive tissues (testes, epididymides, prostate, seminal vesicles) was conducted only for the control and high dose groups and

for gross lesions noted in other dose groups. The detailed findings on the male reproductive system are provided in Tables 12 (above) and 15.

Table 15. Findings on the Male Reproductive System from the Multigeneration Reproduction study in Rats with Diuron Technical (97.1 % purity) (Haskell Laboratory, 1990)

Parameter	Dosage (ppm)			
	0	10	250	1750
Parental Mean Absolute Organ Weights (g)				
F0 Adults				
Testes	3.775	3.883	3.836	3.820
F1 Adults				
Testes	3.896	4.174*	3.969	3.898
Parental Mean Relative Organ Weights (% of Body Weight)				
F0 Adults				
Testes	0.5675	0.5703	0.5689	0.6278*
F1 Adults				
Testes	0.5435	0.5718	0.5728	0.6611*
Microscopic observations (F0)				
Testes :				
Seminiferous tubular atrophy (bilateral)	-	1/1	-	0/30
Seminiferous tubular atrophy (unilateral)	1/30	-	-	0/30
Epididymides:				
Hypospermia (unilateral)	1/30	-		0/30
Spermatic granuloma (unilateral)	2/30	-	1/1	1/30
Prostate (inflammation)	1/30	-	-	0/30
Microscopic observations (F1)				
Testes :				
Seminiferous tubular atrophy (bilateral)	1/30	-	-	0/29

*significantly different from control by Dunnett's test ($p<0.05$).

The increase in relative testis weight in the F0/F1 high-dose males was judged by the author to be due to decreased body weight. Body weights of the high-dose F0 and F1 males were significantly less than controls throughout most of the study, with an overall gain of F0 and F1 males being 82 and 83% of control. The increased absolute testis weight of the F1 males in the 10 ppm group was considered to be an incidental finding.

This study was determined to meet with the FIFRA guidelines and found to be acceptable to CDPR with no adverse effects on reproduction. The parental and reproductive NOELs were both 250 ppm with LOELs of 2500 ppm based on reduction in parental and pup body weights. U.S. EPA appears to have also reviewed this two-generation reproductive toxicity study in rats, and determined the parental (systemic) NOAEL was 16.9 (males) and 20.3 (females) mg/kg/day, based on decreased body weight, body weight gain and

food consumption in both sexes at the LOAEL of 120 (males) and 144 (females) mg/kg/day and the developmental (pup) NOAEL was 20.3mg/kg/day, based on decreased pup body weight during the lactation period for both sexes and generations at the LOAEL of 144 mg/kg/day. The reproductive NOAEL for males was 120 mg/kg/day, the highest dose tested. The toxicological data base evaluating pre- and postnatal toxicity for diuron is considered complete with respect to current data requirements and it was determined that there were no male reproductive effects (U.S. EPA, 1999).

E. 2.2. Dominant lethal studies

No study on dominant lethal effects was found.

E. 2.3. Oncogenicity study

No effects on the male reproductive system were noted in the mouse oncogenicity study.

E. 2.4. Rat chronic study

Bayer (1985a).

The design of this study is discussed above in section B.5.2. While this study was conducted primarily in accordance with protocols determining the chronic toxicity potential of compounds regulated under FIFRA, the following findings in male animals were noted. No significant effects in the reproductive system were noted in the animals (Wistar rats) at the interim sacrifice (12 months). The tumor incidences in the rat bioassay at terminal sacrifice are shown in Table 3, while the incidence of histopathological findings are summarized in Table 16 below. An increase in the incidence of some effects on the male reproductive system was observed. These include a report of "interstitum loosened" in the testes of the animals at the high dose level that died or were killed in extremis and a slight increase in unilateral Leydig cell tumors in sections of the testes at the high dose at terminal sacrifice. The term "interstitum loosened" was not defined (details on the method of fixation have been included in section B.5.2.). However, the biological significance of these findings is unclear.

Table 16. Selected Results from the Two-year Chronic/oncogenicity Study in Rats (Bayer 1985a)

Organ	Dosage ppm			
	0	25	250	2500
Histopathological findings of animals that died or were killed *in extremis*				
Testes:				
Interstitium loosened	6/10	-	-	10/10
Epididymides- round cell infiltration	0/10	-	-	1/10
Prostate – calculus	3/10	-	-	2/10

E.3. Integrative evaluation.

The two-generation reproduction study in rats (Haskell, 1990) did not demonstrate any specific effects on the male reproductive system or on fertility. Compound-related parental toxicity was observed at the high dose of 1750 ppm (120 mg/kg/day in males) as evidenced by decreased body weight gain and food consumption in both sexes and both generations. The lack of a dose-response with effects only at the high dose serves to support a NOEL of 250 ppm (17 mg/kg/day in males). In chronic studies submitted to meet pesticide guideline requirements, an increased incidence of benign unilateral Leydig cell tumors of the testes at the high dose (117 mg/kg/day) in rats was observed at the end of the study, but similar effects were not noted at the interim sacrifice. However, the tumors were not restricted to the reproductive system.

F. SUMMARY

F.1. Developmental toxicity.

Four studies in experimental animals of developmental toxicity of diuron were located, three in rat and one in rabbit (Khera et al., 1979; Talakin et al., 1984; Argus Laboratories 1987a, 1987b). Also, two studies on reproduction and fertility effects in the rat contain information relevant to developmental endpoints (Hodge et al., 1967; Haskell Laboratory, 1990). No studies in humans have been located. In assessing the potential reproductive toxicity of diuron, data from the two-generation reproduction study in the rat and chronic study in rats and mice were reviewed. Findings from these studies indicate that at maternal dose levels above 80 mg/kg/day some amount of developmental toxicity (delayed ossification of the calvarium and wavy ribs at doses of 125 mg/kg/day and above; lower birth weights at 144 mg/kg/day; delayed ossification at 400 mg/kg/day) may be observed in offspring of rats. However, the effects observed do not suggest a specific system being affected or contribute to a pattern of malformations or specific syndrome, although reduced birth weights and delayed ossification are typical indications of growth retardation. The mechanism of action of diuron and related compounds suggests that the developing reproductive system may be particularly susceptible; however, no studies designed to specifically examine such potential effects have been conducted. Data on the effect of diuron during late gestation are not available at this time. The findings from studies on linuron, a structurally similar herbicide demonstrate that the teratology studies (with traditional dosing regimes and assessment periods) and multigeneration reproduction studies fail to clearly identify the hazard of chemicals with antiandrogenic potential while studies specifically designed to evaluate exposures during the development of the reproductive systems do demonstrate antiandrogenic effects (retention of areolae/nipples, epididymidal and testicular lesions and hypospadias in male offspring). Findings from *in vitro* studies evaluating the potency of diuron to displace testosterone bound to the androgen receptor suggest that diuron could have effects *in vivo* similar to that of other known antiandrogens (linuron and flutamide). In evaluating the exposure to diuron, the concentrations of diuron detected in water are probably far below those needed to displace the endogenous ligand of humans and terrestrial mammals in the *in vitro* systems reported (Bauer et al., 1998).

F.2. Female Reproductive Toxicity

The reproduction studies provide information relating to fertility effects and effects on the reproductive system resulting from exposure to the pesticide. In the two-generation reproduction study, decreased F0 and F1 body weights (5% to 20% less than controls) and food consumption were noted at the high dose level of 1750 ppm. Reduced F1 and F2 pup weights were also noted at 1750 ppm (Haskell, 1990). Histopathological examination of reproductive tissues (ovaries, uterus, cervix and vagina) for the control and high dose groups and for gross lesions noted in other doses did not demonstrate adverse effects on the reproductive system. Accordingly, the NOEL and LOEL for reproductive toxicity were 250 ppm (20.3 mg/kg/day) and 1750 ppm (144 mg/kg/day) respectively. Chronic toxicity tests provide an opportunity to evaluate toxic effects of long-term exposures. Data from the interim sacrifices from a chronic study could provide useful information regarding the onset and sequence of toxicity (U.S. EPA, 1996). In the chronic toxicity study in rats (Bayer, 1985a) no significant effects on the female reproductive system were noted in the animals examined at 12 months.

F.3. Male Reproductive Toxicity

The reproduction studies provide information relating to fertility effects and effects on the reproductive system resulting from exposure to the pesticide. In the two-generation reproduction study, decreased F0 and F1 body weights (5% to 20% less than controls) and food consumption were noted at the high dose level of 1750 ppm. Reduced F1 and F2 pup weights were also noted at 1750 ppm (Haskell, 1990). Testicular weights in this study did not exhibit a clear dose-response. Histopathological examination of reproductive tissues (testes, epididymides, prostate, seminal vesicles) for the control and high dose groups and for gross lesions noted in other doses did not demonstrate adverse effects on the reproductive system. Accordingly, the NOEL and LOEL for reproductive toxicity were 250 ppm (16.9 mg/kg/day) and 1750 ppm (120 mg/kg/day) respectively. Chronic toxicity tests provide an opportunity to evaluate toxic effects of long-term exposures. Data from the interim sacrifices from a chronic study could provide useful information regarding the onset and sequence of toxicity (U.S. EPA, 1996). In the chronic toxicity study in rats (Bayer, 1985a) no significant effects on the male reproductive system were noted in the animals examined at 12 months.

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