EVIDENCE ON THE DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF

METRIBUZIN

DRAFT

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Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment
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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 cancer or 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 cancer or reproductive toxicity." 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 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 that 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 metribuzin "has been clearly shown by scientifically valid testing according to generally accepted principles" to cause reproductive toxicity.

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A. ABSTRACT

Metribuzin is a triazine herbicide. It is slightly soluble in water, and somewhat soluble in several organic solvents. It is used in California as a pre- and post-emergence herbicide on grasses and broad-leafed weeds. It is used in the agriculture of various crops including alfalfa, asparagus, corn, potatoes, and tomatoes, as well as in ornamentals and landscape maintenance.

No direct data on absorption of metribuzin were located. In animals, substantial absorption by the oral route can be inferred by the subsequent observation of metribuzin and its metabolites in organs and urine, and metabolites in feces. Metribuzin is metabolized by several routes to a large number of compounds. Metribuzin and/or its metabolites distribute to all organs examined. In several studies, liver and kidney were found to have the highest concentrations. In the only study to examine the thyroid, it was found to have 10-fold higher concentration than the liver. Concentrations in testes and ovaries were relatively low. No information on distribution to the placenta or fetus was found.

Acute oral toxicity varies considerably by species and strain. Acute oral LD_{50}s range from 2,200 mg/kg in the male Wistar rat to 245 mg/kg in the male guinea pig. There were no major differences between sexes. Liver hypertrophy and reduced body weight or weight gain are commonly observed in both chronic and subchronic studies. Alterations of circulating thyroid hormones are a relatively sensitive endpoint, with thyroxine displaying a characteristic inverted "U" concentration-response relationship.

Data on developmental and reproductive toxicity come from developmental studies (with treatment via oral gavage) in Sprague-Dawley and FB 30 rats and New Zealand White and American Dutch rabbits, multigeneration reproductive studies (with treatment via feed) in FB 30 and Sprague-Dawley rats, and mouse dominant lethal studies. In addition there have been numerous subchronic and chronic studies in several species that report on ovarian and testicular weight and pathology following metribuzin treatment. No data on humans were found.

In the FB 30 rat developmental study, mild maternal toxicity and no developmental toxicity were observed. In the Sprague-Dawley rat developmental study, reduced fetal weight was observed at all doses, as was maternal toxicity in the form of reduced food consumption, body weight, and body weight gain. At the high dose, increased incidence of delayed ossification and rib anomalies were observed.

In the developmental study in New Zealand White rabbits, there was an increase in the incidence of total abortions at the high dose. Females lost weight during treatment at this dose. There were also increased resorptions, reduced fetal weight, and an increase in incompletely ossified sternebrae, although none of these effects were statistically significant. In the American Dutch rabbit developmental study, statistically significant reductions in fetal weight and increases in delayed ossification were observed at the

middle dose, but not at the high dose. Maternal weight gain was reduced at the high dose.

Two additional rabbit developmental studies, conducted by Industrial Bio-Test Laboratories, Inc. (IBTLI) were found by the U. S. Environmental Protection Agency (U.S. EPA) to be invalid because the information in the report was not supported by the raw data. These studies were therefore not reviewed in this document.

In the FB 30 rat reproductive study, in the F2 and F3 litters, birth weights were generally somewhat lower than controls in the metribuzin treated groups, but these were not statistically significant and were concentration-related in only one litter out of four. No parental toxicity was observed. In the Sprague-Dawley rat reproductive study, in the F1/F2 litter only, there were reduced implantations and litter sizes at the middle and high concentrations. Male and female weight was reduced at the high concentration in both the F0 and F1 generations. Female weight was also reduced at the middle concentration in the F1 generation.

Two male and one female dominant lethal studies with metribuzin in NMRI mice used single gavage administrations of metribuzin followed by multiple matings. No consistent dominant lethal effects or other indications of reproductive toxicity were found. An additional dominant lethal study in mice conducted by IBTLI was found by U.S. EPA to be invalid.

There have been numerous subchronic and chronic studies in multiple species with metribuzin, not designed as reproductive studies, but which reported on ovarian and/or testicular weight and pathology. In most studies, no effects on reproductive organ weight, gross pathology or histopathology were observed. In two rat inhalation studies, increased relative, but not absolute, testes weight was observed in the presence of reduced body weight. Reduced ovary and testes weight were observed at the high concentration in a chronic dog study. Interpretation of this observation is complicated by severe systemic toxicity, including 75% mortality in both sexes.

Symmetrical triazine herbicides (e.g. atrazine, simazine, ametryn, and prometryn) have characteristic toxicological effects, including induction of mammary tumors in Sprague-Dawley rats, disruption of the estrous cycle, and thyroid effects. The extent to which these observations can be generalized to metribuzin is not clear; metribuzin is an asymmetrical triazine. Differences in study designs limit the ability to compare toxicological results. The limited available evidence suggests that metribuzin and the symmetrical triazines may similarly affect the thyroid, but not the estrous cycle.

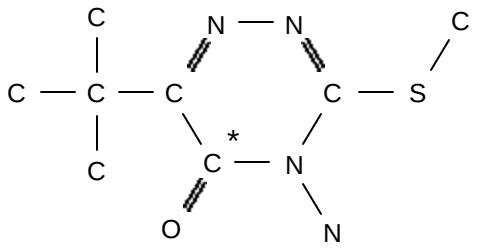
B. INTRODUCTION

B.1 Chemical Structure and Physical Properties

Metribuzin (CAS No. 21087-64-9) is an asymmetrical triazine herbicide. A systematic name is 4-amino-6-*tert*-butyl-3-(methythio)-1,2,4-triazin-5-one. Common synonyms include Sencor, Bay 94337, and DIC 1468. The molecular weight is 214.3. The formula is $C_8H_{14}N_4OS$. The structure is shown in Figure 1. It is a white, crystalline solid with a melting point of 125-126.5 degrees C. It is slightly soluble in water, and somewhat soluble in several organic solvents (NIOSH 2001, NLM 2001).

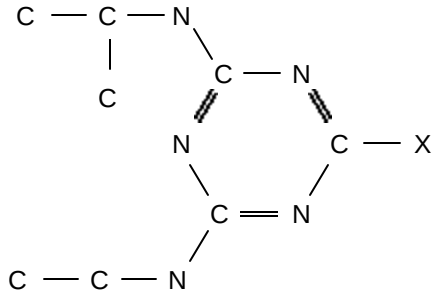
Metribuzin is distinct from the symmetrical triazines such as atrazine, simazine, ametryn, and prometryn (see OEHHA 1999 for a review of the symmetrical triazines). In the symmetrical triazines, the central ring structure has alternating carbon and nitrogen atoms, whereas metribuzin has two nitrogen atoms and two carbon atoms which are adjacent to each other. Additionally, depending upon the specific compound, some or all of the various groups attached to the central ring structure are different (see Figure 1).

Figure 1. Structures of metribuzin, atrazine, and ametryne.



Metribuzin

* Indicates 5 position, commonly used in ^{14}C labeling studies.



Atrazine: X = Cl

Ametryne: X = S-C

B.2 Regulatory History

Metribuzin is not identified as a carcinogen under Proposition 65.

A Reregistration Eligibility Decision for pesticide use has been approved by U.S. EPA (U.S. EPA 1998). Federal drinking water guidelines of 100 ug/L have been set (NLM 2001).

B.3. California Use and Exposure Information

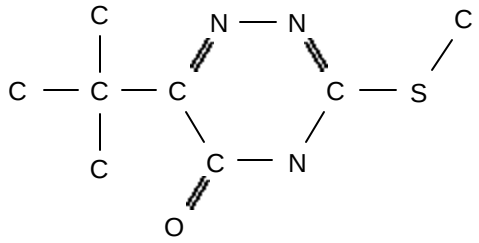
Metribuzin is a pre- and post-emergence herbicide used on grasses and broad-leafed weeds (NLM 2001). It is currently registered for use in California (CDPR 2001). Major uses in California are on agricultural crops including alfalfa, asparagus, corn, potatoes, and tomatoes, as well as ornamentals and landscape maintenance. A total of 28,648 pounds was used in California in 1998 (CDPR 1998). 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.

B.4. Pharmacokinetics

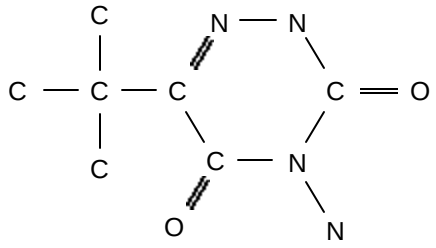
B.4.1. Overview

No studies have been located of pharmacokinetics of metribuzin in humans. There have been several studies in rats, and also studies in mice, dogs, and farm animals. Several metabolites of metribuzin have been identified (see Figure 2).

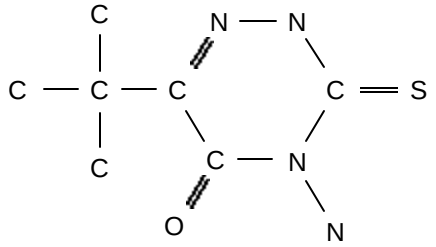
Figure 2. Structures of selected metribuzin metabolites



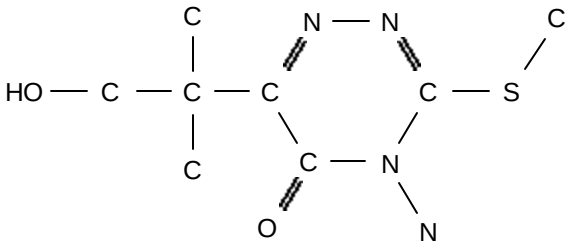
DA
(deaminometribuzin)



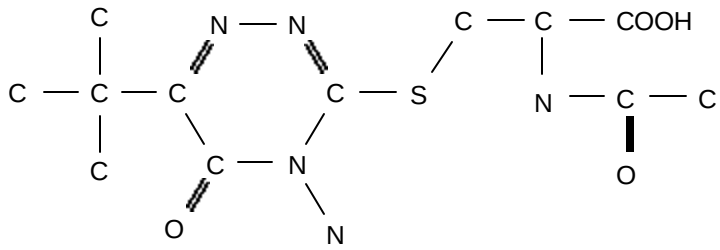
DK
(diketometribuzin)



DM
(demethylmetribuzin)



t-BuOH-metribuzin
(tert-butylhydroxy-
metribuzin)



N-Ac-Cys-
Metribuzin
(N-acetylcysteinyl-
metribuzin)

B.4.2. Pharmacokinetic studies in rats

In a study in Sprague-Dawley rats, one male and one female rat were administered 5-^{14}C-metribuzin at 20 mg/kg by gavage (Baychem 1972b). Approximately 90% of the ^{14}C label was recovered in feces plus urine over 16 days time, the majority in the first two days. No ^{14}C label was found in expired air. In the male, excretion to feces was greater, whereas in the female excretion to urine was greater. In a subsequent experiment, two male rats were administered 5-^{14}C-metribuzin at 100 mg/kg by gavage. Approximately 100% of the ^{14}C label was recovered in the urine and feces within 5 days, the majority in the first two days. Excretion to feces was greater than to urine. In another experiment, groups of males and females were administered 5-^{14}C-metribuzin at 50 mg/kg by gavage. Rats were sacrificed at 4, 24, 48, 72, and 96 hours after treatment (apparently one rat/sex/time point). In the males, peak ^{14}C label tissue levels were found at the 4 hour time point. In the females, approximately equal peak tissue levels were found at the 4 and 24 hour time points. Liver contained the highest concentrations of ^{14}C label, followed by kidney, followed by other tissues (heart, fat, ovaries, brain, muscle, plasma, testes). Tissue half lives were longest in the liver at 30.4 hours for males and 33.6 hours for females. Other tissue half lives ranged from 18.4 to 33.6 hours. Half lives for males were generally slightly shorter than for females. Metabolites identified included deaminometribuzin (DA), diketometribuzin (DK), and deaminodiketometribuzin (DADK) (see Figure 2). There were considerable amounts of unidentified metabolites. Digestion of liver tissue with proteases released considerable amounts of ^{14}C label, suggesting covalent binding of metabolites to proteins. Digestion with B-glucuronidase or aryl sulfatase released small to negligible amounts of ^{14}C label.

In another study in rats, 5-^{14}C-metribuzin was administered to male Sprague-Dawley rats at 5 or 10 mg/kg orally (Bayer 1981b). Liver and thyroid were examined by various analytical techniques for metribuzin and metabolites 24 hours after treatment. Concentrations of ^{14}C label were found to be approximately 10 times higher in thyroid than in liver. The metabolites demethylmetribuzin (DM) and deaminodemethylmetribuzin (DADM) were identified in thyroid and liver.

In a subsequent study in rats, 5-^{14}C-metribuzin was administered to one male Sprague-Dawley rat at 10 mg/kg orally (Bayer 1982b). Feces collected over 24 hours were analyzed using several techniques. Metribuzin, DA, DK, DADK, and several unidentified polar metabolites were found in the feces. DM and DADM were tested for but not found.

In a brief study in rats, 5-^{14}C-metribuzin was administered to male albino rats at 1 or 200 mg/kg by gavage (Bleeke et al. 1985). About 95% of the ^{14}C label was recovered in two days in urine and feces, with about equal amounts in urine and feces. The major urinary metabolite was DA mercapturate. No attempt was made to identify other metabolites. In vitro studies of metribuzin metabolism by rat liver microsomes supplemented with NADPH found considerable amounts of protein binding by metribuzin metabolites (Bleeke and Casida 1984). If the microsomes were additionally supplemented with N-acetylcysteine, protein binding was minimal, and metribuzin mercapturate and/or DA

mercapturate were found. The authors speculated that this could be due to the formation of a reactive sulfoxide intermediate.

Male and female Wistar rats were treated with 5-^{14}C-metribuzin at a single dose of 5 or 500 mg/kg, or with unlabelled metribuzin at 5 mg/kg/d for 14 days followed by 5-^{14}C-metribuzin (Mobay 1987). There were 6 rats/sex/group. The 500 mg/kg dose caused frank toxicity, including labored breathing and the deaths of two females. These rats were replaced for the study. Metribuzin was rapidly excreted in urine and feces, with almost all ^{14}C label excreted by 48 to 72 hours after administration. Rats were sacrificed 96 hours after administration of the labeled metribuzin. Less than 1% of the ^{14}C label remained in the body at this time point. The majority of ^{14}C was found in feces for both sexes. No significant differences between males and females were found, except that excretion was slightly slower for the females than the males in the repeated dose group. Excretion to feces compared to excretion to urine was similar for the single dose and repeated dose groups treated at 5 mg/kg, but excretion to feces was somewhat greater for the 500 mg/kg/ group, suggesting limited absorption. Metribuzin, DA, DK, DADK, DM, t-butylhydroxymetribuzin, t-butylhydoxyDA, t-butylhydroxy-DK, t-butylhydroxy-DADK, 3-amino DA, N-Ac-Cys-metribuzin, and N-Ac-Cys-DA were identified in urine, feces, or tissues. Several other unknown metabolites were also observed. The most prevalent metabolite in feces and urine was DA-N-Ac-Cys (i.e. the mercapturic acid conjugate of DA). Degradation of metabolites was observed in some of the analytical procedures.

B.4.3. Pharmacokinetic studies in other animals

A study was conducted in male Swiss Webster mice treated with metribuzin or ^{14}C-metribuzin by intraperitoneal (i.p.) injection (Bleeke et al. 1985). Pretreatment with piperonyl butoxide (PB), a cytochrome P450 inhibitor, was found to be protective, increasing the LD_{50} by about 3 fold. In contrast, pretreatment with diethyl maleate, which results in depletion of glutathione (GSH), increased the toxicity of metribuzin, reducing the LD_{50} by about 2 fold. At high doses of metribuzin, liver GSH was depleted. Also at high doses, ^{14}C label was found to be covalently bound to liver proteins. PB was protective against both these effects. The primary urinary metabolites were mercapturic acid conjugates, which arise from reaction with GSH. In vitro studies of metribuzin metabolism by mouse liver microsomes supplemented with NADPH found considerable amounts of protein binding by metribuzin metabolites (Bleeke and Casida 1984). If the microsomes were additionally supplemented with N-acetylcysteine, protein binding was minimal, and metribuzin mercapturate and/or DA mercapturate were found. The authors speculated that this could be due to the formation of a reactive sulfoxide intermediate.

A study was conducted in male dogs (one mongrel, three hounds) administered 5-^{14}C-metribuzin at 10 mg/kg orally (capsule) (Baychem 1972a). Blood levels were tested at a number of time points, with peak ^{14}C label levels occurring 4 hours after administration. Tissue levels were generally highest in liver, followed by kidney, followed by other tissues (skin, muscle, heart, fat, brain). The ^{14}C label was excreted in urine and feces, with 80 to 90% excreted in one to three days. Approximately twice as much ^{14}C label

was found in urine as in feces. Digestion of urine with B-glucuronidase and/or aryl sulfatase had no appreciable effect on metabolites.

Studies have also been conducted in goats, pigs, and cows (Baychem 1972c, 1974b, 1974c). All were treated orally with ^{14}C-metribuzin. In two lactating female goats, small amounts of ^{14}C label were found in the milk. Concentrations of ^{14}C label were highest in liver, followed by kidney, followed by other tissues (heart, fat, other muscle). In two male pigs, approximately 100% of ^{14}C label was recovered in urine and feces in three days. Most of the ^{14}C label recovered was in urine. Metribuzin, DA, DK, DADK, and unidentified compounds were found in tissues, urine, or feces. Concentrations of ^{14}C label were highest in liver, followed by kidney, then other tissues (fat, heart, other muscle, brain). In one dairy cow, peak blood ^{14}C label levels occurred 4 hours after administration. Some ^{14}C label was found in milk. Concentrations of ^{14}C label were highest in liver, followed by kidney, followed by fat, and heart and other muscle. Metribuzin, DA, DK, DADK and other compounds were identified in tissues.

B.4.4 Summary of pharmacokinetic studies

Several studies of the pharmacokinetics of metribuzin have been performed in rats and other animals. Many of these studies are limited by having used only one or two animals per experiment or time point. In general, orally administered metribuzin is fairly rapidly absorbed, with peak blood or tissue levels around 4 hours after single administration. It is also fairly rapidly excreted, with the majority of metribuzin or its metabolites appearing in the urine or feces in one to a few days after administration. There appear to be species, strain, and/or sex specific differences as to whether most of the excreted material is in the urine or the feces. There is evidence that cytochrome P450 is involved in initial metabolism. Metribuzin appears to be metabolized by several pathways, some of which can act in combination (see Figure 2). As a result, numerous metabolites of metribuzin have been found in tissues, urine or feces. Generalizations across studies are limited because most studies examined a relatively limited number of metabolites. Several studies indicate that the highest concentrations of metribuzin and/or its metabolites are found in the liver, followed by the kidney, followed by other commonly examined tissues. Concentrations in ovaries and testes were relatively low. One study found that concentrations of metribuzin and/or its metabolites were approximately 10-fold higher in the thyroid than in the liver. Evidence indicates that glucuronidation and sulfation do not play a major role in metabolism or excretion. In contrast, conjugation with GSH followed by conversion to mercapturic acid derivatives appears to play a major role in detoxification and excretion. There is evidence that at very high levels, or in the absence of non-protein sulfhydrils (e.g., glutathione), metribuzin metabolites can bind to proteins.

B.5. Industrial Bio-Test Laboratories Inc. Studies

A number of metribuzin toxicology studies were conducted by Industrial Bio-Test Laboratories, Inc. (IBTLI). IBTLI was subsequently found to have submitted numerous

fraudulent and invalid studies to regulatory agencies, including U.S. EPA. (Marshall 1983, U.S. EPA 1983b). Evaluation by U.S. EPA of the metribuzin studies conducted by IBTLI found four studies that were invalid (IBTLI 1971a, 1971b, 1972a, 1972b), described as "the information in the final report was not supported by the raw data from the study" (U.S. EPA 1983a). These invalid studies are not discussed further in this document. One study, a subchronic study in dogs (IBTLI 1970) was valid.

B.6 Non-DART Toxicities

No studies have been located of human effects of metribuzin 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.6.1. Acute Toxicity

Acute LD_{50}s of metribuzin have been reported for several species (Baychem 1974a, Bayer 1969a, Bleeke et al. 1985). Acute LD_{50}s vary by species, strain and route of exposure, as summarized in Table 1. Of the several species tested, rats had the highest LD_{50}s, and guinea pigs the lowest by oral gavage. LD_{50}s from intraperitoneal injection were considerably lower than for gavage. Symptoms observed to follow high, acute exposures include a profound, non-arousable sedation, labored breathing, and coarse coat. See also section B.5.4, Neurobehavioral toxicity, below. Dermal application of 500 mg/kg for four hours or seven days caused no lethality in rats. Inhalation concentrations of 860 to 892 mg/m^3 for four hours caused no lethality in male or female rats or male mice.

Table 1. Acute LD_{50}s for metribuzin.

Species, strain	Sex	LD_{50} (mg/kg)	
		Oral (gavage)	Intraperitoneal injection
Rat, Wistar	Male	2200 (1)	363 (1)
Rat, Wistar	Female	2345 (1)	363 (1)
Rat, Sprague-Dawley	Male	1090 (2)	
Rat, Sprague-Dawley	Female	1206 (2)	
Mouse, CF1	Male	698 (1)	247 (1)
Mouse, CF1	Female	711 (1)	275 (1)
Mouse, Swiss Webster	Male		210 (3)
Guinea pig, Pirbright	NR	250 (1)	
Guinea pig (albino)	Male	245 (2)	
Guinea pig (albino)	Female	274 (2)	
Rabbit (not pure breed)	NR	>500 (1, 4)	
Cat (not pure breed)	NR	>500 (1, 4)	

NR – not reported
(1) Data from Bayer 1969a.
(2) Data from Baychem 1974a.
(3) Data from Bleeke et al. 1985
(4) No lethality at indicated dose.

B.6.2. Subchronic and chronic toxicity

There have been a number of subchronic toxicity studies. Rats have been treated by the oral, inhalation, and dermal routes (Baychem 1972d, Bayer 1969b, 1970, 1981a, 1982a, 1983, 1999b, Porter et al. 1993, University of Chicago 1969). There have also been a guinea pig gavage study (Tomazszewski et al. 1985, 1986), a rabbit dermal study (Bayer 1989), and a dog oral study (IBTLI 1970). In rats, reduced body weight or body weight gain were commonly found (oral at 300 to 1,500 ppm in food, 90 to 720 mg/m^3 by inhalation, and 700 mg/kg dermal). Increased liver weight has also been observed, at lower levels of exposure than body weight effects for the oral route but not the inhalation or dermal routes (35 to 1,500 ppm in food, 720 mg/m^3 by inhalation, and 700 mg/kg dermal). Increased mortality was not observed in these rat studies. Guinea pigs treated by gavage at 83 mg/kg/d for 30 or 90 days also displayed loss of body weight (no quantitative data presented), fatigue, histopathological effects in liver, and altered liver function (Tomaszewski et al. 1985, 1986). Rabbits treated at up to 1000 mg/kg dermally for 5 days/week for 3 weeks showed no effects on mortality, body weight, or organ weight. However, only 5 animals/sex/group were used (Bayer 1989). Dogs treated at up to 500 ppm in food for 90 days showed no effects on mortality, body weight, or organ weight. However, only 4 animals/sex/group were used (IBTLI 1970).

A number of subchronic studies have found effects on circulating thyroid hormones, thyroid weight, or thyroid histology. In a study in rats, increased thyroid weight and histological evidence of increased thyroid activity was observed at 1,500 ppm in food

(Bayer 1969b). An extensive study in rats found increased circulating levels of T4 (thyroxine) at 35, 100, 300 and 900 ppm in food, with an inverted "U" concentration-response relationship (Bayer 1982a). Inconsistent changes in T3 (triiodothyronine) levels were observed. Thyroid weights were reduced early in the study (7 days), nearly normal near the middle of the study (21 days), and increased at the end of the study (63 days). Changes in histological staining of the thyroid were also reported. This was attributed by the authors to an altered functional state of the thyroid. Increased T4 was also found in rat inhalation studies, with an inverted U concentration-response relationship over the range of 31 to 720 mg/m^3 (Bayer 1981a). A study in rats treated at 10 ppm in water found increased "free thyroxine index" (not defined by the authors) at all time points (2 and 6 weeks for females and 7, 13, and 16 weeks for males) (Porter et al. 1993). A rabbit dermal study found increased T4 at 200 and 1,000 mg/kg in females, and 1,000 mg/kg in males (Bayer 1989). The authors of one study hypothesized that the thyroid and thyroid hormone effects could be due to reduced peripheral conversion of T4 to T3, resulting in a compensatory increase in thyroid activity (Bayer 1982a).

There have been several chronic studies with metribuzin. There have been one mouse study, two rat studies, and one dog study, all with metribuzin administered in feed. A study in CD1 mice at up to 3,200 ppm found increased kidney and liver weight in females as the only effect (Mobay 1981). A Wistar rat study at 0, 25, 35, 100 or 300 ppm found reduced body weight in females at 300 ppm and increased thyroid weight in males at 35, 100, and 300 ppm. No other effects were reported (Bayer 1974b). A later study in Fischer 344 rats at 0, 30, 300, or 900 ppm found reduced body weight in males at 900 ppm and in females at 300 and 900 ppm. Increased thyroid weight in males and females was found at 300 and 900 ppm. Thyroid hyperplasia was found in males at 300 and 900 ppm. This study also found increased levels of circulating T4 and reduced levels of T3 at 30, 300 and 900 ppm. The concentration-response relationships were inverted "U" and "U" shaped, respectively, for these observations. No mortality was found (Miles 1993). The study in Beagle dogs at 0, 25, 100, or 1,500 ppm found severe effects at the high concentration, including increased mortality (3 out of 4 animals), reduced weight or weight gain, reduced food consumption, anemia, kidney damage, and increased thyroid weights (Bayer 1974d).

B.6.3. Carcinogenicity

No evidence of carcinogenic effects from metribuzin was found in studies in Wistar rats (Bayer 1974b), Fischer 344 rats (Miles 1993), and CD1 mice (Mobay 1981).

B.6.4. Neurobehavioral toxicity

There have been three neurobehavioral toxicity studies. A subchronic study treated food-restricted male Sprague-Dawley rats with metribuzin at 0 or 10 ppm in water, with six rats per group (Boyd et al. 1990). There were no ad-lib food controls. The duration of treatment was reported as 90 days in the abstract, and 108 days in the text. In T-maze, food-reward training trials, metribuzin treated rats took longer (statistically significant)

than controls to learn the first reversal. Three subsequent reversals found no significant difference between metribuzin treated rats and controls. The number of days of treatment at the time of trials was not reported: the initial training began one week after the beginning of exposure, and treatment was terminated after all rats completed four reversals. Upon sacrifice, metribuzin treated rats were found to have elevated (statistically significant) levels of choline in the hippocampus, but acetylcholine was not affected. Levels in the cortex and neostriatum were not affected.

An acute study treated Fischer 344 rats with metribuzin by gavage at 0, 2, 5, 20, or 100 mg/kg (Bayer 1999a). There were no treatment related deaths or effects on body weight. Neurobehavioral observations included clinical effects, functional observational battery, and motor and locomotor testing. Numerous effects were observed at 100 mg/kg, including eyelid ptosis, lacrimation, salivation, pinpoint pupils, decreased activity, decreased response to stimulus, and reduced body temperature. Effects were reduced at the 20 mg/kg dose level, and only minimal effects were observed at 5 mg/kg. Effects were most pronounced shortly after treatment. Most effects resolved by the next test day (day 7), although reduced activity was observed in males after 14 days. No gross pathology or histopathology of brain or nerve tissues was found.

A subchronic study treated Fischer 344 rats at 0, 30, 300, or 900 ppm in food for 13 weeks (Bayer 1999b). These concentrations corresponded to 0, 1.92, 21.2, and 62.3 mg/kg/d, respectively, for males, and 0, 2.19, 23.9, and 70.1 mg/kg/d, respectively, for females. There were no treatment related deaths. Females had reduced (statistically significant) food consumption and body weight at 300 and 900 ppm, but there were no statistically significant effects in males. Neurobehavioral observations included clinical effects, functional observational battery, and motor and locomotor testing at 4, 8, and 13 weeks. No neurobehavioral effects were observed. No gross pathology or histopathology of brain or nerve tissues was found.

In a developmental toxicity study in Sprague-Dawley rats (Miles 1986), hypoactivity and ptosis were observed during the early days of treatment in females at 25, 70 and 200 mg/kg, and ataxia at 70 and 200 mg/kg (see Section C.2.1.2).

B.6.5. Summary of non-DART toxicity

In chronic and subchronic studies, treatment with metribuzin has resulted in mortality, reduced body weight and increased liver weight, as well as effects on circulating thyroid hormones and thyroid function. Transient neurobehavioral effects have been observed. The mechanism of these effects has not been elucidated.

Thyroid hormone effects have also been observed following exposure to atrazine (reviewed in OEHHA 1999), although differences in study designs preclude detailed comparisons of results. Mammary tumor incidence was found to be increased in female Sprague-Dawley and male Fischer rats treated with atrazine, although there was weak to no induction of mammary tumors in female Sprague-Dawley rats with thiomethyl-s-triazines, such as ametryn or prometryn (reviewed in OEHHA 1999). Metribuzin has a

thiomethyl group, as opposed to the chloro group in atrazine. No carcinogenicity studies of metribuzin in Sprague-Dawley rats have been located. Fischer rats treated with metribuzin displayed no increase in mammary tumor incidence.

C. DEVELOPMENTAL TOXICITY

C.1. Overview

Four studies in experimental animals of developmental toxicity of metribuzin were located, two in rat and two in rabbit (Bayer 1972, Miles 1986, 1989, 1991, MRI 1981). Also, two rat reproductive studies contain information relevant to developmental endpoints (Bayer 1974a, Miles 1988, 1990). No studies of human exposure and developmental effects were located.

C.2. Animal developmental toxicity studies

C.2.1. Rats

C.2.1.1. Developmental study in FB 30 rats: Bayer 1972

Mated female FB 30 rats were treated with metribuzin by gavage on gestation days (gd) 6-15 at 0, 5, 15, 50, or 100 mg/kg/d, with 22-24 inseminated females/group (Bayer 1972). The purity of the test compound was stated to be 99.5%. Animals were sacrificed on gd 20. Maternal results reported included survival and body weight. Fetal results reported included number of resorptions, body weight, malformations and variations in bone development.

All animals survived treatment. One female at 50 mg/kg/kd and two at 100 mg/kg/d displayed "ill-effects" which included ruffled coats, dispend, and reduced general activity. Also, females treated with 100 mg/kg/d displayed a slight, non-statistically significant reduction in weight gain during gd 6-15 and during gestation. These results are summarized in Table 2.

The percentage of females showing evidence of fertilization or pregnancy at termination was not reduced in the metribuzin treated groups. The numbers of implants, resorptions, or fetuses/litter were not affected by treatment. Fetal weight was not affected by treatment, but placental weight was reduced (statistically significant) in the 100 mg/kg/d group. There was no increase in malformations or variations in bone development (sternum, hyoid, vertebral column, ribs, extremities, or skull) in the treated groups. These results are summarized in Table 2.

Table 2. Results of FB 30 rat developmental study with metribuzin (Bayer 1972) [(1)]

Metribuzin dose (mg/kg/d)		0	5	15	50	100
Maternal deaths		0	0	0	0	0
Body weight gains of pregnant rats (g) [(2)]	gd 6-15	44.3	42.4	41.5	42.9	38.0
	gestation	118.1	121.1	117.5	119.7	109.8
Fertilized/ inseminated females [(3)] [No. (%)]		21/24 (87.5%)	22/22 (100%)	22/23 (95.7%)	22/24 (91.7%)	21/24 (87.5%)
Pregnant/ fertilized females [(3)] [No. (%)]		20/21 (95.2%)	22/22 (100%)	21/22 (95.5%)	22/22 (100%)	21/21 (100%)
Number of implantations/litter		11.8	10.9	11.4	11.2	11.9
Number of resorptions/litter		1.4	1.1	0.9	1.2	1.7
Number of fetuses/litter		10.4	9.8	10.5	10.0	10.2
Fetal weight (g)		3.90	4.11	3.98	3.98	3.98
Placental weight (g)		0.542	0.548	0.543	0.513	0.502*
Malformations/litter		0.05	0.05	0	0	0
Percentage of fetuses with "slight variation in bone development."		50.0%	42.4%	42.2%	48.1%	50.3%

(1) Values are numbers, means, or percentages. No indices of variation (e.g. standard deviation) were reported by the authors.
Wilcoxon test for weight gains, implantation quota, number of fetuses, resorption quota, fetal weight, placental weight.
Chi^2 test for fertilization quota, fetuses with bone alterations, malformations.
* Significantly different from controls by Wilcoxon test at $p < 0.05$.

(2) Females were reported to weigh between 200 and 250 g at the beginning of the study.

(3) Fertilized appears to refer to females with any implants at sacrifice.
Pregnant appears to refer to females with fetuses at sacrifice.

C.2.1.2. Developmental study in Sprague-Dawley rats: Miles 1986

Inseminated female Sprague-Dawley rats were treated with metribuzin by gavage at 0, 25, 70, or 200 mg/kg/d for gd 6-15, with 33 rats/group (Miles 1986). The purity of the test compound was stated to be 92.6%. Five animals from each group were sacrificed on gd 16, and the rest were sacrificed on gd 20. Maternal animals sacrificed on gd 16 were examined for thyroid weight and serum T3 and T4 concentrations. Results reported from the animals sacrificed on gd 20 included maternal clinical observations, food consumption, body weight, gross external and visceral changes, thyroid weight, serum T3 and T4, pregnancy rates, corpora lutea, implantations, resorptions, litter size, fetal weight and viability, placental weight, uterine weight, fetal sex, and fetal external, visceral, and skeletal alterations.

All females survived treatment. Hypoactivity and ptosis were noted at all dose levels. Ataxia was noted at the mid and high dose levels. Food consumption and body weight were reduced (statistically significant) during metribuzin treatment at all dose levels. Food consumption after the end of metribuzin treatment was similar among groups. Final body weight and net weight (the final body weight minus the weight of intact uterus) were reduced in a dose-related manner in all groups (statistically significant in the mid and high dose groups). The final body weight and net body weight were reduced by about 10% and 9%, respectively, in the high dose group compared to controls. Body weight gain and net body weight gain during gestation were reduced in a dose-related manner in all metribuzin treated groups (all statistically significant). There was a statistically significant increase in thyroid weight at the high dose. T3 concentrations were not significantly affected. T4 levels were reduced on gd 16, and increased on gd 20, in the mid and high dose groups. These results are summarized in Table 3.

Metribuzin treatment had no effect on the fraction of females pregnant, number of implantations, number of resorptions, litter size, percentage pre-implantation loss, or percentage post-implantation loss. Median fetal weight was reduced (statistically significant) at all dose levels. The authors report median fetal weights for historical controls of 3.4-4.0 g for 15 studies in their laboratory from 1980 to 1986. The authors did not report the mean fetal weights. RCHAS staff calculated the mean fetal weights, which were reduced at all doses ($p<0.01$, Student's T test), and decreased with increasing dose. Median placental weight was reduced (statistically significant) at the high dose level. There were no increases in gross, visceral, or skeletal malformations attributable to metribuzin treatment. Increased incidences (statistically significant) of delayed ossification of skull, ribs, vertebrae, sternebrae, pelvis, and appendages were observed at the high dose level. Also, increased incidence (statistically significant) of wavy, curved, and/or bulbous ribs was observed at the high dose level. These results are summarized in Table 4.

Table 3. Results of Sprague-Dawley rat developmental study with metribuzin (Miles 1986) [1]

Metribuzin dose (mg/kg/d)		0	25	70	200
Maternal deaths		0	0	0	0
Food consumption by pregnant rats (g)	gd 1	21.6 ± 0.6	21.1 ± 0.7	21.3 ± 0.6	21.6 ± 0.6
	gd 6	24.2 ± 0.5	24.3 ± 0.8	22.9 ± 0.8	23.4 ± 0.6
	gd 8	24.4 ± 0.5	20.2 ± 0.6*	15.4 ± 0.7*	14.3 ± 0.8*
	gd 12	27.5 ± 0.5	23.2 ± 0.6*	23.1 ± 0.6*	21.4 ± 0.7*
	gd 15	25.5 ± 0.5	23.0 ± 0.5*	22.5 ± 0.4*	21.1 ± 0.7*
	gd 20	27.7 ± 0.6	28.1 ± 0.6	27.8 ± 0.8	26.2 ± 0.7
Body weight of pregnant rats (g)	gd 0	238.5 ± 2.3	240.8 ± 2.9	234.9 ± 3.0	233.4 ± 2.8
	gd 6	266.6 ± 3.0	270.2 ± 4.2	260.9 ± 3.5	262.0 ± 3.0
	gd 15	315.0 ± 4.0	300.8 ± 4.6*	290.6 ± 4.2**	283.2 ± 3.5**
	gd 20	384.0 ± 5.2	371.7 ± 4.9	359.9 ± 4.7**	346.5 ± 3.6**
Weight gain gd 0-20 (g)		145.5 ± 3.9	130.9 ± 2.8*	125.0 ± 2.9**	113.0 ± 3.1**
Net weight gd 20 [2] (g)		306.9 ± 4.3	297.3 ± 4.6	288.6 ± 3.9**	279.6 ± 3.3**
Net weight gain gd 20 [2] (g)		68.4 ± 3.3	56.5 ± 2.5**	53.7 ± 1.9**	42.6 ± 2.8**
Serum T3 (ug/dl)	gd 16 [3]	78.3 ± 4.8	80.5 ± 5.2	80.4 ± 7.7	60.6 ± 12.8
	gd 20	90.1 ± 4.8	81.3 ± 3.7	85.1 ± 4.5	82.5 ± 3.8
Serum T4 (ug/dl)	gd 16 [3]	2.24 ± 0.05	2.20 ± 0.16	1.08 ± 0.18*	0.34 ± 0.08*
	gd 20	1.33 ± 0.07	1.61 ± 0.10	1.67 ± 0.10*	1.97 ± 0.10*
Thyroid weight (g)	gd 16 [3]	0.021 ± 0.004	0.022 ± 0.002	0.024 ± 0.003	0.033 ± 0.003*
	gd 20	0.018 ± 0.001	0.020 ± 0.001	0.020 ± 0.001	0.024 ± 0.001*

(1) Values are numbers or averages ± SE, except where indicated otherwise.
* Significantly different from controls at $p < 0.05$ by Dunett's test.
** Significantly different from controls at $p < 0.01$ by Dunett's test.

(2) Net weight is weight of pregnant female minus weight of intact uterus.

(3) There were n = 5 pregnant animals per group sacrificed on gd 16, except for the 70 mg/kg/d group, where one of the animals sacrificed was found not to be pregnant, resulting in n = 4 for that group.

Table 4. Results of Sprague-Dawley rat developmental study with metribuzin (Miles 1986) [1]

Metribuzin dose (mg/kg/d)		0	25	70	200
Number pregnant/inseminated		32/33	31/33	31/33	31/33
Number of litters at gd 20 sacrifice [2]		27	26	27	26
Corpora lutea/litter [3]		15.5	15.7	15.1	15.5
Number of implantations/litter [3]		14.6	14.8	14.1	14.3
Number of resorptions/litter [3]		1.0	1.2	1.1	1.2
Number of fetuses/litter [3]		13.6	13.6	13.0	13.1
Fetal weight [median] (g)		3.8	3.6##	3.6##	3.1##
Fetal weight (mean ± SD) [4] (g)		3.79 ± 0.21	3.58 ± 0.26@@	3.56 ± 0.28@@	3.19 ± 0.35@@
Placental weight [median] [3] (g)		0.53	0.51	0.51	0.49##
Ribs: wavy, curved, and/or bulbous	No. fetuses (%)	2 (1.0%)	0 (0%)	4 (2.2%)	13* (7.3%)
	No. litters (%)	1 (3.7%)	0 (0%)	2 (7.4%)	6 (23.1%)

(1) Values are numbers or averages ± SE, except where indicated otherwise.
* Significantly different from controls at $p < 0.05$ by Fisher's Exact test.
Significantly different from controls at $p < 0.01$ by Healy method.

(2) There were 5 animals per group sacrificed on gd 16. All were found to be pregnant, except for one animal in the 70 mg/kg/d group.

(3) The authors did not report measures of variation, e.g. standard deviation, for these results.

(4) Fetal weight mean ± standard deviation and statistical significance calculated by RCHAS staff from individual litter data. Original data to two significant digits. Results of RCHAS staff calculations are reported to 3 significant digits to avoid rounding errors.
@@ Significantly different from controls at $p < 0.01$ by Student's T-test.

C.2.2 Rabbits

C.2.2.1. Developmental study in New Zealand White rabbits: MRI 1981

Mated female New Zealand White rabbits were treated with metribuzin by gavage at 0, 15, 45, or 135 mg/kg/d for gd 6-18, with 19-21 animals per group (MRI 1981). The purity of the test compound was stated to be 93.0%. Animals were sacrificed on gd 30. Results reported included mortality, female body weight, clinical signs, abortions, corpora lutea, implantations, resorptions, live and dead fetuses, fetal weight, and gross, soft tissue, and skeletal anomalies.

Several females died during the test period. In the control group, one female died following dosing in the lung, two died from disease diagnosed as pneumonitis, and one died of unknown causes. In the 15 mg/kg/d group, one female died following dosing in the lung, one died from disease diagnosed as pneumonitis, and one died of unknown causes. In the 135 mg/kg/d group, one female died following dosing in the lung and one died from disease diagnosed as pleuritis. The authors stated that qualitatively reduced water and feed intake, and reduced fecal and urine output were observed, with the appearance of dose-dependency. However, no quantitative data on these endpoints were reported. Females dosed at 135 mg/kg/d lost weight during the treatment period. Mean body weights were reduced from controls on gds 13, 18 and 20 (statistically significant). At this dose, females gained more weight than controls during the post-treatment period (statistically significant). The weight of the females at 135 mg/kg/d was about 12% and 5% lower than controls on gd 18 and 30 respectively. These results are summarized in Table 5.

One control female, one 45 mg/kg/d female, and four 135 mg/kg/d females aborted their litters. The authors attributed abortions at the high dose to maternal toxicity. These results are summarized in Table 5. There were no statistically significant differences observed in number of copora lutea, implantations, resorptions, or litter size. However, there was an increased frequency of nonviable implants/pregnant female at 135 mg/kg/d (not statistically significant), mainly due to increased frequency of early resorptions. One control and two 135 mg/kg/d females had completely resorbed litters. Fetal weight was reduced at 45 and 135 mg/kg/d, but the differences were not statistically significant. The magnitude of the fetal weight reduction at 135 mg/kg/d was about 14%. Gross and soft-tissue examination found no indication of metribuzin treatment-related anomalies. Skeletal examination found an increase in percentage of incompletely ossified sternebrae at 135 mg/kg/d, but the increase was not statistically significant. There was no effect on completely unossified sternebrae. The percentages of extra ribs were increased, and the percentages of rib buds (less than one-half of normal size) were reduced in all metribuzin-treated groups, but the changes were not statistically significant. These results are summarized in Table 6.

Table 5. Results of New Zealand White rabbit developmental study with metribuzin (MRI 1981) [1]

Metribuzin dose (mg/kg/d)		0	15	45	135
Females mated		21	20	19	19
Females pregnant		17	17	17	16
Female deaths [2]		4	3	0	2
Females sacrificed due to premature delivery [3]		0	1	1	0
Females sacrificed subsequent to total abortions		1	0	1	4
Pregnant females sacrificed on scheduled date		13	14	15	10
Non-pregnant females sacrificed on scheduled date		3	2	2	3
Body weight of pregnant females sacrificed on scheduled date (kg)	gd 0	3.79 ± 0.10	3.81 ± 0.10	3.87 ± 0.06	3.75 ± 0.16
	gd 6	4.02 ± 0.08	4.02 ± 0.11	4.11 ± 0.06	3.94 ± 0.15
	gd 18	4.15 ± 0.09	4.13 ± 0.11	4.19 ± 0.05	3.63 ± 0.16*
	gd 30	4.33 ± 0.07	4.25 ± 0.12	4.35 ± 0.06	4.11 ± 0.12
Net weight gd 30 [4] (kg)		3.86 ± 0.05	3.77 ± 0.09	3.80 ± 0.08	3.66 ± 0.11
Weight gain gd 6-18 (kg)		0.13 ± 0.04	0.11 ± 0.04	0.07 ± 0.03	-0.31 ± 0.11*
Weight gain gd 0-30 (kg)		0.54 ± 0.06	0.44 ± 0.08	0.47 ± 0.06	0.36 ± 0.10
Net weight gain gd 0-30 (kg)		0.07 ± 0.08	-0.04 ± 0.08	-0.07 ± 0.07	-0.09 ± 0.09

(1) Values are numbers or means ± SE.
* Statistically significant difference from controls using Dunnett's procedure at $p < 0.05$.
(2) Includes pregnant and non-pregnant females.
(3) Premature delivery due to failure to observe successful mating, followed by subsequent observation of successful mating.
(4) Net weight is final body weight minus weight of reproductive tract and fetuses.

Table 6. Results of New Zealand White rabbit developmental study with metribuzin (MRI 1981) [(1)]

Metribuzin dose (mg/kg/d)		0	15	45	135
Pregnant females sacrificed on scheduled date		13	14	15	10
Corpora lutea/ pregnant female		9.9 ± 0.8	9.1 ± 0.7	11.9 ± 0.7	10.4 ± 0.5
Implantations/ pregnant female		8.8 ± 1.0	7.8 ± 1.0	10.8 ± 1.0	10.0 ± 0.4
Resorptions (%)	Early	16 ± 8	5 ± 2	3 ± 1	26 ± 3
	Late	4 ± 1	3 ± 2	9 ± 4	5 ± 2
Dead fetuses (%)		1 ± 1	0	1 ± 1	3 ± 2
Non-viable implants/pregnant female (resorptions plus dead fetuses)		1.8 ± 0.6	0.6 ± 0.2	1.7 ± 0.8	3.3 ± 1.0
Live fetuses/pregnant female		6.9 ± 0.9	7.2 ± 0.9	9.1 ± 1.0	6.7 ± 1.2
Fetal body weight (g)		45. 9 ± 1.9	47.7 ± 2.0	41.6 ± 1.7	39.6 ± 2.2
Skeletal examination [No. fetuses (No. litters)]		47 (12)	51 (13)	72 (15)	37 (8)
Unossified sternebrae [% fetuses (No. affected litters)] [(4)]		17.4 (5)	13.1 (4)	1.1 (1)	15.2 (4)
Incompletely ossified sternebrae [% fetuses (No. affected litters)] [(4)]		16.0 (6)	5.7 (3)	7.6 (5)	26.5 (5)
Extra ribs [% fetuses (No. affected litters)] [(4)]		18.6 (7)	47.3 (9)	38.9 (11)	45.4 (6)
Rib buds (< 1/2 normal size) [% fetuses (No. affected litters)] [(4)]		20.4 (6)	3.8 (2)	16.3 (8)	0 (0)

(1) Values are numbers, percentages, or means ± SE.
No statistically significant differences from controls were found using Dunnett's procedure at $p < 0.05$.
(2) Includes pregnant and non-pregnant females.
(3) Premature delivery due to failure to observe successful mating, followed by subsequent observation of successful mating.
(4) Mean of the percent of fetuses with the indicated anomaly calculated on a litter basis.

C.2.2.2. Developmental study in American Dutch rabbits: Miles 1989, 1991

Inseminated female American Dutch rabbits were treated with metribuzin by gavage at 0, 10, 30, or 85 mg/kg/d for gd 6-18, with 17 animals per group (Miles 1989, 1991). The purity of the test compound was stated to be 92.7%. Animals were sacrificed on gd 28. Results reported included mortality, female body weight, food consumption, clinical signs, abortions, number of corpora lutea, implantations, resorptions, live and dead fetuses, fetal weight, and gross, soft tissue, and skeletal anomalies.

Three females died during the test period. In the 10 mg/kg/d group, one died and was found upon necropsy to have purulent peritonitis. In the 30 mg/kg/d group, one died and was found to have lung changes indicative of dosing trauma. In the 85 mg/kg/d group, one died and was found to have infections including pulmonary changes and hemorrhagic enteritis. No gross clinical findings were observed. Females dosed at 85 mg/kg/d had reduced (statistically significant) food intake and gained less weight than controls (statistically significant) during the treatment period. Increased food consumption and body weight gain compared to controls were observed in this group after cessation of metribuzin treatment. At sacrifice, there were dose-related reductions in body weight in all treated groups, but these were not statistically significant. In the 85 mg/kg/d group, maternal body weights were reduced by about 5% on both gd 18 and 28. These results are summarized in Table 7.

In the 85 mg/kg/d group, one abortion was observed. The female which aborted was found upon necropsy to have pneumonia. The authors state that this was an incidental finding, not related to metribuzin treatment. There were no statistically significant differences observed in number of corpora lutea, implantations, resorptions, or litter size. Fetal weight was reduced (statistically significant) at 30 mg/kg/d, but was not reduced at 85 mg/kg/d. The authors reported historical control ranges for their laboratory of 34.6 to 40.5 g for 8 studies between 1985 and 1988. Gross and visceral examination found no indication of metribuzin treatment-related malformations. Skeletal examination found sporadic increases in delayed ossification, primarily in the 10 and 30 mg/kg/d groups. The authors state that the lack of dose-response indicates that these were incidental findings, not related to metribuzin treatment. These results are summarized in Table 8.

Table 7. Results of American Dutch rabbit developmental study with metribuzin (Miles 1989, 1991) [(1)]

Metribuzin dose (mg/kg/d)		0	10	30	85
Females inseminated		17	17	17	17
Pregnant females		15	15	15	16
Females died		0	1	1	1
Females with total abortions		0	0	0	1
Pregnant females with fetuses on gd 28		15	14	14	14
Body weight of pregnant females sacrificed on scheduled date (kg)	gd 0	2.82 ± 0.04	2.84 ± 0.05	2.78 ± 0.05	2.78 ± 0.05
	gd 6	2.90 ± 0.03	2.88 ± 0.04	2.85 ± 0.05	2.86 ± 0.05
	gd 18	3.09 ± 0.04	3.04 ± 0.04	3.02 ± 0.06	2.94 ± 0.06
	gd 28	3.24 ± 0.03	3.15 ± 0.05	3.11 ± 0.07	3.09 ± 0.06
Weight gain gd 6-18 (kg)		0.19 ± 0.01	0.16 ± 0.02	0.18 ± 0.02	0.08 ± 0.03**
Weight gain gd 0-28 (kg)		0.42 ± 0.02	0.31 ± 0.05	0.33 ± 0.05	0.31 ± 0.03

(1) Values are numbers or means ± SE.

** Statistically significant difference from controls using Dunnett's test at $p < 0.01$.

Table 8. Results of American Dutch rabbit developmental study with metribuzin (Miles 1989, 1991) [(1)]

Metribuzin dose (mg/kg/d)	0	10	30	85
Pregnant females with fetuses on gd 28	15	14	14	14
Corpora lutea/pregnant female	6.3	7.2	6.4	5.8
Implantations/pregnant female	5.9	6.5	6.4	5.8
Resorptions/pregnant female	0.2	0.5	0.3	0.3
Number of dead fetuses	0	0	0	0
Live fetuses/pregnant female	5.7	6.0	6.1	5.5
Fetal body weight (g)	39.9	38.6	35.0**	39.4
Skeletal examination [No. fetuses (No. litters)]	85 (15)	84 (14)	86 (14)	77 (14)
Skull bones incompletely ossified [% fetuses (% litters)]	20.0 (66.7)	36.9[#] (78.6)	44.2[##] (78.6)	20.8 (50.0)
Skull fontanelle enlarged [% fetuses (% litters)]	15.3 (66.7)	35.7[##] (78.6)	41.9[##] (78.6)	20.8 (50.0)
Unossified 5th sternebrae [% fetuses (% litters)]	7.1 (20.0)	6.0 (14.3)	22.1[#] (57.1)	7.8 (35.7)
Incompletely ossified 5th sternebrae [% fetuses (% litters)]	63.5 (100)	61.9 (85.7)	61.6 (100)	66.2 (100)
Extra ribs [% fetuses (% litters)]	15.3 (46.7)	21.4 (50.0)	16.3 (57.1)	22.1 (42.9)

(1) Values are numbers, percentages, or means. Indices of variation (e.g. standard deviation) were not provided by the authors.

** Statistically significant difference from controls using Healy's test at $p < 0.01$.

[#] Statistically significant difference from controls using Pair-Wise Comparisons at $p < 0.05$.

[##] Statistically significant difference from controls using Pair-Wise Comparisons at $p < 0.01$

C.3 Developmental Endpoints From Reproductive Studies

C.3.1. Three Generation Reproductive Study in FB 30 rats: Bayer 1974a

Male and female FB-30 rats were treated with metribuzin for three generations at 0, 35, 100, or 300 ppm in food (Bayer 1974a). The purity of the test compound was stated to be 99.5%. There were two litters per generation. The second litter of each generation became the parental group of the subsequent generation. There were 10 male and 20 female rats per group. For mating, two females were caged with one male for 19 to 20 days: males were interchanged so that females were caged with three different males for longer than one estrous cycle. The F0 generation was 33 days old at the beginning of treatment. This generation was treated for 70 days before first mating. The F1b and F2b generations were first mated at 100 days of age. Litters were culled to 10 on the fifth day after birth. Pups were allowed to nurse for up to 4 weeks. Results reported included parental mortality and body weight, fertility, litter size, pup weight and growth, and gross observations of the F3b litters. The authors indicated that the results were tested statistically using the Wilcoxon non-parametric test. However, at no point in the report were statistically significant results indicated: only non-statistically significant results were stated to be so.

In the F0 generation, one female in the 100 ppm group died after the second mating. One female in the 300 ppm group was sacrificed due to severe inflammation of the middle ear. In the F1b generation, one female in the control group died during the first mating. One female in the 300 ppm group died after the first mating. In the F2b generation, one male each died in the control and 35 ppm groups after the second and before the first mating, respectively. These deaths were ascribed to pneumonia. One female in the 35 ppm group and two females in the 300 ppm group died before the first mating. One of the deaths in the 300 ppm group was ascribed to pneumonia. No statistically significant or concentration-related differences in body weight between metribuzin treated and control groups were found in any generation. The authors reported average body weights only in figures: no numerical averages were reported. No alterations of behavior were observed.

Fertility (number pregnant/number mated) was lower in the 300 ppm group than in the controls in the second F0 mating (78.9% compared to 90%). There was no reduction in fertility in the metribuzin treated groups compared to control in the other five matings. In both F2b matings, the fertility of the controls was low compared to other matings and groups. Litter size was not reduced (statistically significant or concentration-related) in metribuzin treated groups compared to controls in any mating. No statistically significant reduction in birth weights was observed in metribuzin treated groups compared to controls in any mating. Birth weights in metribuzin treated groups were generally somewhat lower than controls in the F2a, F2b, F3a and F3b litters. This was concentration-related in one litter (F3a) but not in the other three. No gross external abnormalities were observed in any litter. Gross pathological examination of the F3b litters found no abnormalities. Histopathological examination of this litter found an increase in pneumonia with lymphocytic infiltrates in the lung, and lymphocytic and/or lymphocytic/histiocytic infiltrates in the liver of metribuzin treated groups compared to

controls. Also, increased fatty infiltration was observed in the liver of metribuzin treated groups compared to controls. Concentration-response for these observations was inconsistent. These results are summarized in Table 9.

Table 9. Results from three generation reproductive study in FB 30 rats (Bayer 1974a). [(1)]

Metribuzin concentration in food (ppm)		0	35	100	300
Fertility [no. pregnant/ no. mated (%)]	F0/F1a	20/20 (100%)	20/20 (100%)	19/20 (95%)	20/20 (100%)
	F0/F1b	18/20 (90%)	18/20 (90%)	19/19 (94.7%)	15/19 (78.9%)
	F1b/F2a	17/19 (89.5%)	20/20 (100%)	20/20 (100%)	19/20 (95%)
	F1b/F2b	16/19 (84.2%)	20/20 (100%)	18/20 (90%)	18/19 (94.7%)
	F2b/F3a	8/20 (40%)	15/19 (78.9%)	18/20 (90%)	16/18 (88.9%)
	F2b/F3b	4/20 (20%)	11/19 (57.9%)	16/20 (80%)	11/18 (61.1%)
Litter size at birth (no. pups/ litter)	F1a	11.4	10.8	11.9	11.9
	F1b	9.2	9.6	11.7	10.1
	F2a	11.5	12.0	12.5	12.1
	F2b	11.9	11.9	11.1	10.6
	F3a	11.4	9.6	10.5	9.9
	F3b	9.8	8.4	9.7	8.5
Birth weight (g)	F1a	6.38	6.32	6.38	6.28
	F1b	6.34	6.21	6.42	6.88
	F2a	6.54	6.08	6.22	6.01
	F2b	6.69	5.90	7.03	6.36
	F3a	6.75	6.55	6.24	6.18
	F3b	7.08	6.46	5.99	6.40
F3b animals examined histopathologically		10	10	10	10
F3b lung: minimal to medium grade pneumonia		6	8	9	10
F3b liver: low grade lymphocytic and/or lymphocytic/histiocytic infiltrates		5	10	6	10
F3b liver: low grade fatty infiltration		2	5	7	6

(1) Data are numbers, means, or percentages. No indices of variation (e.g. standard deviation) were reported. No indications of statistical significance were reported for tabulated results.

C.3.2. Two Generation Reproductive Study in Sprague-Dawley Rats: Miles 1988, 1990

Sprague-Dawley rats were treated for two generations with metribuzin at 0, 30, 150, or 750 ppm in food (Miles 1988, 1990). The purity of the test compound was stated to be 92.6%. There was one litter per generation. There were 30 males and 30 females per group. F0 animals were treated for 10 weeks before mating. Mating was performed by cohabiting one male per female overnight (separated during the day) for a 21 day mating period. If insemination did not occur within 21 days, females were cohabited with a different proven male for 7 days. Results reported included mortality, parental food consumption and body weight, general behavior, estrous cycling, fertility, litter size, birth weight, pup growth, clinical pathology and histopathology.

The authors of the study did not provide estimates of the doses resulting from the exposures used. RCHAS staff have estimated the doses based on food consumption and body weight data in the report. These results are summarized in Table 10.

No deaths occurred in the parental animals. Food consumption was reduced (statistically significant) in males and females of the F0 and F1 generations at 750 ppm. At 750 ppm body weight and body weight gain were reduced (statistically significant) in males of the F0 and F1 generations and females of the F0 and F1 generations during the premating phase and gestation (by 10-14% during gestation). At 150 ppm body weight was also reduced (statistically significant) during gestation in the females of the F1 generation at 150 ppm by 7-9%. Body weight gain during gestation was reduced (statistically significant) in the F0 generation at 30 ppm but not other concentrations, and in the F1 generation at 150 and 750 ppm. Body weight at the end of lactation was reduced (statistically significant) in the F1, but not the F0 generation at 750 ppm. The authors reported that no behavioral effects or signs of toxicity were attributable to metribuzin treatment, although some incidental findings were observed in all groups. Increased (statistically significant) serum gamma glutamyl transferase (GGT) was found in the F1 females at 150 and 750 ppm, possibly indicative of liver effects. A concentration related increase in the incidence of mild hepatocyte hypertrophy was also found in the F1 generation. These results are summarized in Table 11.

There was no effect on estrous cycling, time required for insemination, fertility (number pregnant/number inseminated), number of implantations, litter size, number of dead pups, or birth weight in the F0/F1 generation. No gross or histopathological effects on ovaries were observed. There was a reduction (statistically significant) in the number of implantations and litter size in the F1/F2 generation at 150 and 750 ppm. Historical control data provided by the authors for their laboratory for 20 studies from 1982 to 1988 displayed a range of 13.1 to 16.1 implantations per litter and litter sizes from 12.0 to 16.8 pups per litter. There was no effect on fertility, number of dead pups, or birth weight in the F1/F2 generation. No gross or histopathological effects on ovaries were observed. No abnormalities attributable to metribuzin treatment were observed. Pup weight and weight gain during lactation were reduced (statistically significant) in the F1 and F2 generations at 750 ppm. This effect was first observed on lactation day 14, after, the authors report,

pups began consuming the test diets. Pup weight and weight gain during lactation were also reduced (statistically significant) in the F2 generation at 30 ppm, also after lactation day 14. These results are summarized in Table 12.

Table 10. Doses in two generation Sprague-Dawley rat reproductive study (Miles 1988, 1990). [(1)]

Metribuzin concentration in food (ppm)		0	30	150	750
Female dose: F0 (mg/kg/d)	Premating	0	2.23 ± 0.36	11.2 ± 1.4	52.8 ± 10.9
	Gestation	0	2.00 ± 0.11	10.2 ± 0.3	50.0 ± 4.9
Female dose: F1 (mg/kg/d)	Premating	0	2.74 ± 0.41	13.8 ± 2.4	68.0 ± 8.8
	Gestation	0	2.24 ± 0.16	11.2 ± 0.9	56.2 ± 3.0

(1) Doses estimated by RCHAS staff from food consumption and bodyweight data. Data are mean ± SD

Table 11. Parental data in two generation Sprague-Dawley rat reproductive study (Miles 1988, 1990). [1]

Metribuzin concentration in food (ppm)		0	30	150	750
Female body weights: F0 (g)	Day 0	176.5 ± 2.2	174.1 ± 2.5	172.0 ± 1.9	174.7 ± 2.0
	Day 69 [2]	270.3 ± 4.4	265.3 ± 5.5	258.9 ± 3.6	235.8 ± 3.1**
	gd 0	269.7 ± 4.1	267.7 ± 5.7	261.9 ± 4.1	233.5 ± 2.9**
	gd 20	376.4 ± 5.8	364.6 ± 6.5	360.9 ± 5.3	338.7 ± 4.6**
	ld 0	301.4 ± 5.3	290.6 ± 5.7	280.6 ± 5.1	265.0 ± 3.0**
	ld 21	304.9 ± 4.8	299.1 ± 4.0	306.9 ± 3.8	302.7 ± 3.2
Female body weight gain during gestation (gd 0-20): F0 (g)		106.8 ± 3.3	96.8 ± 2.7*	99.0 ± 3.0	105.2 ± 2.4
Female body weights: F1 (g)	Day 0	142.9 ± 3.9	139.8 ± 2.6	137.1 ± 3.7	135.6 ± 5.3
	Day 70 [2]	269.9 ± 5.9	258.4 ± 4.5	254.2 ± 4.5	234.3 ± 4.3**
	gd 0	272.1 ± 6.8	260.9 ± 4.4	253.9 ± 4.7*	233.0 ± 4.2**
	gd 20	382.9 ± 8.6	362.7 ± 5.3	349.5 ± 4.9**	331.8 ± 6.0**
	ld 0	298.0 ± 7.4	286.4 ± 4.7	281.6 ± 4.8	260.8 ± 4.1**
	ld 21	307.9 ± 4.4	300.1 ± 3.2	302.1 ± 3.8	292.5 ± 4.3*
Female body weight gain during gestation (gd 0-20): F1 (g)		110.8 ± 3.5	101.7 ± 2.5	95.7 ± 3.1**	98.9 ± 2.9*

(1) Data are mean ± SE

* Statistically significant difference from control by Dunnett's test at p = 0.05.

** Statistically significant difference from control by Dunnett's test at p = 0.01.

(2) End of premating phase.

Table 12. Reproductive and developmental data in two generation Sprague-Dawley rat reproductive study (Miles 1988, 1990). (1)

Metribuzin concentration in food (ppm)		0	30	150	750
Number of times estrus achieved in 11 day period (10 rats monitored)	F0	20	23	24	17
	F1	24	25	24	23
No. days to insemination (median)	F0	2	2	2	2
	F1	3	3	3	2
Fertility (no. pregnant/no. inseminated) (%)	F0	96.7%	96.7%	93.1%	100%
	F1	83.3%	96.7%	96.7%	93.3%
Number of implantations	F0/F1	13.55 ± 2.18	13.14 ± 2.75	13.44 ± 3.02	13.17 ± 1.62
	F1/F2	15.00 ± 1.68	14.03 ± 2.06	13.03 ± 3.22**	13.54 ± 2.03
Litter size	F0/F1	12.79 ± 2.35	12.34 ± 2.73	12.19 ± 3.34	12.33 ± 1.88
	F1/F2	14.12 ± 1.62	13.31 ± 2.21	11.97 ± 3.40**	12.32 ± 2.47*
Dead pups/litter	F0/F1	0.6	0.7	0.9	1.0
	F1/F2	0.4	0.6	0.4	0.2
Birth weight (g)	F1	6.041 ± 0.508	6.045 ± 0.477	6.093 ± 0.539	5.945 ± 0.449
	F2	5.908 ± 0.416	5.766 ± 0.527	5.903 ± 0.644	5.861 ± 0.491
Pup weight lactation day 7 (g)	F1	15.63 ± 1.60	15.49 ± 1.70	15.20 ± 1.84	14.64 ± 1.61
	F2	15.45 ± 1.52	14.86 ± 1.74	15.00 ± 1.94	14.62 ± 1.71
Pup weight lactation day 14 (g)	F1	32.22 ± 2.91	31.14 ± 2.89	30.84 ± 3.54	30.16 ± 2.42*
	F2	32.13 ± 2.61	30.34 ± 2.93	30.28 ± 2.90*	29.76 ± 2.73**
Pup weight lactation day 21 (g)	F1	52.14 ± 5.45	50.84 ± 5.00	51.07 ± 5.10	47.58 ± 3.34**
	F2	52.03 ± 4.23	48.57 ± 4.50*	49.24 ± 4.82	47.04 ± 3.85**

(1) Data are percentages, means or mean ± SD, except medians as indicated.
*Statistically significant difference from controls using Dunnett's test at $p \leq 0.05$.
**Statistically significant difference from controls using Dunnett's test at $p \leq 0.01$.

C.4. Integrative evaluation – Developmental toxicity.

The above sections describe the design and results of four developmental studies in experimental animals: two in rats (Bayer 1972, Miles 1986) and two in rabbits (Miles 1989, 1991, MRI 1981), as well as two available multigeneration reproductive studies in rats (Bayer 1974a, Miles 1988, 1990). No studies in humans have been located.

In the earlier rat developmental study (Bayer 1972), FB 30 rats were treated with metribuzin at 0, 5, 15, 50, or 100 mg/kg/d for gd 6-15 by gavage. No indications of developmental toxicity were observed. Maternal toxicity was mild, with a slight, non-statistically significant reduction in weight gain during treatment and during gestation.

In the later rat developmental study (Miles 1986) Sprague-Dawley rats were treated with metribuzin at 0, 25, 70, or 200 mg/kg/d for gd 6-15 by gavage. There were dose-related and statistically significant reductions in fetal weight in metribuzin-treated groups. Historical control data provided by the authors indicated that, although reduced from concurrent controls, the fetal weights in the 25 and 70 mg/kg/d groups were within the historical control range for that laboratory. Fetal weight was reduced by about 16% in the 200 mg/kg/d group, and was outside of the historical control range. Additionally, increased incidences of delayed ossification of several bones, and wavy, curved or bulbous ribs were observed in the 200 mg/kg/d group. No other indications of developmental toxicity were observed. Some maternal toxicity was observed at all doses. There were dose-related and usually statistically significant reductions in maternal food consumption, body weight, and weight gain in all metribuzin treated groups. At sacrifice on gd 20, maternal body weight was reduced by about 10% in 200 mg/kg/d group. Serum T4 was reduced on gd 16 and increased on gd 20 in the 70 and 200 mg/kg/d groups. Thyroid weights were increased in the 200 mg/kg/d group.

In the first of the rat reproductive studies (Bayer 1974a), FB 30 rats were treated with metribuzin at 0, 35, 100, or 300 ppm in food for three generations, with two litters per generation. In the F2 and F3 litters, birth weights were generally somewhat lower in the metribuzin treated groups than controls. However, these were not statistically significant and were concentration-related in only one litter out of four. In F3b animals examined histopathologically, increased pneumonia with lymphocytic infiltrates of the lung and lymphocytic/histiocytic infiltrates of the liver, as well as low grade fatty infiltration of the liver in metribuzin treated groups were observed. No other indications of developmental toxicity were observed. No parental toxicity was observed.

In the second rat reproductive study (Miles 1988, 1990), Sprague-Dawley rats were treated with metribuzin at 0, 30, 150, or 750 ppm in food for two generations, with one litter per generation. There was no effect on fertility, number of dead pups, or birth weight in either generation. In the F1/F2 generation only, at 150 and 750 ppm, there were reduced (usually statistically significant) numbers of implantations and litter size. Historical control data provided by the authors indicated that, although reduced from concurrent controls, the numbers of implantations and litter sizes were within historical control ranges for that laboratory. At 750 ppm, maternal weight was reduced

(statistically significant) by 10-14% during gestation in both the F0 and F1 generations. At 150 ppm, maternal weight was reduced (statistically significant) by 7-9% during gestation in the F1 generation only. Serum gamma glutamyl transferase (GGT) was increased in the F1 females at 150 and 750 ppm, possibly indicative of liver effects. Also, a concentration related increase in mild liver hypertrophy was observed in the F1 generation.

In summary, the earlier rat developmental and reproductive studies (Bayer 1972, 1974a) did not use doses or concentrations which resulted in significant maternal or systemic toxicity. No consistent indications of fetal toxicity were observed. The later rat developmental study (Miles 1986) used doses which resulted in dose-related and usually statistically significant reductions in maternal food consumption, body weight gain, and body weight at all doses. The magnitude of the effects was not large, with females at the high dose weighing about 10% less at sacrifice than controls in the high dose group. There were dose-related and statistically significant reductions in fetal weight in all groups. The magnitude of the fetal weight reduction was about 15% in the high dose group. The later rat reproductive study (Miles 1988, 1990) used concentrations which resulted in reduced maternal weight at the high concentration in the F0 and the middle and high concentrations in the F0 and F1 parents. No differences in birth weights were observed in this study. The high concentration used in this study resulted in doses similar to the mid dose in the later rat developmental study. In the later rat reproductive study, reduced implantations and litter size (usually statistically significant, but not dose-related) were observed in the mid and high doses of the F1/F2 generation.

In the earlier rabbit study (MRI 1981), New Zealand White rabbits were treated with metribuzin at 0, 15, 45, or 135 mg/kg/d by gavage for gd 6-18, with sacrifice on gd 30. There was an increased incidence of total abortions in the 135 mg/kg/d group, which the authors attributed to maternal toxicity. In the 45 mg/kg/d group, fetal weight was slightly reduced, but the difference was not statistically significant. In the 135 mg/kg/d group there were increased incidence of early resorptions, reduced fetal weight, and an increase in incompletely ossified sternebrae, none of which were statistically significant. There were also increases in the frequency of extra ribs, and decreases in the frequency of rib buds (less than one-half of normal size), neither of which was statistically significant. There was no indication of maternal toxicity at 15 or 45 mg/kg/d. At 135 mg/kg/d, the females lost weight during treatment (statistically significant difference from controls). The females in this group gained more weight than controls during the period after metribuzin treatment and before sacrifice (statistically significant).

In the later rabbit study (Miles 1989, 1991), American Dutch rabbits were treated with metribuzin at 0, 10, 30, or 85 mg/kg/d by gavage for gd 6-18, with sacrifice on gd 28. There were reduced fetal weight and delayed ossification in the 30 mg/kg/d group (statistically significant), but not in the 85 mg/kg/d group. There was reduced maternal weight gain at the high dose.

In summary, the earlier rabbit study (MRI 1981) used doses which resulted in maternal weight loss during treatment at the high dose (statistically significant difference from

controls). This study also observed increased early resorptions, reduced fetal weight, and indications of delayed ossification at the high dose. Although these effects were not statistically significant, the observation of multiple effects suggests that they may be biologically significant. The later study (Miles 1989, 1991) used a different strain of rabbits, and used doses which resulted in reduced maternal weight gain during treatment at the high dose (statistically significant). No indications of fetal toxicity were observed at the high dose, although there were some indications at the low and mid doses, which were not dose-related.

D. FEMALE REPRODUCTIVE TOXICITY

D.1. Overview

There are two rat reproductive studies in which both males and females were treated with metribuzin (Bayer 1974a, Miles 1988, 1990). Also, there is a female dominant lethal study (Bayer 1974c). Additionally, there are several subchronic and chronic studies which contain information on female reproductive endpoints. No studies of human exposure and female reproductive effects were located.

D.2 Animal reproductive toxicity studies

D.2.1. Three Generation Reproductive Study in FB 30 rats: Bayer 1974a

This three-generation reproductive toxicity study in FB-30 rats treated with metribuzin at 0, 35, 100, or 300 ppm in food has been described previously in Section C.3.1 (Bayer 1974a).

As previously described, several animals died or were sacrificed in each of the generations. No statistically significant or concentration-related differences in body weight between metribuzin treated and control groups were found in any generation. Parameters assessed that are relevant to female reproductive toxicity included fertility (number pregnant/number mated), which was reduced in the 300 ppm group compared to controls in the second F0 mating (78.9% compared to 90%). There was no reduction in fertility in the metribuzin treated groups compared to control in the other five matings. In both F2b matings, the fertility of the controls was low compared to other matings and groups. Litter size was not reduced (statistically significant or concentration-related) in metribuzin treated groups compared to controls in any mating. No statistically significant reduction in birth weights was observed in metribuzin treated groups compared to controls in any mating. Birth weights in metribuzin treated groups were generally somewhat lower than controls in the F2a, F2b, F3a and F3b litters, although this was not generally concentration-related. These results are summarized in Table 9.

D.2.2. Two Generation Reproductive Study in Sprague-Dawley Rats: Miles 1988, 1990

This two generation reproductive toxicity study in Sprague-Dawley rats treated with metribuzin at 0, 30, 150, or 750 ppm in food has also been described previously, in Section C.3.2 (Miles 1988, 1990). Results reported that are relevant to assessment of female reproductive toxicity included mortality, food consumption, body weight, general behavior, estrous cycling, fertility, litter size, birth weight, pup growth, clinical pathology, and histopathology.

No deaths occurred in the parental animals. Food consumption was reduced (statistically significant) in males and females of the F0 and F1 generations at 750 ppm. At 750 ppm body weight and body weight gain were reduced (statistically significant) in males of the F0 and F1 generations at 750 ppm and females of the F0 and F1 generations during the premating phase and gestation (by 10-14% during gestation). At 150 ppm body weight was also reduced (statistically significant) during gestation in the females of the F1 generation at 150 ppm by 7-9%. Body weight gain during gestation was reduced (statistically significant) in the F0 generation at 30 ppm but not other concentrations, and in the F1 generation at 150 and 750 ppm. Body weight at the end of lactation was reduced (statistically significant) in the F1, but not the F0 generation at 750 ppm. The authors reported that no behavioral effects or signs of toxicity were attributable to metribuzin treatment, although some incidental findings were observed in all groups. Increased (statistically significant) serum gamma glutamyl transferase (GGT) was found in the F1 females at 150 and 750 ppm, possibly indicative of liver effects. A concentration related increase in the incidence of mild hepatocyte hypertrophy was also found in the F1 generation. These results are summarized in Table 11.

There was no effect on estrous cycling, time required for insemination, fertility (number pregnant/number inseminated), number of implantations, litter size, number of dead pups, or birth weight in the F0/F1 generation. No gross or histopathological effects on ovaries were observed. There was a reduction (statistically significant) in the number of implantations and litter size in the F1/F2 generation at 150 and 750 ppm. Historical control data provided by the authors for their laboratory for 20 studies from 1982 to 1988 displayed a range of 13.1 to 16.1 implantations per litter and litter sizes from 12.0 to 16.8 pups per litter. There was no effect on fertility, number of dead pups, or birth weight in the F1/F2 generation. No gross or histopathological effects on ovaries were observed. Pup weight and weight gain during lactation were reduced (statistically significant) in the F1 and F2 generations at 750 ppm. This effect was first observed on lactation day 14, after, the authors report, pups began consuming the test diets. Pup weight and weight gain during lactation were also reduced (statistically significant) in the F2 generation at 30 ppm, also after lactation day 14. These results are summarized in Table 12.

D.3.1. Female Dominant Lethal Study in NMRI Mice: Bayer 1974c

Female NMRI mice were treated with metribuzin by gavage in proestrus at 0 or 300 mg/kg with 37 or 38 animals per group (Bayer 1974c). The purity of the test compound was stated to be 99.5%. Females were then mated overnight at a ratio of two females per one male. Females were sacrificed on gd 14. Results reported included mortality, clinical observations, corpora lutea, resorptions, and live and dead implantations.

The dose chosen was based upon a smaller pilot study. In this study, female mice were treated by gavage at 200, 300, 400, or 500 mg/kg. Over the next five days, it was observed that 0/5, 0/5, 2/5, and 4/5 of the treated females died. Additional symptoms included mild drowsiness at all doses, ruffled coat at 400 and 500 mg/kg, and ataxia at 500 mg/kg.

In the main study, no maternal deaths were observed. Mild drowsiness was observed in the metribuzin treated females. No effect of metribuzin was observed on corpora lutea or implantations per female. Pre-implantation loss and post-implantation losses were lower (statistically significant) in metribuzin treated females than controls. These results are summarized in Table 13.

Table 13. Results of female dominant lethal study in NMRI mice (Bayer 1974c) (1)

Metribuzin dose (mg/kg)	0	300
No. females	37	38
No. fertilized (%)	32 (86.5%)	33 (86.8%)
Corpora lutea/female (2)	12.3	12.3
Implantations/female (2)	11.1	11.8
Pre-implantation loss/female (2) (%)	1.2 (9.9%)	0.4** (3.7%)
Living embryos/female (2)	10.3	11.4
Post-implantation loss/female (2) (%)	0.9 (8.4%)	0.5* (4.4%)

(1) Values are numbers, averages or percentages.
* Statistically significant difference from control at $p < 0.05$ by Wilcoxon test.
** Statistically significant difference from control at $p < 0.01$ by Wilcoxon test.
(2) Fertilized females only

D.4. Subchronic and chronic studies with female reproductive endpoints

While not designed as female reproductive toxicity studies, a number of subchronic and chronic studies have reported ovarian weights and histology findings following treatment with metribuzin. Species studied include mice (Mobay 1981), rats (Baychem 1972d, Bayer 1969b, 1970, 1974b, 1981a, Miles 1993), rabbits (Bayer 1989), and dogs (Bayer 1974d, IBTLI 1970). These results are summarized in Table 14.

There are some limitations to these studies. Many of these studies, especially the earlier ones, did not achieve systemically toxic doses, or did not provide statistical analyses of study findings. In most studies, ovary weight, and ovary gross and histopathology were not found to be affected by metribuzin treatment. In an inhalation study in Wistar rats, relative ovary weight was increased at the high concentration (720 mg/m^3). At this concentration, absolute ovary weight was not affected, and body weight was reduced. In one chronic feeding study in Beagle dogs, reduced ovary weight was observed at the high concentration (1,500 ppm) (Bayer 1974d). Increased mortality (3/4 females), reduced weight or weight gain, reduced food consumption, anemia, and kidney damage were also observed at this concentration. None of these effects were observed at lower concentrations.

Table 14. Subchronic and chronic studies reporting on ovarian weight and pathology. (1)

Reference	Study Description	Systemic toxicity	Ovarian weight and pathology
	Mouse		
Mobay 1981	CD1 mice treated at 0, 200, 800, or 3,200 ppm in food for 2 years. Equivalent to 0, 35, 139, and 567 mg/kg/d. N = 50/group.	**Effects:** Increased absolute and relative liver and kidney weights at 3,200 ppm. **No effect:** Mortality. Body weight. Other organ weights. Organ gross or histopathology.	**No effect:** Ovary weight. Ovary gross or histopathology.
	Rat		
Baychem 1972d	Sprague-Dawley rats treated with a 50% wettable powder formulation of metribuzin at 0 or 5 mg/L by inhalation for 1 hour/day, 5 days/week, 2 weeks. N = 5/group. Note: no statistical tests reported.	**Effects:** Reduced female body weight gain. **No effects:** Food consumption. Organ weights. Organ gross and histopathology.	**No effect:** Ovary weight. Ovary gross and histopathology.

(1) Effects noted if finding is statistically significant ($p \leq 0.05$) or noted as biologically significant by authors.

Table 14 continued. Subchronic and chronic studies reporting on ovarian weight and pathology. (1)

Reference	Study Description	Systemic toxicity	Ovarian weight and pathology Female reproductive toxicity
	Rat continued		
Bayer 1969b	Wistar rats treated at 0, 50, 150, 500, or 1,500 ppm in food for 3 months. N = 15/group (treated), 30/group (control).	**Effects:** Reduced body weight at 1,500 ppm. Increased absolute liver weight at 150, 500, 1,500 ppm, relative liver weight at all concentrations. Increased thyroid weight at 1,500 ppm. Increased relative kidney, spleen weight at 1,500 ppm. Histological evidence of thyroid hyperactivity. **No effect:** Mortality. Food consumption. Other organ weights. Gross organ pathology.	**Effects:** Increased relative ovary weight at 1,500 ppm. **No effect:** Ovary absolute weight. Ovary histopathology.
Bayer 1970	Wistar rats treated at 0, 10, 25, or 60 ppm in food for 3 months. N = 30 controls, others 15/group.	**Effect:** Increased absolute and relative liver weight at 60 ppm. **No effect:** Mortality. Food consumption. Body weight. Other organ weights. Organ gross or histopathology.	**No effect:** Ovary weight. Ovary gross or histopathology.

(1) Effects noted if finding is statistically significant ($p \leq 0.05$) or noted as biologically significant by authors.

Table 14 continued. Subchronic and chronic studies reporting on ovarian weight and pathology. (1)

Reference	Study Description	Systemic toxicity	Ovarian weight and pathology Female reproductive toxicity
	Rat continued		
Bayer 1974b	Wistar rats treated at 0, 25, 35, 100, or 300 ppm in food (equivalent to 0, 1.68, 2.28, 6.53, and 20.38 mg/kg/d) for up to 2 years. N = 80/group controls, 40/group others.	**Effects:** Reduced body weight at 300 ppm for parts of study, but not at end. **No effect:** Mortality. Body weight. Food consumption. Organ weights. clinical chemistry.	**No effect:** Ovary weight. Ovary histopathology.
Bayer 1981a	Rat subchronic. Study 1: Wistar rats treated at 0, 93, 219, or 720 mg/m^3 (analytical concentration) by inhalation for 6 hours/day, 5 days/week, for 3 weeks. N = 10/group.	**Effects:** Reduced body weight at all concentrations. Increased relative liver weight at 720 mg/m^3 Increased T4 at 93, 219 mg/m^3 **No effect:** Mortality. Other organ weights.	**Effects:** Ovary relative weight "slightly high" at 720 mg/m^3. **No effect:** Ovary absolute weight. Ovary histopathology.
	Study 2: Wistar rats treated as in Study 1 except at 0, 31 or 90 mg/m^3 (analytical concentration)	**Effects:** Reduced body weight at 90 mg/m^3 Increased T4 at 93 mg/m^3 **No effect:** Mortality. Organ weights. Organ histopathology.	**No effect:** Ovary weight. Ovary histopathology.

(1) Effects noted if finding is statistically significant ($p \leq 0.05$) or noted as biologically significant by authors.

Table 14 continued. Subchronic and chronic studies reporting on ovarian weight and pathology. (1)

Reference	Study Description	Systemic toxicity	Ovarian weight and pathology Female reproductive toxicity
	Rat continued		
Miles 1993	Fischer 344 rats treated at 0, 30, 300, or 900 ppm in food (equivalent to 0, 1.6, 17.7, and 53.6 mg/kg/d) for 1 or 2 years. N = 10/group (1 year group) N = 50/group (2 year group)	**Effects:** Reduced body weight at 300 and 900 ppm (multiple time points). Reduced body weight gain at 30, 300, 900 ppm (multiple time points). Increased thyroid weight at 300 and 900 ppm in 2 year group. Increased T4, reduced T3 at 30, 300, 900 ppm (multiple time points). **No effects:** Mortality. Food consumption. Other organ weights, other organ gross or histopathology.	**No effects:** Ovary weight. Ovary gross or histopathology.
	Rabbit		
Bayer 1989	New Zealand White rabbits treated at 0, 40, 200, or 1000 mg/kg dermally for 5 days/week for 3 weeks. Additional groups at 0 or 1000 mg/kg were treated similarly, and allowed to recover for 2 weeks. N = 5/group.	**No effects:** Mortality. Body weight. Organ weights. Organ gross or histopathology.	**No effects:** Ovary weight. Ovary gross or histopathology.

(1) Effects noted if finding is statistically significant ($p \leq 0.05$) or noted as biologically significant by authors.

Table 14 continued. Subchronic and chronic studies reporting on ovarian weight and pathology. (1)

Reference	Study Description	Systemic toxicity	Ovarian weight and pathology Female reproductive toxicity
	Dog		
Bayer 1974d	Dog chronic. Beagle dogs were treated at 0, 25, 100 or 1,500 ppm in food for 2 years. N = 4/group. Note: no statistical tests were reported.	**Effects:** Increased mortality (3/4), reduced weight or weight gain, reduced food consumption, anemia, kidney damage at 1,500 ppm.	**Effects:** Reduced absolute and relative ovary weight at 1,500 ppm. **No effects:** Ovary histopathology.
IBTLI 1970 (note: this study was considered to be valid by U.S. EPA)	Dog subchronic. Beagle dogs were treated at 0, 50, 150, or 500 ppm in food for 90 days. N = 4/group Note: no statistical tests were reported.	**No effect:** Mortality. Body weight. Food consumption. Organ weights. Organ gross and histopathology.	**No effect:** Ovary weight. Ovary gross and histopathology.

(1) Effects noted if finding is statistically significant ($p \leq 0.05$) or noted as biologically significant by authors.

D.5. Integrative evaluation – Female reproductive toxicity.

There have been two multigeneration reproductive studies in rats (Bayer 1974a, Miles 1988, 1990), and a female dominant lethal study in mice (Bayer 1974c). Additionally, there have been several chronic or subchronic studies reporting ovarian weights and pathology in mice (Mobay 1981), rats (Baychem 1972d, Bayer 1969b, 1970, 1974b, 1981a), rabbits (Bayer 1989), and dogs (Bayer 1974d, IBTLI 1970).

In the earlier rat reproductive study (Bayer 1974a), FB 30 rats were treated with metribuzin at 0, 35, 100, or 300 ppm in food for three generations, with two litters per generation. No parental toxicity was observed. In the F2 and F3 litters, birth weights were generally somewhat lower in the metribuzin treated groups than controls. However, these were not statistically significant and were concentration-related in only one litter out of four. No other indications of female reproductive toxicity were observed.

In the later rat reproductive study (Miles 1988, 1990), Sprague-Dawley rats were treated with metribuzin at 0, 30, 150, or 750 ppm in food for two generations, with one litter per generation. At 750 ppm, maternal weight was reduced (statistically significant) by 10-14% during gestation in both the F0 and F1 generations. At 150 ppm, maternal weight was reduced (statistically significant) by 7-9% during gestation in the F1 generation only. Serum gamma glutamyl transferase (GGT) was increased in the F1 females at 150 and 750 ppm, possibly indicative of liver effects. Also, a concentration related increase in mild liver hypertrophy was observed in the F1 generation. There was no effect on fertility, number of dead pups, or birth weight in either generation. In the F1/F2 generation only, at 150 and 750 ppm, there were reduced (usually statistically significant) numbers of implantations and litter size. Data provided by the authors indicated that, although reduced from concurrent controls, the numbers of implantations and litter sizes were within the range of historical controls in that laboratory.

Female NMRI mice were treated with metribuzin by gavage in proestrus at 0 or 300 mg/kg (Bayer 1974c). They were then mated, and sacrificed on gd 14. Mild drowsiness was observed. No maternal mortality was observed. No adverse effects on corpora lutea, implantations, pre-implantation losses, or post-implantation losses was observed.

Numerous chronic and subchronic studies in mice, rats, rabbits, and dogs found no effect on ovary weight, gross pathology or histopathology. An inhalation study in rats found increased relative, but not absolute ovary weights, in the presence of reduced body weight, at the high concentration (Bayer 1981a). One chronic study in Beagle dogs treated with metribuzin in food found reduced ovary weight at 1,500 ppm (the high concentration) (Bayer 1974d). Severe systemic toxicity also occurred at this concentration, including mortality (3/4 females), reduced weight or weight gain, reduced food consumption, anemia, and kidney damage.

Thus, from the studies reviewed in this section, there emerge two possible indications of female reproductive toxicity. A two-generation rat study found reduced implantations and litter size at the middle and high concentrations in the second generation. Maternal

weight was reduced at these concentrations. The number of implantations and litter sizes was within historical control ranges for that laboratory. A chronic dog study found reduced ovary weight at a severely maternally toxic concentration. However, numerous other studies in multiple species did not find effects on ovarian weight or abnormal ovarian pathology subsequent to metribuzin exposure. Such studies are not specifically designed to assess reproductive effects, however.

Treatment with atrazine has been found to produce alterations of the estrous cycle in specialized studies in rats (reviewed in OEHHA 1999). A two-generation reproduction study with atrazine did not report results for estrous cycling (Ciba-Geigy 1987). No alterations of the estrous cycle were found in a two-generation reproductive study in rats with metribuzin, although this endpoint was not studied in detail.

E. MALE REPRODUCTIVE TOXICITY

E.1. Overview

There are two rat reproductive studies in which both males and females were treated with metribuzin (Bayer 1974a, Miles 1988, 1990). Results of these studies relevant to male reproductive toxicity are reviewed in this section. Also, there are two male dominant lethal studies in mice (Bayer 1975, 1976). Additionally, there are several subchronic and chronic studies which contain information on male reproductive endpoints, specifically testes weight and gross and histopathology. No studies of human exposure and male reproductive effects were located.

E.2 Animal reproductive toxicity studies

E.2.1. Three Generation Reproductive Study in FB 30 rats: Bayer 1974a

The study design and many of the results for this study have previously been presented in Section C.3.1. Briefly, male and female FB-30 rats were treated with metribuzin for three generations at 0, 35, 100, or 300 ppm in food (Bayer 1974a).

As previously noted, several animals in each generation died or were sacrificed for health reasons, and there were no statistically significant or concentration-related differences in body weight between metribuzin treated and control groups were found in any generation. No alterations of behavior were observed.

Fertility (number pregnant/number mated) was reduced in the 300 ppm group compared to controls in the second F0 mating (78.9% compared to 90%). There was no reduction in fertility in the metribuzin treated groups compared to control in the other five matings. In both F2b matings, the fertility of the controls was low compared to other matings and groups. Litter size was not reduced (statistically significant or concentration-related) in metribuzin treated groups compared to controls in any mating. These results are included in Table 9.

E.2.2. Two Generation Reproductive Study in Sprague-Dawley Rats: Miles 1988, 1990

As described earlier in Section C.3.2, Sprague-Dawley rats were treated for two generations with metribuzin at 0, 30, 150, or 750 ppm in food (Miles 1988, 1990). Further information on study design is provided in Section C.3.2. Doses to male rats estimated by RCHAS staff from food consumption and body weight data are provided in Table 15.

There was no increase in mortality in male rats treated with metribuzin. Food consumption and body weight were reduced (statistically significant) in both generations. These results are summarized in Table 16.

There was no effect on frequency of successful copulation, median number of days to inseminate, fertility (number pregnant/number inseminated), number of implantations, litter size, or number of dead pups, in the F0/F1 generation. No effects on testes gross or histopathology were observed. As discussed previously, there was a reduction (statistically significant) in the number of implantations and litter size in the F1/F2 generation at 150 and 750 ppm. Historical control data provided by the authors for their laboratory for 20 studies from 1982 to 1988 displayed a range of 13.1 to 16.1 implantations per litter and litter sizes from 12.0 to 16.8 pups per litter. There was no effect on frequency of successful copulation, median number of days to inseminate, fertility, or number of dead pups in the F1/F2 generation. No effects on testes gross or histopathology were observed. These results are summarized in Table 17.

Table 15. Doses in two generation Sprague-Dawley rat reproductive study (Miles 1988, 1990). [1]

Metribuzin concentration in food (ppm)	0	30	150	750
Male dose: F0 premating (mg/kg/d)	0	2.01 ± 0.45	9.9 ± 2.2	47.2 ± 10.9
Male dose: F1 premating (mg/kg/d)	0	2.33 ± 0.51	11.7 ± 2.6	59.1 ± 11.8

(1) Doses estimated by RCHAS staff from food intake and body weight data provided in the report. Data are mean ± SD

Table 16. Parental data in two generation Sprague-Dawley rat reproductive study (Miles 1988, 1990). [1]

Metribuzin concentration in food (ppm)		0	30	150	750
Male body weights: F0 (g)	Day 0	217.8 ± 2.2	216.1 ± 2.0	215.7 ± 1.9	214.9 ± 2.1
	Day 72 [2]	475.8 ± 7.8	456.9 ± 7.6	473.5 ± 6.5	438.2 ± 6.3**
Male body weights: F1 (g)	Day 0	194.5 ± 3.3	187.1 ± 4.2	181.2 ± 6.9	176.8 ± 7.5
	Day 70 [2]	487.7 ± 8.6	466.0 ± 8.3	477.9 ± 9.8	440.1 ± 7.5**

(1) Data are mean ± SE

* Statistically significant difference from control by Dunnett's test at p = 0.05.

** Statistically significant difference from control by Dunnett's test at p = 0.01.

(2) End of premating phase.

Table 17. Reproductive data in two generation Sprague-Dawley rat reproductive study (Miles 1988, 1990). (1)

Metribuzin concentration in food (ppm)		0	30	150	750
Copulation index (no. copulated/no cohabited) (%)	F0	100	100	96.7	100
	F1	100	100	100	100
No. days to inseminate (median)	F0	2	2	2	2
	F1	3	3	3	2
Fertility (no. pregnant/no. inseminated) (%)	F0	96.7%	96.7%	93.1%	100%
	F1	83.3%	96.7%	96.7%	93.3%
Number of implantations	F0/F1	13.55 ± 2.18	13.14 ± 2.75	13.44 ± 3.02	13.17 ± 1.62
	F1/F2	15.00 ± 1.68	14.03 ± 2.06	13.03 ± 3.22**	13.54 ± 2.03
Litter size	F0/F1	12.79 ± 2.35	12.34 ± 2.73	12.19 ± 3.34	12.33 ± 1.88
	F1/F2	14.12 ± 1.62	13.31 ± 2.21	11.97 ± 3.40**	12.32 ± 2.47*
Dead pups/litter	F0/F1	0.6	0.7	0.9	1.0
	F1/F2	0.4	0.6	0.4	0.2

(1) Data are percentages, means or mean ± SD, except median as indicated.
*Statistically significant difference from controls using Dunnett's test at $p \leq 0.05$.
**Statistically significant difference from controls using Dunnett's test at $p \leq 0.01$.

E.3. Male Dominant Lethal Studies

E.3.1. Male Dominant Lethal Study in NMRI Mice: Bayer 1975

Male NMRI mice were treated with metribuzin by gavage at 0 or 300 mg/kg with 20 animals per group (Bayer 1975). The purity of the test compound was stated to be 99.5%. After administration, males were caged with three untreated females each for one week, after which the females were removed. This was repeated for eight weeks. Females were sacrificed on gd 14. Results reported included mortality, clinical observations, corpora lutea, resorptions, and live and dead implantations.

The dose chosen was based upon a smaller pilot study. In this study, male mice were treated by gavage at 200, 300, 400, or 500 mg/kg. Over the next five days, it was observed that 0/5, 0/5, 2/5, and 4/5 of the treated males died. Additional symptoms included somnolence at all doses, ruffled coat at 400 mg/kg, and ataxia at 500 mg/kg. In the main study, no male deaths were observed. Light somnolence was observed in the metribuzin treated males.

No statistically significant effect of metribuzin was observed on fertility, pre-implantation loss, or living or dead implantations. These results are summarized in Table 18. The authors concluded that no dominant lethal effects were observed as a result of metribuzin treatment.

Table 18. Results of male dominant lethal study in NMRI mice (Bayer 1975) [1]

Metribuzin dose (mg/kg)		0	300
No. males		20	20
No. females per mating period		60	60
No. Females fertilized (%)	week 1	48 (80.0%)	47 (78.3%)
	week 2	49 (81.7%)	47 (78.3%)
	week 3	46 (76.7%)	49 (81.7%)
	week 4	50 (83.3%)	52 (86.7%)
	week 5	45 (75%)	48 (80.0%)
	week 6	42 (70.0%)	48 (80.0%)
	week 7	52 (86.7%)	42 (70.0%)
	week 8	43 (71.7%)	46 (76.7%)
	Total	375 (78.1%)	379 (79.0%)
Pre-implantation loss/female [2]	week 1	0.35	0.53
	week 2	0.59	0.43
	week 3	0.37	0.53
	week 4	0.40	0.17
	week 5	0.33	0.27
	week 6	0.33	0.31
	week 7	0.23	0.29
	week 8	0.40	0.20
	Average	0.38	0.34

(1) Values are numbers, averages or percentages.
No statistically significant differences were found by 2-factor ANOVA (fertilization) or Kolmogorov-Smirnov test (non-parametric: pre-implantation loss)

(2) Fertilized females only

Table 18 (continued). Results of male dominant lethal study in NMRI mice (Bayer 1975) [1]

Metribuzin dose (mg/kg)		0	300
No. males		20	20
No. females per mating period		60	60
Living implantations/ female [2]	week 1	9.8	9.3
	week 2	9.9	9.9
	week 3	10.3	9.8
	week 4	10.5	10.4
	week 5	10.2	10.2
	week 6	10.2	10.4
	week 7	10.1	9.9
	week 8	10.1	10.4
	Average	10.1	10.0
Dead implantations/ female [2]	week 1	0.38	0.38
	week 2	0.37	0.66
	week 3	0.17	0.47
	week 4	0.40	0.73
	week 5	0.38	0.38
	week 6	0.48	0.58
	week 7	0.56	0.57
	week 8	0.35	0.50
	Average	0.39	0.54

(1) Values are averages. The authors did not report indices of variation (e.g. standard deviation).
No statistically significant differences were found by Kolmogorov-Smirnov test (non-parametric).
(2) Fertilized females only

E.3.2. Male Dominant Lethal Study in NMRI Mice: Bayer 1976

Male NMRI mice were treated with metribuzin by gavage at 0 or 300 mg/kg with 50 animals per group (Bayer 1976). The purity of the test compound was stated to be 99.5%. After administration, males were caged with one untreated female each for four days, after which the females were removed. This was repeated for four additional mating periods of four days each. Females were sacrificed on gd 14. Results reported included mortality, clinical observations, corpora lutea, resorptions, and live and dead implantations.

Two metribuzin treated males died, both from pneumonitis. The first died during the first mating period, and the second died during the second mating period. Mild drowsiness was observed in the metribuzin treated males.

The overall percentage (results of all mating intervals combined) of females pregnant after cohabitation with the metribuzin treated males was higher than for controls (statistically significant). There were no overall effects on total, living, or dead implantations. The number of living implantations was decreased (statistically significant) in the third mating period and increased (statistically significant) in the fourth mating period in the metribuzin treated group compared to controls. The authors attributed this to random variations. Dead implantations were increased (statistically significant) in the metribuzin treated group for the fourth mating interval. The authors noted that number of dead implantations per litter in the control group was particularly low and that there were more living implantations in the metribuzin treated group than the control group. These results are summarized in Table 19.

Table 19. Results of male dominant lethal study in NMRI mice (Bayer 1976) [1]

Metribuzin dose (mg/kg)	No. males (initial)	0	300
		50	50
No. Females fertilized/ No. females mated [2] (%)	mating 1 (days 1-4)	45/50 (90.0%)	45/49 (91.8%)
	mating 2 (days 5-8)	43/50 (86%)	45/48 (93.8%)
	mating 3 (days 9-12)	38/50 (76.0%)	41/48 (85.4%)
	mating 4 (days 13-16)	41/50 (82.0%)	37/48 (77.1%)
	mating 5 (days 17-20)	36/50 (72.0%)	42/48 (87.5%)
	Total	203/250 (81.2%)	210/241* (87.1%)
Total implantations/ female [2]	mating 1 (days 1-4)	12.3	12.3
	mating 2 (days 5-8)	11.9	11.7
	mating 3 (days 9-12)	12.8	11.9*
	mating 4 (days 13-16)	10.8	12.2*
	mating 5 (days 17-20)	11.9	11.3
	Average	12.0	11.9
Living implantations/ female [2]	mating 1 (days 1-4)	11.6	11.7
	mating 2 (days 5-8)	11.4	11.3
	mating 3 (days 9-12)	12.2	11.5
	mating 4 (days 13-16)	10.3	11.4
	mating 5 (days 17-20)	11.4	10.9
	Average	11.4	11.4
Dead implantations/ female [2]	mating 1 (days 1-4)	0.76	0.62
	mating 2 (days 5-8)	0.56	0.53
	mating 3 (days 9-12)	0.63	0.44
	mating 4 (days 13-16)	0.49	0.86*
	mating 5 (days 17-20)	0.64	0.48
	Average	0.62	0.58

(1) Values are numbers, averages or percentages. The authors did not report indices of variation (e.g. standard deviation).
* Statistically significant difference at $p < 0.05$ (methods not clear: may include 2-way ANOVA, Tukey, and/or Kolmogorov-Smirnov [non-parametric] tests).
(2) Each mating period was four days. Fertilized females only

E.4. Subchronic and chronic studies with male reproductive endpoints

A number of subchronic and chronic studies have provided data on testicular weight and pathology. Study designs are indicated in Table 14. Species studied include mice (Mobay 1981), rats (Baychem 1972d, Bayer 1969b, 1970, 1974b, 1981a, Miles 1993, University of Chicago 1969), rabbits (Bayer 1989), and dogs (Bayer 1974d, IBTLI 1970). There are some limitations to these studies. Many of these studies, especially the earlier ones, did not achieve systemically toxic doses, or did not report statistical results.

In most studies, testes weight, and testes gross and histopathology were not found to be affected by metribuzin treatment. Relative, but not absolute, testes weight was increased in two experiments conducted via the inhalation route in Wistar rats (Bayer 1981a). In the first experiment, groups of 10 animals were treated via inhalation at 0, 93, 219 or 720 mg/m^3 for 6 hours per day, five days per week for 3 weeks. The increase in relative testes weight occurred in the presence of reduced body weight. Increased relative liver weight in the highest dose group and increased T4 in the low and mid dose groups were also observed. The second study utilized the same study design as the first, but at lower doses of 31 and 90 mg/m^3, as well as a control group. Increased relative testes weight was observed at both dose levels. The authors noted reduced body, heart, liver, spleen, and kidney weight in the high dose group and increased T4 in high and low dose groups. These results are given in greater detail in Tables 20 and 21. One study in Beagle dogs treated with metribuzin in food found reduced absolute testes weight and "immature" testes and prostate at the high concentration (Bayer 1974d). Severe male toxicity also occurred at this concentration, including mortality (3/4 males), reduced weight or weight gain, reduced food consumption, anemia, increased liver weight, and kidney damage.

Table 20 Results of first rat inhalation study with metribuzin (Bayer 1981a) (1)

Metribuzin concentration (mg/m3)		0	93	219	720
Body weight (g)	Week 0	198	200	197	199
	Week 3	240	225	229	225
Testes weight week 3	Absolute (mg)	2904	2936	3020	2979
	Relative (mg/100g)	1213	1305	1320	1325

(1) Data are means. No indices of variation (e.g. standard deviation) were provided by the authors.

Table 21 Results of second rat inhalation study with metribuzin (Bayer 1981a) (1)

Metribuzin concentration (mg/m3)		0	31	90
Body weight (g)	Week 0	206	206	203
	Week 3	240	237	223
Testes weight week 3	Absolute (mg)	2787	2939	2928
	Relative (mg/100g)	1162	1240	1317

(1) Data are means. No indices of variation (e.g. standard deviation) were provided by the authors.

E.6. Integrative evaluation – Male reproductive toxicity.

There have been two multigeneration reproductive studies in rats (Bayer 1974a, Miles 1988, 1990), and two male dominant lethal studies in mice (Bayer 1975, 1976). Additionally, there have been several chronic or subchronic studies in mice (Mobay 1981), rats (Baychem 1972d, Bayer 1969b, 1970, 1974b, 1981a, University of Chicago 1969), rabbits (Bayer 1989), and dogs (Bayer 1974d, IBTLI 1970).

In the earlier rat reproductive study (Bayer 1974a), FB 30 rats were treated with metribuzin at 0, 35, 100, or 300 ppm in food for three generations, with two litters per generation. No parental toxicity was observed. No indications of male reproductive toxicity were observed.

In the later rat reproductive study (Miles 1988, 1990), Sprague-Dawley rats were treated with metribuzin at 0, 30, 150, or 750 ppm in food for two generations, with one litter per generation. Body weight in males was reduced (statistically significant) at 750 ppm. At the beginning of mating, this reduction was 8% in the F0 and 10% in the F1 generations. Also, a concentration related increase in mild liver hypertrophy was observed in the F1 generation. There was no effect on fertility or number of dead pups in either generation. In the F1/F2 generation only, at 150 and 750 ppm, there were reduced (usually statistically significant) numbers of implantations and litter size. The authors of the report asserted that the results were within historical control ranges. Data provided by the authors indicated that, although reduced from concurrent controls, the numbers of implantations and litter sizes were within the range of historical controls in that laboratory.

There have been two male dominant lethal studies in mice. All involved single treatment of males with metribuzin, followed by multiple matings with untreated females. The first used gavage of NMRI mice at 0 or 300 mg/kg (Bayer 1975) followed by 8 mating periods of one week each. The second used the same strain and dose, but five mating periods of four days each (Bayer 1976). In the first study, there were no statistically significant differences in fertility, pre-implantation loss, living implants, or dead implants. In the second study, total implantations were reduced (statistically significant) for mating period three (days 9-12) and increased (statistically significant) for mating period four (days 13-16). Dead implantations were reduced for mating period three (not statistically significant) and were increased (statistically significant) for mating period four. There were no statistically significant differences in living implantations.

Numerous chronic and subchronic studies in mice, rats, rabbits, and dogs found no effect on testes weight, gross pathology or histopathology. Relative, but not absolute, testes weight was increased in two subchronic rat inhalation studies (Bayer 1981a). This occurred in the presence of reduced body weight. A chronic study in Beagle dogs treated with metribuzin in food found reduced absolute testes weight and "immature" testes and prostate at the high concentration (1,500 ppm) (Bayer 1974d). Severe male toxicity also occurred at this concentration, including mortality (3/4 males), reduced weight or weight gain, reduced food consumption, anemia, increased liver weight, and kidney damage.

Thus, from the studies reviewed in this section, there emerge three possible indications of male reproductive toxicity. A two-generation rat study found reduced implantations and litter size at the middle and high concentrations in the second generation. These were, however, within the historical control range for that laboratory as reported by the study authors. Additionally, interpretation of these findings is complicated by the exposure of both males and females in this study. As discussed in the Female Reproductive Toxicity section, maternal weights were reduced. One dominant lethal study in mice found no indication of effects on pre-implantation or post-implantation losses. Another dominant lethal study in mice found sporadic increases and reductions in total and dead implantations, but no effect on living implantations.

A pair of rat subchronic studies found increased relative testes weight. However, body weight was reduced, and absolute testes weight was not affected. A Beagle dog chronic study found reduced absolute testes weight and "immature" testes and prostate at the high concentration. Interpretation of this observation is complicated by increased mortality (3/4 males) and other severe systemic toxic effects at the same concentration.

F. Summary of Developmental and Reproductive Toxicity

F.1. Developmental Toxicity

Data on the possible developmental toxicity of metribuzin come from developmental studies in Sprague-Dawley rats (Miles 1986), FB 30 rats (Bayer 1972), New Zealand White rabbits (Miles 1989, 1991), and American Dutch rabbits (MRI 1981). These data are supplemented with results from two multigeneration reproductive studies in Sprague-Dawley rats (Miles 1988, 1990) and FB 30 rats (Bayer 1974a).

Indications of developmental toxicity were primarily found in three of the studies reviewed. In the developmental study in Sprague-Dawley rats, reduced fetal weight was observed at all doses tested, and delayed ossification and rib anomalies at the high dose. Maternal toxicity in the form of reduced food consumption, body weight, and body weight gain was also observed at all doses. In the reproductive study in Sprague-Dawley rats, reduced implantations and litter size were observed at the middle and high concentrations in the second generation only. Reduced body weight was also observed in parental females at these concentrations. In the developmental study in New Zealand White rabbits, increased resorptions, reduced fetal weight, and increased incompletely ossified sternebrae were observed at the high dose, although none of these were statistically significant. Maternal weight loss during treatment and increased abortions were also observed at this dose.

F.2 Female Reproductive Toxicity

Data on the possible reproductive toxicity of metribuzin come primarily from multigeneration reproductive studies in Sprague-Dawley rats (Miles 1988, 1990) and in FB 30 rats (Bayer 1974a). Both sexes were treated in both studies. These data are supplemented with a female dominant lethal study in mice (Bayer 1974c). Additionally, there have been several subchronic or chronic studies in mice (Mobay 1981), rats (Baychem 1972d, Bayer 1969b, 1970, 1974b, 1981a), rabbits (Bayer 1989), and dogs (Bayer 1974d, IBTLI 1970) which reported on ovarian weight and pathology.

No dominant lethal or other reproductive effects were observed in the female mouse dominant lethal study.

Numerous subchronic and chronic studies found no effect on ovary weight, gross pathology or histopathology. A chronic study in dogs found reduced ovary weight at the high concentration (Bayer 1974d). Severe systemic toxicity was also found at this concentration, including 75% mortality.

The study with the strongest indication of female reproductive toxicity was the reproductive study in Sprague-Dawley rats (Miles 1988, 1990). In this study, reduced implantations and litter size were observed at the middle and high concentrations in the second generation. Reduced female weight was also observed at these concentrations.

F.3. Male Reproductive Toxicity

The two multigeneration reproductive studies in Sprague-Dawley rats (Miles 1988, 1990) and FB 30 rats (Bayer 1974a) also provide data on the possible male reproductive toxicity of metribuzin. These data are supplemented with two male dominant lethal studies in mice (Bayer 1975, 1976). Several chronic or subchronic studies in mice (Mobay 1981), rats (Baychem 1972d, Bayer 1969b, 1970, 1974b, 1981a, University of Chicago 1969), rabbits (Bayer 1989), and dogs (Bayer 1974d, IBTLI 1970) reported on testicular weight and pathology.

In general, the chronic and subchronic studies found no effect on testes weight, gross pathology or histopathology. Relative, but not absolute, testes weight was increased in two subchronic rat inhalation studies (Bayer 1981a). This occurred in the presence of reduced body weight. A chronic study in dogs treated with metribuzin in food found reduced absolute testes weight and "immature" testes and prostate at the high concentration (Bayer 1974d). This occurred in the presence of severe systemic toxicity, including 75% mortality.

The strongest indication of male reproductive toxicity was found in the reproductive study in Sprague-Dawley rats (Miles 1988, 1990):reduced implantations and litter size were found at the middle and high concentrations in the second generation. Interpretation of this study is complicated by the exposure of both males and females, and reduced body weight in females at the same concentrations. No consistent dominant lethal or other male reproductive effects were found in two dominant lethal studies in mice.

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