TOXICOLOGICAL REVIEW

OF

MIREX

(CAS No. 2385-85-5)

In Support of Summary Information on the Integrated Risk Information System (IRIS)

April 2003

NOTICE

This document is an external review draft. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency position on this chemical. It is being circulated for review of its technical accuracy and science policy implications.

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This report is an external draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to mirex. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of mirex.

In Section 6, U.S. EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to U.S. EPA's Risk Information Hotline at 513-569-7254.

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This document and summary information on IRIS have received peer review both by U.S. EPA scientists and by independent scientists external to U.S. EPA. Subsequent to external review and incorporation of comments, this assessment has undergone an Agencywide review process whereby the IRIS Program Manager has achieved a consensus approval among the Office of Research and Development; Office of Air and Radiation; Office of Prevention, Pesticides, and Toxic Substances; Office of Solid Waste and Emergency Response; Office of Water; Office of Policy, Planning, and Evaluation; and the Regional Offices.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in the Appendix.

1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries related to human health in the U.S. Environmental Protection Agency (U.S. EPA) Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC), and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC is analogous to the oral RfD. The inhalation RfC considers toxic effects for the respiratory system (portal of entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates for linear dose-response model are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for mirex has followed the general guidelines for risk assessment as set forth by the National Research Council (NRC, 1983). U.S. EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b); *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c); *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991); *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a); Draft Revised *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1999a); *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b); Science Policy Handbook: Risk Characterization

(U.S. EPA, 2000a); Benchmark Dose Technical Guidance Document (U.S. EPA, 2000b); *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 2000c) and a memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategies employed for these compounds were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document. The relevant literature was reviewed through 09/03.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Mirex (1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-metheno-1H-cyclobutal[cd]pentalene) is a fully chlorinated organic compound, based on two linked five-member carbon rings. For mirex, the IUPAC designation is perchloropentacyclo-($5.2.1.0^{2,6}.0^{3,9}.0^{5,8}$)decane. Common synonyms include hexachloropentadiene dimer, hexachlorocyclopentadiene dimer, dodecachloropentacyclodecane, perchloropentacyclodecane, Dechlorane®, and Ferriamicide®.

Mirex is usually seen as a snow-white, odorless, free-flowing, crytalline solid. Mirex is extremely stable; it does not burn easily or react readily with acids, bases, chlorine, or ozone (Weinberg Consulting Group, 1992). Ultraviolet radiation causes the slow decomposition of mirex into photomirex (8-monohydromirex); 10-monohydromirex; 5,10-dihydromirex; 2,8-dihydromirex; and chlorodecone (kepone) (WHO, 1984; Carlson et al., 1976). The melting point for mirex is 483-487ºC, and decomposition occurs between 485 and 525ºC (Weinberg Consulting Group, 1992; ATSDR, 1995). Combustion products of mirex include carbon dioxide, carbon monoxide, hydrogen chloride, chlorine, carbon tetrachloride, phosgene, and hexachlorobenzene (Weinberg Consulting Group, 1992). Some relevant physical and chemical properties of Mirex are listed below (compiled by ATSDR, 1995):

CASRN:	2385-85-5
Empirical formula:	$C_{10}Cl_{12}$
Molecular weight:	545.59 g/mol
Vapor pressure:	3×10^{-7} mm Hg at 25ºC
Water solubility:	insoluble, practically insoluble, 0.60 mg/L, 0.2 mg/L at 24ºC
Organic solubility:	dioxane (15.3%), xylene (14.3%), benzene (12.2%), carbon tetrachloride (7.2%), methyl ethyl ketone (5.6%)
Log K_{ow}:	5.28
Log K_{oc}:	3.763

Mirex is considered by the U.S. EPA (2001) to be a persistent, bioaccumulative, and toxic chemical. Mirex is not known to occur naturally and was first synthesized in the mid-1940s. It was first marketed commercially in the U.S. in the 1950s, and has been used as a fire-retardant in thermoplastics, paper, paint, rubber, adhesive, textiles and electrical equipment (Weinberg Consulting Group, 1992). Use of mirex as an insecticide to control fire ants in the Southeastern United States began in 1962, and all uses of mirex as pesticide were cancelled in 1978 (U.S. EPA, 2001).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION

No data were located regarding absorption efficiency of mirex in humans after inhalation, oral, or dermal exposure, although mirex has been detected in human adipose tissue (Kutz et al., 1974, 1985). Ingestion appears to be the most important route of absorption (ATSDR, 1995).

3.1.1. Gastrointestinal Absorption

Mirex is partially absorbed following oral exposure (reviewed ATSDR, 1995). Byrd et al. (1982) compared differences in excretion after oral and intravenous dosing to determine that 70.8% of orally administered mirex was absorbed by rats. Other studies have assumed that fecal excretion of radiolabelled mirex within 24-48 hours of ingestion represents the portion of mirex not absorbed by the alimentary tract (ATSDR, 1995; Gibson et al., 1972; Mehendale et al., 1972; Chambers et al., 1982). Absorption efficiencies of 45% (Mehendale et al., 1972), 82-85% (Gibson et al., 1972), 75% (Ivie et al., 1974; Wiener et al., 1976), 69-75% (Chambers et al., 1982) or 90% (Pittman et al., 1976) have been calculated.

Byrd et al. (1982) collected data enabling gastrointestinal absorption to be calculated by comparing fecal excretion following oral and intravenous dosing. A dose of 10 mg/kg [^{14}C]mirex in corn oil was administered to four female albino rats. In the first 48 hours, 32.9% (range 15.7-42%) of the administered radiolabel was excreted in feces. Of the total administered dose, 36.1% (range 18.1-44.6%) was excreted in feces and less than 0.1% was excreted in urine within 21 days. In female albino rats dosed intravenously with 10 mg/kg radiolabelled mirex, 7% was eliminated in feces within 21 days. The difference in excretion, 29.2%, is attributed to the transport of mirex unabsorbed through the alimentary tract; therefore, 70.8% was absorbed gastrointestinally.

A 6 mg/kg dose of [^{14}C]mirex in corn oil was administered to five male CD-1 rats by gavage (Mehendale et al., 1972). In the first 48 hours, 55% of administered radiolabel was excreted in feces, indicating that 45% was absorbed gastrointestinally. The authors indicate that similar results were observed after administration of a 1.5 mg/kg dose of mirex.

A single 0.2 mg/kg dose of [^{14}C]-mirex was administered by gavage to an unspecified number of mature female Sprague Dawley rats (Gibson et al., 1972). Of the total dose eliminated in feces, 6.74% was eliminated in the first day, 8.52% in the second day, 1.28% in the third day and 0.82% in the fourth day. It therefore appears that mirex was absorbed within 48-72 hours and that gastrointestinal absorption was less than 85%. Absorption was decreased slightly by pre-feeding with 250 ppm (20 mg/kg [1]) unlabeled dietary mirex. In pre-fed animals, total

[1]Gibson et al. (1972) administered 250 ppm mirex to mature female CD rats in the diet for 7 days. Assuming a body weight of 0.35 kg and a food consumption rate of 0.028 kg/day (U.S. EPA, 1988), a dose of 20 mg/kg-day was calculated. Sample calculation: 250 ppm/1E-6 x 0.028 kg food/day x 1E6 mg/kg / 0.35 kg body weight = 20 mg/kg.

fecal excretion in for 7 days was 24.96% compared to 18.4% in non-prefed animals, a difference of 6.56%.

Groups of male or female Sprague Dawley rats were administered a single oral dose of approximately 0.15 to 0.3 mg/kg [^{14}C]-mirex in soybean oil (Chambers and Yarbrough, 1979; Chambers et al., 1982). Fecal excretion during the first two days of treatment was 28.85% for males and 21.2% for females; fecal excretion during the next 12 days was 2.6% for males and 3.3% for females. Gastrointestinal absorption was therefore determined to be approximately 71-79%.

Groups of 12 female Sprague Dawley rats and groups of 50 Japanese quail were fed diets containing 0, 0.3, 3.0 or 30 ppm [^{14}C]mirex for various periods of time (Ivie et al., 1974). Percentages of elimination were reportedly similar among different dose levels. Rats eliminated 24.2% of mirex within 24 hours and an additional 5.9% during the next 6 days, indicating that approximately 75.2% was absorbed gastrointestinally. Quail eliminated 15.8% within 24 hours and an additional 5.0% during the next 6 days, indicating that approximately 84.2% was absorbed gastrointestinally.

One female rhesus monkey received a single 1 mg/kg dose of [^{14}C]mirex in ethanol orally, in a gelatin capsule (Wiener et al., 1976). Approximately 25% of the radiocarbon dose appeared in the feces in the first 48 hours, indicating that gastrointestinal absorption was approximately 75%.

Doses of 0, 0.25 or 1.0 mg/kg of mirex were administered orally to male and female rhesus monkeys for 510 days (Pittman et al., 1976). At an unspecified time near study termination, a 24-hour fecal sample was analyzed. Recovery was approximately 10% suggesting that gastrointestinal absorption was 90%.

Rozman et al. (1984) administered [^{14}C]mirex to 3 lactating dairy goats intraruminally. Fecal elimination was not significant on day 1, and on days 2, 3, and 4 was 13.89%, 6.41% and 4.87% of the total administered dose. Total fecal elimination over 14 days was 44.5%. Peak elimination in milk was on day 3. Therefore, gastrointestinal absorption in sheep was approximately 80%.

Absorption of mirex following milk ingestion is a source of mirex exposure. After giving birth, unspecified numbers of CD rat dams received 0, 1, or 10 mg/kg [^{14}C]mirex on postnatal days 2-5; single pups were sacrificed on postnatal days 3, 5, 7, 9, 12, 15 and 17. Mirex was detected in maternal milk, milk samples taken from pups' stomachs, in pup brain and liver tissue (Kavlock et al., 1980).

3.1.2. Other Routes of Absorption

One study was identified demonstrating absorption of mirex by inhalation. Research cigarettes, 85 mm, were impregnated with 100 ppm [^{14}C]mirex in acetone; lightly anesthetized

female Sprague Dawley rats were exposed to eight 5-mL puff volumes of mainstream smoke at 15-second intervals (Atallah and Dorough, 1975; Dorough and Atallah, 1975). Within 4 minutes, 47% of the mirex was exhaled, 36% remained in the lungs, and 14.4% was present in other tissues of the body.

Mirex administered to pregnant or nursing animals has been detected in their offspring, indicating that neonates may absorb mirex through the placenta (Kavlock et al., 1980; Smrek et al., 1978). Kavlock et al. (1980) administered 5 mg/kg [^{14}C]mirex in corn oil by gavage to unspecified numbers of pregnant CD rats on days 15, 18 or 20 of gestation; animals were sacrificed 4, 24 and 48 hours after dosing (4 hours and parturition in the day 20 group). Mirex was detected in maternal plasma, liver, and kidneys and in fetal placenta, brain, heart, and kidneys at 4 hours.

Smrek et al. (1978) conducted pharmacokinetic experiments in groups of 5 male and 5 female goats. Authors report that mirex was detected in young fetuses harvested from mirex-treated mothers, and conclude that mirex was absorbed transplacentally.

Although no data was found regarding dermal exposure of mirex leading to systemic absorption, one laboratory has reported that mirex promotes formation of skin tumors in female CD-1-mice initiated with 200 nm 7,12-dimethylbenz(a)anthracene (Moser et al., 1992, 1993; Meyer et al., 1993, 1994; Kim and Smart, 1995; Kim et al., 1997). These observations appear to indicate that mirex is locally absorbed following dermal exposure.

3.2. DISTRIBUTION

Mirex is highly stable and rapidly partitions into fat (ATSDR, 1995). Mirex has been detected in human adipose tissues (Kutz et al., 1974, 1985; Burse et al., 1989). Testing of adipose tissues found mirex concentrations of 0.16 to 5.94 ppm in men and 0.3 to 1.13 ppm in women who lived in areas of high insecticidal usage of mirex (Kutz et al., 1974). In residents of Memphis, Tennessee, living near a waste site, adipose mirex levels were as high as 3.72 ppm (Burse et al., 1989); serum levels were predictive of adipose levels.

A 6 mg/kg dose of [^{14}C]mirex in corn oil was administered to five male CD-1 rats by gavage (Mehendale et al., 1972). After 7 days, all animals were sacrificed. Of the 93.88% total recovery, 58.5% was present in feces, 0.69% in urine, 27.8% in fat, 1.75% in liver, 0.76% in small intestine, 3.20% in muscle, 0.23% in large intestine, 0.06% in stomach, 0.03% in heart, 0.09% in kidney, 0.07% in brain, 0.12% in testes, and 0.08% in lung. On a percent of total administered mirex per gram of tissue basis, mirex was most concentrated in fat at 1.54%, kidneys at 0.25%, and liver at 0.15%; all other tissues were 0.10% or less.

A single 0.2 mg/kg dose of [^{14}C]-mirex was administered by gavage to an unspecified number of mature female Sprague Dawley rats (Gibson et al., 1972). Tissue distribution was measured in four tissues, in descending order of mirex concentration, fat, liver, kidney, muscle and brain. Animals were sacrificed and measured 1, 2, 7, 14, and 28 days after mirex

administration; mirex concentrations increased in fat and decreased in other tissues over the 28 days.

A single dose of approximately 1 mg/kg [^{14}C]mirex was given intravenously to 2 female rhesus monkeys and orally to 1 female rhesus monkey (Kennedy et al., 1975; Wiener et al., 1976). In injected animals, plasma mirex levels were initially high and decreased rapidly within hours and were not accurately detectable after 9 days; in the orally dosed animal, radiolabel was not detected in plasma prior to 2 hours and reached a maximum at 5 hours. Route of administration did not otherwise significantly affect tissue distribution or excretion rates. The highest concentration was in fat, approximately 80% of the retained radiolabel. In decreasing order of magnitude, mirex was found in the adrenal, peripheral nerve, thyroid, and skin. Low levels were detected in all 38 tissues and the 7 types of fat tested.

Groups of male or female Sprague Dawley rats received a single oral dose of approximately 0.15 to 0.3 mg/kg [^{14}C]mirex (Chambers et al., 1982). Mirex accumulated in fat at high levels, in nervous tissues at low to moderate levels, and in the testes and the gastrointestinal tract at low levels. In females, mirex concentrations in the ovaries were more than 5-fold higher than in other organs at 1 week after dosing but were similar to other organs at 2 weeks after dosing; a similar response was seen with 2,8-dihydromirex.

Groups of 12 female Sprague Dawley rats and groups of 50 new-hatched Japanese quail were fed diets containing 0, 0.3, 3.0 or 30 ppm [^{14}C]mirex for up to 16 months (Ivie et al., 1974), pairs of animals were sacrificed periodically to measure tissue concentrations. In both rats and quail, mirex accumulated rapidly in adipose tissue and was detectable in all tissues measured; no plateau of residue accumulation was observed in any tissue. Average tissue distributions of mirex in rats were: brain 1.0%, muscle 1.0%, liver 3.0%, kidney 8.0%, fat 1.0%, skin 0.5%, feces 8.0%, other 0.3%. After cessation of dosing, mirex was more persistent in fat and skin than in other tissues. In quail, average mirex tissue distributions were: muscle 1.0%, liver 0.3%, fat 0.3%, skin 0.8%, feces 6.8%, egg yolk 1.0%, other 0.5%. Brain and kidney concentrations were not measured in quail. After cessation of dosing, mirex was more persistent in fat, skin, and feathers than in other tissues.

Studies have investigated the distribution of mirex in livestock. In male and female goats receiving mirex orally for 61 weeks, a steady-state was reached in plasma but not adipose tissue after 5 months of exposure (Smrek et al., 1977). Mirex was detected in the fat, liver, and brain of goats chronically dosed with mirex (Smrek et al., 1978). Mirex accumulated in the fat of Pitman-Moore minipigs fed 50 mg/day mirex for 7 days (calculated by ATSDR, 1995 as 3-4.5 mg/kg/day) (Morgan et al., 1979). Samples of back-fat were taken at the end of dosing and 9 days after dosing, mirex levels were 39.8 ppm and 41.5 ppm, respectively (Morgan et al., 1979); the increase over time suggests a slow redistribution of mirex. Other porcine tissues were also measured 9 days after dosing; mirex levels were 0.34 ppm in plasma, 0.12 ppm in red blood cells, 1.24 ppm in liver, 0.44 ppm in kidneys, and 0.62 ppm in the brain (Morgan et al., 1979).

Several studies have specifically investigated the distribution of mirex into the nervous system. Following oral dosing of male rats, mirex was detected in the brain within 30 minutes to 2 hours; at 4 days mirex was present in the cerebral cortex, cerebellum, brain stem and spinal cord, as well as the liver, kidneys, testes, and omental fat (Brown and Yarbrough, 1988). In chronic dosing experiments with 0, 89 or 890 μg/kg/day mirex, a dose-dependent accumulation was observed in the rat brain (Thorne et al., 1978). In mice, mirex accumulated in the brain; at 48 hours mirex concentrations were higher in the medulla, pons and striatum compared to the cortex, midbrain and cerebellum but no differences within the brain were observed 6, 12, or 48 hours after dosing (Fujimori et al., 1982). Khera et al. (1976) detected 3.5-fold higher mirex concentrations in fetal brain tissue compared to adult tissue.

Studies with pregnant animals have demonstrated that mirex accumulates in maternal and fetal tissues. Pregnant rats received oral doses of 0, 1.5, 3.0, 6.0, or12.5 mg/kg mirex on days 6-15 of gestation (Khera et al., 1976). In adult females, mirex concentrations were highest in adipose tissue. Mirex was detected in maternal and fetal tissues at all dose levels, except the control; fetal brain levels of mirex were higher than maternal brain levels, but lower concentrations of mirex were present in other fetal tissues compared to their mothers (Khera et al., 1976). Another study observed that tissue levels were 4- to 5-fold higher in mothers than in fetuses, with mirex concentrating in the liver, brain, heart and kidneys (3:2:2:1 ratio) in fetuses (Kavlock et al., 1980). Smrek et al. (1978) detected mirex in fetal goat tissues following chronic maternal exposure.

Chernoff et al. (1979b) administered 0 or 25 ppm [^{14}C]mirex in the diet to groups of at least 17 CD rat dams on gestational day 4 onward, and pups were switched to mothers of different groups at birth. Control pups were not exposed pre- or postnatally to mirex. Pups in the prenatal group were born to mothers who received 25 ppm mirex from day 4 of gestation onward; they were switched after birth to control-treated mothers for lactation. Pups in the postnatal group were not exposed prenatally, but were switched after birth to mothers who had been and continued to receive 25 ppm mirex in the diet; they were also weaned onto feed containing 25 ppm mirex. Pups in the perinatal group were exposed both pre- and postnatally to mirex, as described above. In newborn mice exposed prenatally to 25 ppm mirex, 2 ppm was detected in the brain and 10 ppm in the liver. In the postnatal group, 1.9 ppm mirex was detected in the liver at lactational day 9 and trace amounts of mirex were detected in other tissues; after day 31, trace levels of mirex (<1 ppm) were only detected in fat. For the postnatal and perinatal pups, 35-80 ppm mirex was detected in the milk of dams exposed during lactation to 25 ppm mirex. Mirex distribution in prenatal and postnatal pups was similar and concentrations peaked at day 21, when brain, kidney and liver concentrations were approximately 10, 20 and 160 ppm. Small amounts of mirex were detected in the eyes. After weaning (day 21), mirex tissue concentrations decreased; the authors attribute this both to elimination and dilution resulting from rapid growth.

One study investigating mirex distribution following inhalation was identified. Research cigarettes were impregnated with [^{14}C] mirex and lightly anesthetized female Sprague Dawley rats were exposed to mainstream smoke (Atallah and Dorough, 1975; Dorough and Atallah,

1975). Within 4 minutes, 47% of the mirex was exhaled, 36% remained in the lungs, 11.1% was present in the blood, and 1.3% was recovered from the heart tissue.

Following injection, mirex is rapidly cleared from plasma into other tissues. In rats, levels of mirex in plasma were 96% lower 8 hours post-injection compared to 2 minutes post-injection (Byrd et al., 1982). In rhesus monkeys, a single intravenous dose of mirex distributed into the fat (86-87%), skin (3.7-10%), skeletal muscle (0.6-1.7%), and other tissues (Wiener et al., 1976). In mice receiving intraperitoneal injections of mirex, peak concentrations in plasma were observed at 3 hours and in the liver at 6 hours. The first two half-lives for mirex in plasma were 9.2 and 62.8 hours; the first two half-lives for mirex in the liver were 12.1 and 62.4 hours (Charles et al., 1985).

3.3. METABOLISM

No studies were located regarding the metabolism of mirex in humans. Experiments indicate that animals are not capable of metabolizing mirex, but that gut bacteria may be capable (Stein et al., 1976; Stein and Pittman, 1977). In experiments using radiolabelled mirex to assay for metabolism, no metabolism of mirex was detected in rhesus monkeys (Wiener et al., 1976; Pittman et al., 1976), rats (Gibson et al., 1972; Ivie et al., 1974), Japanese quail (Ivie et al., 1974), cattle (Dorough and Ivie, 1974), mosquito fish (Ivie et al., 1974) or fathead minnow (Huckins et al., 1982). The potential for conversion of mirex to chlordecone in Pitman-Moore minipigs was assayed; no evidence of metabolism was found (Morgan et al., 1979).

A study of mirex in adult female rhesus monkeys has been reported in four papers (Kennedy et al., 1975; Stein et al., 1976; Wiener et al., 1976; Stein and Pittman, 1977). Two monkeys were given a single intravenous injection of 1 mg/kg [^{14}C]mirex. Chromatographic analyses of identified a nonpolar metabolite as either 10-monohydromirex or 9-monohydromirex in feces but not in fat tissues; authors concluded that gut bacteria may have metabolized mirex; the metabolite was less than 3% of the total administered dose.

Andrade and Wheeler (1975) exposed sewer sludge microorganisms to mirex under anaerobic conditions; using gas chromatography and mass spectrometry, a metabolite was isolated and putatively identified as 10-monohydromirex or 9-monohydromirex.

Mehendale et al. (1972) conducted *in vitro* metabolism experiments with liver preparations in the presence of cofactors from rats, mice, and rabbits and plant root extracts. No evidence of dechlorination of mirex was detected.

3.4. ELIMINATION AND EXCRETION

Fecal elimination of mirex is high during the first 48 hours after oral dosing and is attributed to elimination of unabsorbed mirex (reviewed ATSDR, 1995). Fecal elimination of absorbed mirex is slow and is the primary route of excretion; 1% or less of mirex is eliminated in

urine during the first 48 hours (Gibson et al., 1972; Chambers et al., 1982; Ivie et al., 1974; Wiener et al., 1976).

A 6 mg/kg dose of [^{14}C]mirex in corn oil was administered to five male CD-1 rats by gavage (Mehendale et al., 1972). Within 48 hours, 55% of the dose was excreted in feces; authors consider this to represent the portion of mirex that passed unabsorbed through the alimentary tract. Over a period of 7 days, 58.5% of the total administered dose was excreted in feces and 0.69% in urine. The authors estimate the first half-life of mirex is 38 hours and the second half-life (of absorbed mirex) is in excess of 100 days (Mendendale et al., 1972).

A single 0.2 mg/kg dose of [^{14}C]-mirex was administered by gavage to an unspecified number of mature female Sprague Dawley rats (Gibson et al., 1972). Of the total dose eliminated in feces, 6.74% was eliminated in the first day, 8.52% in the second day, 1.28% in the third day, 0.82% in the fourth day, and 0.67% on days 5-7. Total fecal elimination in 7 days was 18.03%. Between 0.4-0.5% of the total dose of mirex was eliminated in urine each day, 0.26% over 7 days. Absorption was decreased slightly by pre-feeding with a single dose of 250 ppm (20 mg/kg[1]) unlabeled dietary mirex; 12.84% was excreted in feces on the first day, 7.18% on the second day, and 4.94% on days 3-7 (24.96% total for days 1-7).

In female rhesus monkeys, 25% of orally administered mirex was eliminated unabsorbed; subsequently, 7% of the total dose was eliminated in feces over 386 days (Wiener et al., 1976).

In groups of 5 male and 5 female adult common goats dosed orally with 0 or 1 mg/kg-day mirex for 61 weeks (Smrek et al., 1978), the half-life of mirex after cessation of dosing was less than 52 weeks in both males and females.

Ivie et al. (1974) reported the fecal elimination of mirex from groups of 12 female Sprague Dawley rats and groups of 50 Japanese quail were fed diets containing 30 ppm [^{14}C]mirex (Ivie et al., 1974) on days 1 and 7, and after 1, 6, 12 and 16 months. In rats, the average percentage of consumed mirex eliminated in feces was 27.1% (24.2% on day 1, 30.1% on day 7, and 26.3-28.2% thereafter). In quail, the average percentage of consumed mirex eliminated in feces was 24.0% (15.8% on day 1, 20.8% on day 7, and 25.4-27.9% thereafter). In rats treated for 6 months with mirex and monitored for up to 10 months later, less than half of the administered dose was excreted. Therefore the half-life of mirex in rats was greater than 10 months. In female quail, egg laying resulted in dramatic decreases in tissue mirex concentrations compared to male quail. In female quail, a 75% reduction in residual mirex was observed within 3 months after cessation of dosing; in male quail, 40% of residual mirex was eliminated within 10 months. Ivie et al. (1974) also treated mosquito fish with a diet containing 50 ppm [^{14}C]mirex for 15 or 56 days. After cessation of treatment, 50% of mirex was eliminated in 30 days; after 200 days, 25% of the original body burden remained.

Rozman et al. (1984) administered [^{14}C]mirex to three lactating dairy goats intraruminally. Fecal elimination was not significant on day 1, and on days 2, 3, and 4 was 13.89%, 6.41% and 4.87%, respectively, of the total administered dose. Total fecal elimination

over 14 days was 44.5%. Urinary elimination averaged 0.029% per day, 0.41% over 14 days. Elimination in milk peaked on day 3; of the total administered dose of mirex, 14.6% was eliminated in milk in 14 days. After the 14 days of observation, 50 g/kg feed containing light paraffin mineral oil was added to the diet of 2 of the goats for 16 days, followed by basal diet for 16 days, diet plus mineral oil for 14 days, and then basal diet for 14 days. The third goat received 50 g/kg feed of hexadecane on days 32-46 (14 days). Goats ate all feed provided; no laxative effects were observed. Mineral oil increased the rate of fecal excretion of mirex 2-fold, but did not significantly alter milk or blood levels of mirex. The authors conclude that light paraffin mineral oil enhances the removal of body stores of mirex from lactating goats.

Mirex is eliminated into milk and eggs. Mirex was detected in 3 of 14 human milk samples tested (Mes et al., 1978). Following oral dosing, mirex has been detected in the milk of a cow (Dorough and Ivie, 1974), rats (Kavlock et al., 1980), and goats (Smrek et al., 1977). Mirex has been detected in eggs of Japanese quail (Ivie et al., 1974).

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS — EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

Mirex has been detected in human tissue and milk samples by multiple studies, many of which were directed toward determining body burdens of chlorinated pesticides. However, studies designed to associate toxic effects in humans to mirex exposure in the absence of complex mixtures were not identified.

Moysich et al. (1998) performed a case-control study assessing a wide variety of potential factors in 154 primary postmenopausal breast cancer cases and 192 postmenopausal community controls. They reported that among parous women who had never breast-fed, the detection of mirex in serum was associated with increased breast cancer risk, odds ratio 2.42 (95% confidence intervals 0.98-4.32). For this subgroup, increased risk was also associated with detection of polychlorinated biphenyls. In the total population sample, detectable tissue mirex was not associated with increased incidence of breast cancer, odds ratio 1.37 (95% confidence intervals 0.78-2.39).

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS — ORAL AND INHALATION

4.2.1. Subchronic Toxicity

No studies were located in which health effects were evaluated in animals following inhalation exposure to mirex.

Subchronic oral exposure to mirex has been associated with increased liver weight or liver lesions in Charles River or Sprague Dawley rats at doses as low as 0.3 mg/kg/day (Chu et al., 1981a; Larson et al., 1979; Yarbrough et al., 1981), decreased sperm counts in male rats at doses as low as 0.05 mg/kg/day and testicular degeneration at higher doses of 5 or 10 mg/kg/day (Yarbrough et al., 1981); and thyroid lesions (such as follicular epithelial thickening and follicular collapse) in Sprague Dawley rats at doses as low as 0.7 mg/kg/day (Chu et al., 1981a; Yarbrough et al., 1981). Similar lesions were not found in a study of beagle dogs provided dietary doses as high as 4 mg/kg/day for 13 weeks (Larson et al., 1979).

Larson et al. (1979) administered 0, 4, 20 or 100 ppm mirex (reported purity 98%) (0, 0.2, 0.8, or 4 mg/kg/day for males and females[2]) in the diet to groups of 2 male and 2 female

[2]Larson et al. (1979) administered 0, 4, 20, or 100 ppm mirex to dogs in the diet for 13 weeks. Based on averages of reported body weights (10.8 kg for male and 8.8 kg for female dogs) and using U.S. EPA (1988) allometric equations for all species combined, food consumption rates of 0.428 and 0.364 kg food/day were calculated for males and females, respectively. Calculated doses were 0, 0.2, 0.8, or 4 mg/kg-day for both males and females. Sample calculation: 4 mg/kg diet x 0.98 purity x 0.428 kg diet/day ÷ 10.8 kg = 0.2 mg/kg-day.

purebred beagle dogs for 13 weeks. Body weight was measured weekly; food consumption was measured daily. Hematology and blood chemistry (glucose, urea nitrogen, alkaline phosphatase, glutamine oxaloacetic transaminase, cholinesterase, and sulfobromophthalein retention) were measured prior to dosing and during week 13. Histopathology was conducted for the adrenal gland, bone marrow, brain, cecum, gonad, heart, kidney, liver, lung, pancreas, pituitary, small and large intestine, spleen, stomach, urinary bladder and thyroid. Two dogs receiving 4 mg/kg mirex died: one female during week 10 and one male during week 13. The male dog that died exhibited elevated hematocrit, white blood cell count, and sulfobromophthalein retention. In dogs receiving 4 mg/kg mirex, body weight was decreased and serum alkaline phosphatase was markedly elevated; statistical significance was not reported for these endpoints. No adverse histopathological effects were attributed to mirex treatment.

The same report (Larson et al., 1979) describes the dietary administration of 0, 5, 20, 80, 320 or 1280 ppm mirex (reported purity 98%) to groups of 10 male and 10 female Charles River rats for 13 weeks (0, 0.3, 1.2, 5.6, 22, or 93 mg/kg-day for males and 0, 0.3, 1.3, 6.5, 26, or 94 mg/kg-day for females[3]). During weeks 4 and 13, food consumption was measured, and unspecified urinalysis and hematology were performed on 4 male or 5 female rats, respectively, per group. Histopathology was performed for the adrenal gland, bone marrow, brain, cecum, gonad, heart, kidney, liver, lung, pancreas, pituitary, small and large intestine, spleen, stomach, urinary bladder and thyroid. One of 10 female rats in the 26 mg/kg dose-group died; all female rats and 5 of 10 male rats in the highest dose-group died. Statistically significant decreases in average body weight were observed in females at 94 mg/kg-day and males at both 22 and 93 mg/kg-day. Dose-dependent decreases in food consumption were observed in both sexes, and were statistically significant at the lowest doses tested. Mirex caused a dose-responsive increase in relative and absolute liver weight, statistically significant in female rats at all doses except 6.5 mg/kg and in male rats at 1.2 mg/kg and above. Liver vacuolation and swelling were observed in rats receiving at least 1.2 mg/kg mirex; the incidence was not reported. No liver necrosis or inflammation was reported, and no abnormalities were detected in other organs. This study does not identify a NOAEL, and identifies a LOAEL of 0.3 mg/kg-day (5 ppm in the diet) based on altered liver weight in female rats with changes in liver histopathology noted at higher doses.

Yarbrough et al. (1981) exposed groups of 10 male Sprague Dawley rats to 0, 0.5, 5.0, 50 or 75 ppm mirex (0, 0.05, 0.5, 5, or 7.1 mg/kg-day)[4] in the diet for 28 days. Food and water consumption, and urinary and fecal output were determined weekly. At study termination,

[3]Larson et al. (1979) administered 0, 5, 20, 80, 320, or 1280 ppm mirex to rats in the diet for 13 weeks. Based on the average of food consumption reported at 4 and 13 weeks (as g food per kg body weight), calculated doses were 0, 0.3, 1.2, 5.6, 22, or 93 mg/kg-day for males and 0, 0.3, 1.3, 6.5, 26, or 94 mg/kg-day for females. Sample calculation: 5 mg/kg diet x 0.98 purity x 70.5 g/kg ÷ 1000 mg/g = 0.3 mg/kg-day).

[4]Yarbrough et al. (1981) treated male rats with 0, 0.5, 5.0, 50, or 75 ppm mirex in the diet for 28 days. Based on a mean starting weight of 72.5 g (the average of the reported range of 60-85 g), and a reported weight gain of 150 g, a mean weight of 0.223 kg was calculated. Using the U.S. EPA (1988) allometric equations for laboratory mammals, a food consumption rate of 0.021 kg food/day was calculated. Calculated doses were 0, 0.05, 0.5, 5, or 7 mg/kg-day. Sample calculation: 0.5 mg/kg diet x 0.021 kg diet/day x 1/.223 kg body weight = 0.05 mg/kg-day mirex.

endpoints included body and organ weights, blood parameters, plasma chemistry, sorbitol dehydrogenase, hepatic microsomal parameters, sperm counts, and histopathology of the liver, thyroid and testis. No mortalities were observed; food and water consumption and fecal output were not affected by treatment. A dose-dependent increase in the incidence of liver lesions was statistically significant at 0.5 mg/kg and above: cytoplasmic changes were identified in 4 of 10 livers of rats treated with 0.5 mg/kg mirex and in all livers of rats dosed with 5 or 7.1 mg/kg mirex. In livers of rats treated with 0.05 mg/kg mirex, mild and subtle cytoplasmic "alterations" were noted, but pathologists were unable to consistently separate these from control livers. Four hepatic symptoms of mirex treatment were identified: increased cytoplasmic density with cell swelling (either hypertrophy or hyperplasia), homogenous eosinophilic cytoplasmic densities centered around the nucleus, hyaline or myelin cytoplasmic inclusions, and lipidosis. Measured at sacrifice, the following hepatic effects were statistically significant: increased relative liver weight at 0.5, 5, and 7.1 (but not 0.05) mg/kg mirex; decreased hepatic sorbitol dehydrogenase at all mirex dose-levels; increased total hepatic microsomal protein at 5 and 7.1 mg/kg; increased hepatic cytochrome P450 levels at 0.5 mg/kg and above; increased hepatic aminopyrine N-demethylase at 0.05 and 7.1 mg/kg; increased hepatic aniline hydroxylase at 5 mg/kg. Severe testicular lesions were observed in 1 of 10 rats dosed with 5 mg/kg mirex and in 2 of 9 rats dosed with 7.1 mg/kg mirex: hypocellularity of the seminiferous tubules, decreased spermatogenesis, and testicular degeneration (characterized by the presence of luminal nucleated cells and giant cells). Decreases in sperm counts were statistically significant at 0.05, 0.5, and 5 (but not 7.1) mg/kg. Prominent thyroid lesions were observed in 4 of 9 rats treated with 5 mg/kg mirex and in 4 of 9 rats treated with 7.1 mg/kg mirex: hypertrophy and elongation of follicular lining cells, lining cell proliferation causing papillary formations, follicular atrophy, focal epithelial exfoliation, and reduced colloid density. Less prominent thyroid lesions were reportedly visible in some rats treated with 0.05 mg/kg mirex. Statistically significant decreases in serum T3 concentrations were observed in rats treated 5 and 7.1 mg/kg and in serum T4 concentrations in rats treated with 0.5 and 7.1 mg/kg mirex. No other consistent, exposure-related effects were observed on evaluated endpoints.

Chu et al. (1981a) treated groups of 40 male Sprague-Dawley rats with 0, 5, or 50 ppm mirex (reported purity 98%) in the diet for 28 days (0, 0.7, or 7 mg/kg-day [5]). Ten animals per group were sacrificed immediately after the 28-day exposure. At 12, 24, and 48 weeks post-exposure, 10 animals per group were sacrificed. Endpoints evaluated were hematologic analysis, serum chemistry, measurements of testicular sorbitol dehydrogenase and hepatic mixed function oxidase activity, serum T3 and T4 levels, and histopathology of the adrenal gland, bone marrow, brain, esophagus, heart, kidney, liver, lung, trachea and bronchi, lymph node (mesenteric and mediastinal), pancreas, parathyroid, pituitary, skeletal muscle, small and large intestine, spleen, stomach, testis and epididymis, thoracic aorta, thymus and thyroid. No animals died following

[5]Chu et al. (1981a) treated groups of male Sprague Dawley rats with 0, 5, or 50 ppm mirex for 28 days. The reported starting weight range was 60-80 g (mean 75 g) and the final body weights were 201.8, 201.3, and 163.9 g, respectively. Average body weights are calculated as 138.4, 138.15, and 119.45 g. Food consumption during treatment was reported as 19.9, 18.3, and 17.1 g/rat/day. Doses are calculated as 0, 0.7, or 7 mg/kg-day. Sample calculation: 5 mg/kg food x 0.0193 kg food/day ÷ 0.138 kg body weight = 0.7 mg/kg-day.

28 days of exposure, or during the first 24 weeks of recovery. One rat in the 7 mg/kg mirex group died before 48 weeks but the cause was not determined. Treatment with 7 mg/kg mirex induced statistically significant decreases in food-intake and body weight and an increase in relative and absolute liver weight observed immediately after and 12 weeks after treatment; these effects had reversed by 24 and 48 weeks recovery. Liver congestion was seen in rats treated with 7 mg/kg mirex immediately following treatment, and liver mottling was observed in mirex-treated rats at all timepoints. The incidence of liver and thyroid lesions are presented in Table 1. In the livers of rats treated with mirex, pericentral fatty vacuolation, "cytoplasmic vacuolation, reduction in aggregated basophilia, nuclear anisokaryosis and hyperchromicity of hepatocytes" were observed. Livers of rats dosed with 0.7 mg/kg mirex appeared to have recovered by 48 weeks, but lesions still were apparent at 48 weeks in rats exposed to 7 mg/kg/day (see Table 1). Mirex induced thyroid lesions including "a reduction in colloid density, angular collapse of follicles and an increase in epithelial height with nuclear vesiculation." Thyroid lesions from treatment with 0.7 or 7 mg/kg mirex appeared to have repaired within 12 weeks (Table 1). Elevated incidence of testicular lesions (aspermia or inhibited spermatogenesis) was also observed in mirex-treated rats, but the elevation was not statistically significant. Treatment with mirex induced statistically significant changes in serum biochemistry: immediately after the 28-day exposure, 0.7 or 7 mg/kg mirex increased serum calcium, uric acid, alklaine phosphatase, and inorganic phosphorus and decreased glutamic oxalacetic transaminase, total protein, bilirubin and glucose. Twelve-weeks post exposure, inorganic phosphorous levels remained elevated and glutamic oxalacetic transaminase activity remained lower in rats treated with 0.7 or 7 mg/kg mirex; in the 7 mg/kg group, lactic dehydrogenase was decreased and sorbitol dehydrogenase activity was increased. After 24 weeks post-exposure, lactic dehydrogenase was significantly lower in rats treated with 0.7 or 7 mg/kg mirex, and aminopyrine –demethylase activity was elevated in the 50 ppm mirex group. All parameters had returned to normal by 48 weeks post-exposure. No other consistent and statistically significant adverse exposure-related effects were found. The lowest exposure level, 0.7 mg/kg-day (5 ppm in diet), was an adverse effect level for reversible liver and thyroid lesions in rats. The liver lesions were more persistent than the thyroid lesions, and showed no evidence of recovery at the higher dose level of 7 mg/kg/day.

4.2.2. Chronic Toxicity and Cancer Bioassay

There are three adequate studies that have investigated the toxicity or carcinogenicity of mirex in rats and mice orally exposed for chronic periods (Innes et al., 1969; NTP, 1990; Ulland et al., 1977). Two other chronic duration oral exposure studies have been published that focus on liver responses to mirex (Abraham et al., 1983; Fulfs et al., 1977), but they are of restricted usefulness because they are only focused on liver endpoints and incidence data for liver lesions were not adequately reported.

Table 1. Incidence of Histological Changes in Rats Fed Mirex for 4 Weeks Followed by up to 48 Weeks of a Mirex-free Diet
Source: Chu et al., 1981a

Dietary Concentration (mg/kg-day)	Sacrifice at End of Exposure	Recovery Period		
	28 Days	12 Weeks	24 Weeks	48 Weeks
Thyroid lesions[a]:				
0	0/7	0/10	0/7	0/10
0.7	8/10 [c]	0/10	1/7	1/9
7	10/10 [c]	1/10	1/5	1/9
Liver lesions[b]:				
0	0/7	2/10	0/9	0/10
0.7	10/10 [c]	5/10	8/10 [c]	1/9
7	10/10 [c]	10/10 [c]	8/8 [c]	7/9 [c]

[a]Thyroid lesions consisted of follicular epithelial thickening, colloid loss, and follicular collapse.

[b]Liver lesions consisted of fatty infiltration, cytoplasmic vacuolation, anisokaryosis, and hepatocyte necrosis.

[c]Significantly different from controls, $p < 0.05$ (Fisher Exact test performed by Syracuse Research Corporation).

NTP (1990) fed groups of 52 male and 52 female F344/N rats (initial body weight 120 and 100 g, respectively) 0, 0.1, 1, 10, 25, or 50 ppm mirex (reported purity >96%) in the diet for 104 weeks. A second study treated groups of 52 female F344/N rats with 0, 50, or 100 ppm mirex in the diet for 104 weeks, because early toxicity was not observed. Based on food consumption and body weight data, the NTP (1990) estimated these doses to be 0, 0.007, 0.07, 0.7, 1.8, and 3.8 mg/kg-day for males and 0, 0.007, 0.08, 0.7, 2.0, and 3.9 mg/kg/day for females. Doses were estimated at 0, 3.9, and 7.7 mg/kg-day for female rats in the second study. Average doses for male and female rats, thus, were 0, 0.007, 0.08, 0.7, 1.9, and 3.9 mg/kg/day in the first study. The following toxicological parameters were assessed: clinical signs, body weight, survival, gross pathology and histologic examination of adrenal gland, bone marrow, brain, esophagus, heart, kidney, liver, lung and bronchi, mammary gland, lymph nodes (submandibular and/or mesenteric), pancreas, parathyroid gland, pituitary gland, prostrate/testis or ovary/uterus, salivary glands, skin, small and large intestine, spleen, stomach, thymus, thyroid gland, trachea and urinary bladder.

No clinical signs of toxicity were reported for male or female treated rats. Statistically significant reductions in survival were seen in male rats treated with 1.9 or 3.9 mg/kg mirex (19/52 and 15/52, respectively, versus 44/52 for controls). The statistical significance of reported body weight changes were not provided. Male rats in the 1.9 and 3.9 mg/kg-day dose groups gained less weight than controls during the first 70 weeks of exposure and lost weight between 70 and 104 weeks of exposure; body weights after 104 weeks of exposure were 11% (1.9 mg/kg-day) and 18% (3.9 mg/kg-day) less than controls. In the first study, female rats in the 3.9 mg/kg-day group gained less weight than controls; body weights after 104 weeks of exposure were 8% less than controls. In the second study, females in the 3.9 and 7.7 mg/kg-day groups gained less weight than controls; body weights after 104 weeks of exposure were 8% (3.9 mg/kg-day) and 18% (7.7 mg/kg-day) less than controls.

NTP (1990) concluded that this study provided *clear evidence of carcinogenic activity* for male and female F344/N rats, based on "marked increased incidences of benign neoplastic nodules of the liver, as well as by increased incidences of pheochromocytomas of the adrenal glands and transitional cell papillomas of the kidney in males and by increased incidences of mononuclear cell leukemia in females." The dose-responsive increases in the incidences of hepatic neoplastic nodules were statistically significant ($p<0.05$) in mirex-treated male rats at and above 0.7 mg/kg and in female rats of the second study at 3.9 and 7.7 mg/kg mirex. No statistically significant increase in the incidences of hepatic carcinomas were observed in the exposed groups compared with controls. The combined incidences for benign pheochromocytomas or malignant pheochromocytomas were statistically significantly elevated in male rats exposed to 1.9 or 3.9 mg/kg/day, in female rats exposed to 3.9 mg/kg/day in the first study, but not in females exposed to 3.9 or 7.7 mg/kg/day in the second study (see Table 2). A statistically significant trend was found for increased incidence of transitional cell papillomas of the kidney in males rats, but the incidence for these neoplasms was not significantly elevated in the highest dose groups compared with controls (Table 2). Incidences of mononuclear cell leukemia were significantly elevated in male rats exposed to 1.9 mg/kg/day (but not at 3.9 mg/kg/day), in female rats exposed to 1.9 and 3.9 mg/kg/day in the first study, and in female rats

exposed to 7.7 mg/kg/day in the second study (Table 2). Combined analysis of the female groups found statistically significant elevations of incidence of mononuclear cell leukemia at 0.7 mg/kg and above.

The combined incidence of thyroid follicular cell adenomas or carcinomas was statistically significantly increased in male rats exposed to 3.9 mg/kg/day, but NTP (1990) concluded that an "association between follicular cell tumors and exposure to mirex is unlikely", because increased incidence of these tumors were not observed in males exposed to 1.9 mg/kg/day or in any exposed groups of female rats. In addition, no statistically significant exposure-related increases were observed in incidences of non-neoplastic thyroid lesions, although Chu et al. (1981a) reported that 28-day exposure to dietary concentrations as low as 5 ppm produced increased incidence of reversible thyroid lesions in Sprague Dawley rats (follicular epithelial thickening, colloid loss, and follicular collapse).

Non-neoplastic liver effects were dose-dependent and were reportedly dramatic: statistically significant increases in hepatocytomegaly were seen in male rats at 0.007 mg/kg (but not at 0.08 mg/kg) and in male and female rats at and above 0.7 mg/kg; abundant eosinophilic cytoplasm and vacuolated cytoplasm were observed. Other statistically significant increases were: hepatic fatty metamorphosis, at and above 0.7 mg/kg in both sexes; hepatocyte necrosis, in male rats treated with 1.9 and 3.9 mg/kg mirex, in female rats of the first study treated with 0.7 and 3.9 mg/kg, and in female rats of the second study treated with 3.9 mg/kg; and angiectasis (dilated sinusoids) in male rats treated with 0.7 mg/kg or more. Thus, 0.007 and 0.08 mg/kg/day (0.1 and 1 ppm in diet) were no-effect levels for non-neoplastic liver responses in this study.

Other statistically significant elevated incidences of non-neoplastic lesions in the exposed groups compared with controls were (see Table 3 for incidence data):

1) parathyroid hyperplasia in male rats exposed to doses of 0.7 mg/kg/day and higher;

2) nephropathy in female rats exposed to 0.08, 0.7, 1.9 and 3.9 mg/kg/day in the first study (but not in females exposed to 3.9 and 7.7 mg/kg/day in the second study);

3) epithelial hyperplasia of the renal pelvis in male rats exposed to doses of 1.9 mg/kg/day and higher, and

4) splenic fibrosis in male rats exposed to 0.7 mg/kg/day or higher.

Table 2. Incidences for Selected Neoplastic Lesions (In Non-hepatic Tissues) in Male (M) or Female (F) F344/N Rats Fed Mirex in the Diet for 2 Years Incidences for Females in 2nd Study Are Indicated as FII (See Table 4 for Incidences of Hepatic Neoplasms) Source: NTP, 1990

Dietary Concentration	**0 ppm**	**0.1 ppm**	**1 ppm**	**10 ppm**	**25 ppm**	**50 ppm**	**100 ppm**
Dose (mg/kg/day)	0	0.007	0.08	0.7	1.9	3.9	7.7
Lesion	(# Affected Rats/ # Examined Rats)						
Pheochromocytoma (benign or malignant)	10/51 M 1/51 F 3/52 FII	7/52 M 3/52 F	13/52 M 5/52 F	12/52 M 1/51 F	18/51 M[a] 2/51 F	20/51 M[a] 6/52 F[a] 2/52 FII	2/52 FII
Mononuclear cell leukemia	16/52 M 8/52 F 6/52 FII	17/52 M 8/52 F	15/52 M 11/52 F	22/52 M 14/52 F[b]	21/52 M[a] 18/52 F[a]	10/52 M 18/52 F[a] 9/52 FII	14/52 FII[a]
F + FII Combined	14/104	8/52	11/52	14/52[b]	18/52[b]	27/104[b]	14/52[b]
Kidney transitional cell papilloma	0/51 M	0/51 M	0/52 M	0/52 M	1/51 M	3/52 M	

[a]Statistically significant ($p < 0.05$) difference from controls by Incidental Tumor Tests performed by NTP.
[b] Statistically significant ($p < 0.05$) for combined controls (14/104) by Fisher Exact.

Table 3. Incidences for Selected Non-neoplastic Lesions (In Non-hepatic Tissues) in Male (M) or Female (F) F344/n Rats Fed Mirex in the Diet for 2 Years
Source: NTP, 1990
Incidences for Females in 2nd Study Are Indicated by FII

Dietary Concentration	**0 ppm**	**0.1 ppm**	**1 ppm**	**10 ppm**	**25 ppm**	**50 ppm**	**100 ppm**
Dose (mg/kg/day)	0	0.007	0.08	0.7	1.9	3.9	7.7
Lesion	(# Affected Rats/ # Examined Rats)						
Parathyroid hyperplasia	6/32 M	12/39 M	12/39 M	18/40 M*	22/50 M*	24/45 M*	
Nephropathy	50/51M 34/52 F 45/52 FII	50/51 M 35/52 F	45/51 M 44/52 F*	49/52 M 47/51 F *	51/51 M 46/50 F *	52/52 M 42/52 F* 51/52 FII	52/52 FII
Epithelial hyperplasia, renal pelvis (males)	1/52 M	1/52 M	2/52 M	4/52 M	6/52 M *	10/52 M *	
Splenic fibrosis	0/52 M	1/51M	0/50 M	7/51 M*	6/48 M *	9/52 M *	

*Statistically significant ($p < 0.05$) difference from controls by Fisher Exact Test performed by Syracuse Research Corporation

NTP (1990) concluded that nephropathy occurred at similar incidences in control and exposed groups of male and female rats, but that the severity was increased at doses of 1.9 mg/kg/day and greater. Average severity scores (2 = mild; 3 = moderate, and 4 = marked) for control through high-dose male rats were 3.1, 3.0, 3.0, 3.7, 3.5, and 3.9. For females, respective severity scores were 2.5, 2.5, 2.8, 2.6, 3.0, and 2.9 in the first study, and 2.7, 3.0, and 3.2 in the second study.

The liver histopathological slides prepared during the original NTP (1990) study were re-evaluated by a group of pathologists convened by Pathco, Inc. (the "Pathology Working Group"; PWG, 1992) using more current criteria and terminology than those used in the original pathology report. Neoplastic nodules were reclassified as hepatocellular adenomas, eosinophillic foci of cellular alteration, or regenerative hyperplasia. The term toxic hepatitis was used to describe non-neoplastic lesions based on the occurrence of centrilobular hepatocytomegaly, centrilobular fatty change, apoptosis (individual cell necrosis), centrilobular necrosis, and bile duct proliferation. A U.S. EPA-sponsored review by Garman (U.S. EPA, 1999b) of the PWG (1992) report concluded that the re-evaluation was appropriate and that the re-evaluated incidence data for rat liver tumors were valid for risk assessment. The Office of Emergency and Remedial Response recommended that the PWG incidence data be used for risk assessment at Superfund sites (U.S. EPA, 1999a). Table 4 highlights the incidence of liver lesions for male and female rats based on the re-evaluation by the PWG (1992).

The re-evaluation found statistically significant increases in the incidence of hepatocellular adenomas in male rats treated with 1.9 and 3.9 mg/kg/day, and in female rats of the second study exposed to doses of 3.9 and 7.7 mg/kg/day (see Table 4). Thus, the re-evaluation indicated 1.9 mg/kg/day as the lowest dose level inducing a significant tumor response, whereas the NTP (1990) original pathological examination showed statistically significant increased incidence of "neoplastic nodules" at 0.7 mg/kg/day. The incidence of hepatocellular carcinoma was not affected by mirex-treatment (regardless of criteria or terminology used in the pathological exam). Statistically significant increases in the incidences of foci of cellular alteration and toxic hepatitis occurred in both male rats and female rats exposed to 0.7 mg/kg mirex and higher; angiectasis and cystic degeneration were more frequent in male rats exposed to at least 0.7 mg/kg mirex. Regenerative hyperplasia reportedly only occurred in the presence of toxic hepatitis or leukemia.

The PWG (1992) concluded that "increased incidences of hepatocellular adenomas in both sexes were limited to doses of mirex which also induced hepatotoxicity and eosinophillic foci" (see Table 4), that a correlation between the latter two lesions was observed at doses up to 1.9 mg/kg mirex, and that "hepatotoxicity was also observed at doses lower than those with increased incidences of tumors." Despite the strong association between non-neoplastic liver lesions and the hepatocellular adenomas, not all rats with hepatocellular adenomas were diagnosed with non-neoplastic liver lesions. Examination of the PWG's (1992) chairperson's

Table 4. Incidences for Liver Lesions in F344/N Rats Fed Mirex in the Diet for 2 Years Incidence for Females in the Second Study Are Indicated by FII Sources: NTP, 1990; PWG, 1992

Dietary Concentration	0 ppm	0.1 ppm	1 ppm	10 ppm	25 ppm	50 ppm	100 ppm
Dose (mg/kg/day)	0	0.007	0.08	0.7	1.9	3.9	7.7
Lesion	(# Affected Rats/ # Exposed Rats)						
Eosinophilic foci	6/52 M 4/52 F 2/52 FII	4/52 M 4/52 F	5/52 M 5/52 F	14/52 M [a] 13/52 F [a]	17/52 M [a] 20/52 F [a]	21/52 M [a] 21/52F [a] 27/52 FII [a]	26/52 FII [a]
Toxic hepatitis	1/52 M 1/52 F 1/52FII	2/52 M 1/52 F	5/52 M 3/52 F	11/52 M [a] 25/52 F [a]	28/52 M [a] 34/52 F [a]	29/52 M [a] 35/52 F [a] 45/52 FII [a]	37/52 FII [a]
Liver adenomas	1/52 M 3/52 F 0/52 FII	1/52 M 1/52 F	2/52 M 1/52 F	4/52 M 0/52 F	6/52 M[a, b] 2/52 F	10/52 M [a, b] 3/52 F 5/52 FII [a, b]	5/52 FII [a, b]
Liver carcinomas	3/52 M 0/52 F 0/52 FII	0/52 M 0/52 F	2/52 M 0/52 F	1/52 M 0/52 F	2/52 M 1/52 F	3/52 M 1/52 F 0/52 FII	1/52 FII
Liver adenomas or carcinomas	4/52 M 3/52 F 0/52FII	1/52 M 1/52 F	4/52 M 1/52 F	5/52 M 0/52 F	7/52 M 3/52 F	13/52 M [a, b] 4/52 F 5/52FII [a, b]	6/52FII [a, b]

[a]Statistically significant ($p < 0.05$) difference from controls by Fisher Exact Test performed by Syracuse Research Corporation

[b]Statistically significant ($p < 0.05$) difference from controls by Peto Pairwise Test performed by PWG (1992)

Toxic hepatitis refers to findings of centrilobular hepatocytomegaly, centrilobular fatty change, apoptosis, centrilobular necrosis, and bile duct proliferation.

worksheets indicates that among all exposed male rats, 17 of 24 diagnoses of liver adenoma were concurrent with the diagnosis of other liver lesions. In the 1.9- and 3.9-mg/kg/day groups (the groups with significantly increased incidences of liver adenomas), toxic hepatitis or eosinophillic foci were diagnosed in 4/6 and 8/10 male rats with liver adenomas, respectively. At least one pathologist in the PWG diagnosed the presence of non-neoplastic liver lesions (hyperplasia or regeneration) in every rat diagnosed with adenoma, but these incidences reflect consensus opinions that no non-neoplastic lesions were identified in some animals with adenomas.

Summarizing the findings from the NTP (1990) rat bioassay (with liver histopathology re-evaluated by the PWG [1992]), exposure for 2 years to dietary dose levels of 0.007 or 0.08 mg/kg/day produced no consistent statistically significant increased incidence of neoplastic or non-neoplastic lesions in male or female F344/N rats (Tables 2, 3 and 4). In females in the first study, 0.08 mg/kg-day dosage induced a statistically significantly ($p<0.05$) increased incidence of nephropathy, but in females exposed to higher doses (3.9 and 7.7 mg/kg-day) in the second study, no statistically significant increases were found when compared with controls. At 0.7 mg/kg/day (and higher), statistically significant increased incidences of non-neoplastic lesions were found in several organs: toxic hepatitis consisting of centrilobular hepatocytomegaly, fatty change, apoptosis, necrosis, and bile duct proliferation (in both sexes), parathyroid hyperplasia in male rats only, nephropathy in females rats (only in the first study), and splenic fibrosis in male rats only (Tables 3 and 4). Exposure to 1.9 mg/kg/day produced statistically significant incidences of the following tumor types: liver adenomas in male rats, pheochromocytomas or malignant pheochromocytomas in male rats, and mononuclear cell leukemia in male and female rats (Table 2 and 4). At 3.9 mg/kg/day significantly elevated incidences included liver adenomas in male and female rats (second study) (Table 4), liver adenomas or carcinomas in male and female rats (Table 4), benign or malignant pheochromocytomas in male rats (Table 2), and mononuclear leukemia in female rats (first study) (Table 2). In females exposed to 7.7 mg/kg/day in the second study, elevated incidences occurred for mononuclear cell leukemia, liver adenomas, and liver adenomas and carcinomas combined (Tables 2, 3, and 4). Other statistically significant neoplastic responses were observed in several groups of exposed rats, but they were not as strongly associated with mirex exposure as liver neoplasms in both sexes, pheochromocytomas in male rats, or mononuclear cell leukemia in female rats, and are not interpreted as exposure-related. These included a statistically significant trend for increasing incidence of transitional cell papillomas of the renal pelvis in male rats with increasing exposure level, but the highest incidence for this neoplasm (3/52) was found in the highest dose group and was not significantly different from the control incidence by a number of statistical tests.

The Innes et al. (1969) study (also reported as IARC, 1979) reports the effects of lifetime feeding of mirex or 119 other test compounds to mice, starting at 7 days of age. Two strains were used: (C57BL/6 x C3H/Anf)F1 (strain B6C3F1) and (C57BL/6 x AKR)F1 (strain B6AKF1). Four untreated-control groups and one gelatin-control group of each sex and strain (15-18/group) were included in the study. Innes et al. (1969) administered nothing, gelatin, or 10 mg/kg mirex in gelatin to groups of 18 male and 18 female mice of the two strains by gavage from 7-28 days of age; then mice were fed diets containing 0 or 26 ppm mirex for the remainder of their lifetimes. Assuming an average chronic reference body weight of 0.024 kg and food

intake of 0.0063 kg/day for male and female B6C3F1 mice (U.S. EPA, 1988), an estimated daily dietary dose of 7 mg/kg/day is calculated. This was considered a maximum tolerated dose. Mirex-treatment decreased survival: control animals survived to 78-89 weeks; all treated males had died by 59 weeks on study, and all the females had died by 70 weeks. Whether the animals died spontaneously or were terminated is unclear from the report, but it could be inferred that mirex was severely toxic to the mice. Control animals were sacrificed between 78 and 89 weeks. At sacrifice, the thoracic and abdominal cavities were given a "thorough examination" with histological examination of major organs and of all grossly visible lesions. The incidence data for non-neoplastic lesions were not discussed in the report. Statistical analysis of tumor incidence data (for liver tumors, pulmonary tumors, and lymphomas) were performed using separate and combined control group data. The term "hepatoma" was used for all liver tumors regardless of metastasis; the authors reported that metastases were rare.

In mirex-treated animals, the increases in incidence of hepatomas in mirex-treated animals were statistically significant: B6C3F1 males: controls 8/79 (10%), treated 6/18 (33%); B6C3F1 females: controls 0/87 (0%), treated 8/16 (50%); B6AKF1 males: controls 5/90 (6%), treated 5/15 (33%); B6AKF1 females: controls 1/82 (1.2%), treated 10/16 (62.5%). The tumor incidences in all treated groups were significantly elevated relative to the pooled controls. No pulmonary tumors or lymphomas were observed in treated mice. In this study, 10 other compounds of the 120 total tested also induced statistically significant elevations in the incidences of tumor formation.

Groups of 26 male and 26 female Charles River CD rats were fed 40 or 80 ppm mirex in the diet (99% pure) for 10 weeks (Ulland et al., 1977). These doses were the predicted maximum tolerated dose and half maximum tolerated dose. Because no significant adverse effects were observed, the doses were increased to 50 or 100 ppm, respectively. Rats were dosed for a total of 18 months. After cessation of treatment, animals were observed for 6 months. The time weighted doses were calculated to be 4 or 7 mg/kg for males and 4 or 8 mg/kg for females[6]. Groups of 20 male and 20 female rats were maintained as controls. All rats were necropsied and subjected to histopathologic examination of the adrenal glands, cerebrum, cerebellum, esophagus, heart, kidneys, liver (2-3 sections), lungs, pancreas, parathyroid gland, pituitary glands, spinal cord, small and large intestine, spleen, stomach, thymus, thyroid, urinary bladder, and either testes or ovaries and uterus. Food and water consumption data were not reported. Treatment had no effect on body weight. Survival was significantly reduced in both groups of treated males and in high-dose females. Neoplastic and non-neoplastic hepatic lesions occurred at elevated incidences in exposed rats compared with controls (see Table 5).

[6]Ulland et al. (1977) treated male and female CD rats for 10 weeks with 40 or 80 ppm, followed by treatment with 50 or 100 ppm mirex, for a total of 18 months. Time weighted exposures were calculated to be 48.7 or 97.4 ppm. Average body weights of 0.44 for males and 0.33 kg for females were estimated by straight-edge extrapolation from a provided graph of body weight versus time. Using the U.S. EPA (1998b) allometric equations, food intake was calculated to be 0.034 and 0.027 kg/day. Calculated doses were 4 or 7 mg/kg-day for males and 4 or 8 mg/kg-day for females. Sample calculation: 48.7 mg/kg food x 0.99 purity x 0.034 kg food/day ÷ 0.44 kg body weight = 4 mg/kg-day.

Statistically significant increased incidence of hepatic neoplastic nodules occurred only in the high-dose male group, although incidences in both exposed female groups were marginally elevated compared with controls ((p=0.053). Among rats bearing neoplastic nodules, one low-dose and four high-dose males, and one high-dose female also had hepatocellular carcinomas. Carcinomas in male rats occurred with a statistically significant positive trend (Cochran Armitage trend test performed by the Syracuse Research Corporation). The first liver tumor in control males was detected after 80-85 weeks; the first liver tumor in the treated males occurred in the high-dose group after 60-65 weeks. Livers of low-dose females appeared normal by gross examination; other exposed rats showed enlarged, mottled, or spotted livers. Histologic analyses of livers found that mirex induced fatty metamorphosis, megalocytosis, cystic degeneration, necrosis, and biliary hyperplasia with periportal fibrosis. Incidences of megalocytosis were statistically significantly elevated in exposed groups of both sexes (Table 5). Megalocytosis was diffuse and was associated with periportal and perivascular areas; hepatocyte cytoplasm was enlarged with finely granular eosinophilic material. Areas of cellular alteration were characterized by hepatocyte enlargement and cytoplasmic vacuolation with a finely granular eosinophilic material; occasional cystic degeneration was observed. Features of the neoplastic nodules were swollen hepatocytes with vacuolated or granular eosinophilic cytoplasm that occupied the space of several lobules and compressed adjacent tissue. Carcinomas appeared well-differentiated. No other neoplastic or non-neoplastic changes in other tissues were attributed to treatment.

Fulfs et al. (1977) orally administered mirex to mice for 19 months, rats for 8 or 12 months, and monkeys for up to 26 months and examined histological, ultrastructural and biochemical endpoints in the livers; potential toxic or carcinogenic responses in other tissues were not examined. The study provides limited evidence that mice livers may be more sensitive than rat or monkey livers, but it is of restricted usefulness for risk assessment due to its limited focus on the liver and inadequate reporting of incidence data.

Groups of 100 male and 100 female CD-1 mice received 1, 5, 15 or 30 ppm mirex (0.2, 0.9, 3, or 5 mg/kg-day[7]) in the diet for at least 19 months with paired control groups of 100 male and female mice (400 and 400, total) (Fulfs et al., 1977). Groups of 6 were sacrificed at 2, 4, 6, 9, 10, 15, and 18 months. Body weights were reported to be not affected by treatment, but were not otherwise reported. Due to poor survival in the 5 mg/kg group, these animals were removed from the study. Survival was not otherwise reported. Increased relative liver weights were seen in 5-mg/kg mice at 2 months, in 0.9- or 3-mg/kg mice at 6 months and later, and in 1-mg/kg mice (females only) at 18 months. Livers from exposed mice were reportedly enlarged and

[7]Fulfs et al. (1977) did not report body weight or food intake data. Using U.S. EPA (1988) reference values for chronic body weight (0.0373 and 0.0353 kg) and chronic food intake (0.0064 and 0.0061 kg/day) for male and female B6C3F1 mice, respectively, calculated doses were 0, 0.2, 0.9, 3, or 5 mg/kg-day for both males and females. Sample calculation: 1 mg mirex/kg food x 0.064 kg food/day ÷ 0.373 kg body weight = 0.2 mg/kg-day mirex.

Table 5. Incidence of Hepatic Lesions in Charles River CD Rats Exposed Chronically to 0, 50, or 100 PPM Mirex in the Diet
Source: Ulland et al., 1977

Group	Megalocytosis	Foci or Areas of Cellular Alteration[a]	Neoplastic NoDules[b]	Hepatocellular Carcinoma
Controls:				
Male	0/20	3/20	0/20	0/20
Female	0/20	10/20	0/20	0/20
Low-dose: 4 mg/kg/day				
Male	14/26 [c]	6/26 (2)	2/26 (1)	1/26
Female	8/26 [c]	11/26 (3)	4/26 [d]	0/26
High-dose: 7 (M) or 8 (F) mg/kg/day				
Male	12/26 [c]	10/26 (5)	7/26 (4)[c]	4/26 [d]
Female	14/26 [c]	7/26 (2)	4/26 (1) [d]	1/26

[a]Value in parentheses indicate the number of animals also having neoplastic nodule and/or carcinoma

[b]Value in parentheses indicate the number of animals also having carcinoma

[c]Significantly different from controls, $p < 0.05$, Fisher Exact Test performed by Syracuse Research Corporation

[d]Different from controls, $p = 0.053$, Fisher Exact Test performed by Syracuse Research Corporation

showed "nodules" by gross examination. The nodules, which were not otherwise described, were reported to appear after 6 months in the 3-mg/kg mice and after 15 months in the 0.2-mg/kg mice. Centrilobular hypertrophy of the hepatocytes was reported to occur in "most" exposed mice. Individual or multicellular necrosis was observed at dose levels of 0.9 mg/kg/day and higher, but was not observed in the 0.2-mg/kg/day group of mice. Incidence data were not reported. Ultrastructurally, minimal proliferation of smooth endoplasmic reticulum in hepatocytes was reported at the 0.2-mg/kg/day level, with more pronounced proliferation at the higher dose levels.

Sprague Dawley rats were fed 5 ppm mirex for 12 months or 30 ppm mirex for 8 months (estimated doses of 0.3 or 2 mg/kg [8]). Gender, use of controls, and numbers per group were not specified. It was reported that no differences were observed between control versus treated animals for body weight, liver weight, gross pathology, histopathology, or biochemistry (Fulfs et al., 1977).

Groups of 2 male and 2 female rhesus monkeys were treated with 0, 0.25, or 1 mg/kg mirex by gavage, 6 days per week, for up to 26 months. Baseline liver biopsies were taken from all 12 monkeys before administration, from high-dose animals at 16, 19, and 26 months, and from low-dose animals at 36 months. Livers from exposed monkeys were reported to appear normal, except for the occurrence of "occasional focal lymphocytic infiltration" (Fulfs et al., 1977).

Groups of 10 male Sprague Dawley rats were fed 0 or 100 ppm mirex (0 or 8 mg/kg-day[9]) in the diet for 13 months (Abraham et al., 1983). Body weight was measured at study initiation and termination. An unspecified number of animals were injected with tritiated thymidine 1 hour prior to sacrifice; livers were examined for normal, abnormal and adjacent areas; from which samples were taken for analysis of nuclei, histology, and autoradiography. Livers of control-treated rats were reportedly normal; livers of mirex-treated rats exhibited centrilobular and pericentral hepatocyte hypertrophy and necrosis. Hypertrophic nodules, evolved nodules with trabeculi and acini, and advanced carcinomas were observed; however, incidence and statistical significance of these findings were not reported. Mirex treatment caused statistically significant increases in DNA synthesis (measured by thymidine-uptake) in hepatocytes in normal tissue adjacent to carcinomas and in littoral cells in areas of carcinoma.

[8] Using U.S. EPA (1988) reference values for chronic body weight for Sprague Dawley male rats of 0.523 kg and chronic food intake of 0.036 kg/day, doses of 0.3 or 2 mg/kg-day are calculated. Sample calculation: 5 mg mirex/kg food x 0.036 kg food/day ÷ 0.523 kg body weight = 0.3 mg/kg-day mirex.

[9] Using U.S. EPA (1998b) chronic reference values for Sprague Dawley male rat body weight (0.514 kg) and food intake (0.040 kg food/day), a dose of 8 mg/kg/day is calculated.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES — ORAL AND INHALATION

No studies were located regarding the effects of mirex on reproduction or development in humans. Results from animal experiments indicate that mirex adversely affects reproductive performance in both male and female animals and is a developmental toxicant.

4.3.1. Developmental Toxicity

No studies were located regarding developmental effects in humans associated with exposure to mirex.

Developmental effects including cataracts and reduced survival have been observed in the offspring of female rodents exposed during gestation or lactation to orally administered mirex.

Mirex-induced cataracts and reduced survival (Table 6) to weaning were found in rat offspring in an early 2-generation reproductive toxicity study (Gaines and Kimbrough, 1970). Groups of 10 male and 10 female Sherman rats were exposed to 0 or 25 ppm in the diet for 45 or 102 days before they were mated for 7 days to rats without pre-exposure. Females continued to receive their respective pre-mating diet through gestation and lactation. Reported estimates of daily doses ranged from 1.3-3.1 mg/kg-day for males and 1.8-2.8 mg/kg-day for exposed males and females, respectively; the average of the midpoint of these ranges is 2.3 mg/kg-day. Offspring were examined grossly at birth, during lactation, and at weaning for mortality and development of abnormalities, including cataracts. No effects on litter number, number of live pups at birth, pup survival to weaning, or percentage of offspring with cataracts were found in groups in which male rats were exposed for 45 or 102 days. In groups in which female rats were exposed to 2.3 mg/kg/day for 45 or 102 days before mating and during gestation and lactation, pup survival to weaning was statistically significantly reduced to 53% and 61%, respectively,compared with 89% in offspring of non-exposed males and females. Respective percentages of offspring with cataracts in these groups were 33.3% and 46.2%, compared with no cataracts in offspring of non-exposed males and females. No effects on offspring endpoints were found in another group of 10 females exposed to 5 ppm mirex for 102 days prior to mating and during gestation and lactation. Daily doses were reported to range from 0.31-0.49 mg/kg-day; the midpoint of this range is 0.4 mg/kg-day. To determine if *in utero* or lactational exposure to mirex was responsible for cataract formation, one group of 10 female rats received 5 ppm mirex in the diet for 49 days before mating and then throughout gestation and lactation. Three groups of 10 female rats were bred without pre-treatment. At birth, offspring of mirex-treated dams were switched with one of the non-exposed groups, and the offspring of the remaining two non-exposed groups were switched with each other. In offspring treated *in utero* with mirex, cataract incidence was 1.6%. In offspring receiving mirex only in milk, cataract incidence was 37.5%; no offspring of the control-control switched groups exhibited cataract formation (Gaines and Kimbrough, 1970).

Table 6. Developmental Effects in Rats Orally Exposed to Mirex

Strain	Exposure Protocol	Developmental Effects	NOAEL mg/kg/day	LOAEL mg/kg/day	Reference
Sherman	0, 1, 5, or 25 ppm in diet for 45 or 102 days prior to mating with unexposed males, followed by exposure during gestation and lactation. Midpoints of reported dose ranges were 0, 0.07, 0.4, or 2.3 mg/kg/day.	decreased pups/litter & survival of pups to weaning; increased incidence of cataracts in offspring	0.4	2.3	Gaines and Kimbrough, 1970
	Exposure of foster dams to 0 or 5 ppm in diet before mating& during gestation before nursing pups of non-exposed dams.	decreased survival of pups to weaning; increased incidence of cataracts in offspring	None	0.4	
Sherman or Long Evans	0, 1.0, 2.5, 5, or 10 mg/kg on postnatal days 1-4 to lactating dams.	decreased survival of pups to weaning	5	10	Chernoff et al., 1979a
		decreased BW & increased cataracts in offspring	1	2.5	
Sherman	0 or 5 mg/kg on postnatal days 1-5	increased cataracts in offspring	none	5	Scotti et al. 1981
Long Evans	0 or 10 mg/kg on postnatal days 1-4	increased cataracts in offspring	none	10	Rogers and Grabowski, 1984
Long Evans	0, 5, 10, 15 mg/kg on postnatal day 1	increased cataracts in offspring	10	15	Chernoff et al., 1979a
Sprague Dawley	0, 5, 10, 20, or 40 ppm in diet for 13 weeks before mating continuing through mating (males and females) and through gestation and lactation (dams only). Estimated average doses for males and females were 0, 0.4, 0.8, 1.5, or 3 mg/kg/day	decreased pups/litter	none	0.4	Chu et al., 1981b
		decreased survival of pups to weaning	0.8	1.5	
		increased incidence of cataracts in offspring	none (F) 0.8 (M)	0.4 (F) 1.5 (M)	

Strain	Exposure Protocol	Developmental Effects	NOAEL mg/kg/day	LOAEL mg/kg/day	Reference
CD	0, 6, or 12 mg/kg/day on gestation days 7-162	increased external fetal abnormalities Information on maternal effects not reported	none	6	Kavlock et al., 1982
Wistar	0, 1.5, 3, 6, or 12.5 mg/kg/day by gavage (in corn oil) on gestational days 6-15	maternal mortality increased resorptions increased fetal mortality & fetal visceral abnormalities	3 1.5 3	6 3 6	Khera et al., 1976
CD albino	0, 5, 7, 9.5, 19, or 38 mg/kg/day on gestation days 7-16	decreased maternal weight gain or relative liver weight increased edema & delayed ossification in fetuses other abnormalities (enlarged cerebral ventricles & undescended testes) fetal mortality	5 5 7 9.5	7 7 9.5 19	Chernoff et al., 1979a
Long Evans	0, 5, 7, or 10 mg/kg/day on gestation days 6-15	maternal mortality increased fetal mortality altered fetal electrocardiogram patterns	5 5 none	7 7 5	Grabowski and Payne, 1980
Long Evans	0 or 6 mg/kg/day on gestation days 8-15	decreased maternal weight gain increased fetal mortality & edema	none none	6 6	Grabowski, 1981
Long Evans	0 or 6 mg/kg/day on gestation days 8-15	increased fetal mortality, increased edema & altered electrocardiograms, maternal effects not reported	none	6	Grabowski and Payne, 1983a

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Khera et al. (1976) treated groups of 18-20 mated female Wistar rats with 0, 1.5, 3, 6, or 12.5 mg/kg mirex in corn oil by gavage on days 6-15 of gestation, and were euthanized on day 22. Fetuses were examined for weight, viability, and external malformations; one third of each litter was studied for visceral changes and two thirds for skeletal anomalies. Treatment with 12.5 mg/kg caused statistically significant dose-dependent increases in maternal death during gestation and incidence of pregnancy failure, and decreased maternal weight gain. At 12.5 mg/kg, a statistically significant decrease in fetal weight was observed. In animals treated with 6 or 12.5 mg/kg, statistically significant decreases in the number of live fetuses per pregnancy and in the total number of visceral anomalies were detected; visceral anomalies included subcutaneous edema, cleft palate, scoliosis, runts, short tail, and heart defects (Table 6).

Chernoff et al. (1979a) administered 0, 5, 7, 9.5, 19 or 38 mg/kg mirex by gastric gavage to groups of 38, 30, 10, 37, 32 or 10 pregnant CD albino rats, respectively, on days 7-16 of gestation and sacrificed animals on day 21. Maternal deaths occurred in exposed groups but the incidences were not statistically significant or dose-dependent. Maternal weight gain decreased in a dose-dependent fashion and was statistically significant at and above 9.5 mg/kg. Relative maternal liver weight increased in a dose-dependent fashion and was statistically significant at and above 7 mg/kg. No fetuses survived exposure to 38 mg/kg. Statistically significantly increased fetal mortality and decreased birth weight were seen in offspring of the 19-mg/kg dose group (65.1% and 3.46 g for treated versus 4.0% and 3.84 g for controls, respectively). The average number of implants was not affected by treatment. Dose-dependent increased incidence of edematous live fetuses and decreased sternal ossification occurred and were statistically significant at and above 7 mg/kg. Severe edema was frequent in dead fetuses. The incidence of other somatic anomalies, a category including enlarged cerebral ventricles and undescended testes was significantly elevated in offspring of the 9.5- and 19-mg/kg groups.

In a study of cataract formation in postnatally exposed offspring, doses of 0, 1.0, 2.5, 5 or 10 mg/kg mirex (Table 6) were administered to Long-Evans rat and Sherman rat dams on postnatal days 1-4; each group consisted of at least 10 litters (Chernoff et al., 1979a). In Long-Evans rat offspring, statistically significant decreases in offspring body weight were seen at 2.5 mg and above; data were not provided for Sherman rats. Statistically significant decreases in viability were seen in litters of both strains of rats that received 10 mg/kg mirex, on the first day of measurement post-dosing and afterward. No ocular lesions were observed at 1.0 mg/kg mirex; statistically significant dose-dependent changes were observed at 2.5 mg/kg and above in both strains. Cataracts and "an outlining of the lens" were observed when the neonates' eyes opened, on days 12-14. Lens outlining was persistent through weaning and was difficult to discern in mature animals. In a separate experiment, Long-Evans dams received single oral doses of 0, 5, 10 or 15 mg/kg on postnatal day 1 (day of birth); the number of litters were 8, 14, 12 and 20, respectively. No cataracts were detected in the 5-mg/kg group. Increased incidences were observed at 10 and 15 mg/kg, but the increase was statistically significant only at 15 mg/kg.

Chernoff et al. (1979a) also observed mirex-induced developmental cataract formation in CD-1 mice. On postnatal days 1-4, groups of at least 10 nursing mothers were given 0, 1.5, 3.0, 6.0 or 9.0 mg/kg by gavage; animals were observed weekly until postnatal day 37. Statistically

significant decreases in pup survival were seen in the 6.0- and 9.0-mg/kg dose groups. Statistically significant decreases in body weight were seen in all treated litters; this effect was transient below 6.0 mg/kg, but animals treated with 9.0 mg/kg did not recover by 37 days. No mirex-induced ocular lesions were seen at 1.5 mg/kg; statistically significant increases in cataract incidence were observed at 3.0 mg/kg and higher.

Chernoff et al. (1979b) examined the influence of mirex exposure during gestation, during gestation and lactation, and during lactation alone, on survival and development of cataracts in offspring of groups of 17 CD rat dams administered 25 ppm mirex in the diet (2.8 mg/kg-day[10]). Control pups were not exposed pre- or post-natally to mirex. Pups in the prenatal group were born to mothers who received 25 ppm mirex from day 4 of gestation onward; they were switched after birth to control-treated mothers for lactation. Pups in the postnatal group were not exposed prenatally, but were switched after birth to mothers who had been and continued to receive 25 ppm mirex in the diet; they were also weaned onto feed containing 25 ppm mirex. Pups in the perinatal group were exposed both pre- and post-natally to mirex. A statistically significant increase was seen in the incidence of stillborn pups exposed prenatally (3% of controls, 14% of treated). Postnatal survival was significantly decreased in both the prenatally and perinatally exposed animals beginning on lactation day 8 (94% control, 95% postnatal, 74% prenatal, 77% perinatal). Postnatal mirex exposure decreased neonatal body weight gain; these changes were statistically significant from day 8 to 46 in both the postnatal and perinatal groups. Incidences of cataracts and outlined lenses in pups were, respectively: control 0% and 0%; prenatal 5.8% and 0%; postnatal 23.8% and 18.8%, perinatal 18.1% and 12.6%.

Chu et al. (1981b) treated groups of 15 male and 20 female Sprague Dawley rats with 0, 5, 10, 20, or 40 ppm mirex (0, 0.3, 0.7, 1.4, or 2.8 mg/kg-day for males and 0, 0.4, 0.8, 1.6, or 3.2 mg/kg-day for females [11]) in the diet for 91 days prior to mating, and 15 days during mating. Average doses for males and females were 0, 0.4, 0.8, 1.5, or 3.0 mg/kg/day. Females were mated to males of the same treatment group, and females continued to receive treatment during gestation and lactation. When compared to controls, statistically significant decreases in mean litter size were observed at all mirex doses tested. Offspring survival at 21 days was significantly reduced for the 20 ppm group. Fertility index, gestational survival index and 4-day survival were not altered by treatment (Chu et al., 1981b). Statistically significant increases in the incidence of cataracts (Table 6) were seen in female fetuses treated with 0.4 or 1.6 mg/kg-day (insufficient numbers of animals examined prevent evaluation of other treatment groups).

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[10] Chernoff et al. (1979b) administered 25 ppm mirex to female CD rats for the length of gestation, lactation, or both. Assuming subchronic reference body weights of 0.124 kg and food consumption rates of 0.014 kg/day for female F344/N rats (U.S. EPA, 1988), a dose of 2.8 mg/kg-day is calculated.

[11] Chu et al. (1981b) fed male and female Sprague Dawley rats 0, 5, 10, 20 or 40 ppm mirex in the diet. Reference (U.S. EPA, 1988) male and female body weights of 0.523 and 0.338 kg and daily food intakes of 0.036 and 0.027 mg/day were used to calculate doses of 0, 0.3, 0.7, 1.4, or 2.8 mg/kg-day for males and 0, 0.4, 0.8, 1.6, or 3.2 mg/kg-day for females. Sample calculation: 5 mg mirex/kg food x 0.036 kg food/day ÷ 0.523 kg body weight = 0.3 mg/kg-day.

Additional studies have verified the developmental effects of mirex in vehicle delivered to pregnant rats during gestation by gavage. Grabowski and Payne (1980) exposed pregnant Long-Evans rats to 0, 5, 6, 7, or 10 mg/kg mirex by gavage on gestation days 6-15 (Table 6). At 7 and 10 mg/kg, maternal death was elevated and the increased incidence of fetal death was statistically significant. Microphthalmos (abnormally small eye size) and edema were observed in fetuses from all exposed groups. In fetuses with edema compared to those without, the incidence of increased heart rate, and first- and second-degree heart block were statistically significantly increased. Scotti et al. (1981) observed lens swelling in offspring after treating lactating Sherman rats with 5 mg/kg-day on post-natal days 1-5 (Table 6). In fetuses of pregnant Long-Evans rats treated orally with 6 mg/kg-day on gestation days 8-15, the following statistically significant effects were found: increased incidences of cataracts and associated ocular changes (Rogers and Grabowski, 1983); increased plasma glucose and hematocrit (Rogers et al., 1984); and increased incidence of edema with associated altered plasma protein levels, colloid osmotic pressure, and electrocardiograms (Grabowski, 1981; Grabowski and Payne, 1983a,b). Other studies support these findings (Grabowski and Payne, 1983a,b; Rogers and Grabowski, 1984).

Kavlock et al. (1982) treated six pregnant CD rats orally (vehicle was not reported) with 0, 6, or 12 mg/kg/day mirex on gestational days 7-16, and collected fetuses on day 21. The study report contained no information regarding possible maternal toxicity. Mirex treatment caused fetal edema and decreased body weight. The numbers of live fetuses and fetuses with other gross abnormalities were not affected by exposure. Treatment with either 6 or 12 mg/kg day (Table 6) caused statistically significant decreases in fetal: brain weight; liver weight and total glycogen; kidney total protein and alkaline phosphatase. Treatment with 12 mg/kg day also caused statistically significant decreases in fetal: brain total DNA, and protein; lung weight; liver weight and total glycogen; kidney weight, total protein, and alkaline phosphatase activity; and kidney weight. Mirex treatment did not affect lung biochemistry.

Reiter (1977) gavaged nursing Long-Evans rats with 0, 2.5, or 10 mg/kg mirex in corn oil for four days, starting at parturition, and tested pups exposed via milk for neurobehavioral impairment. At 28, 44, and 120 (adult) days of age, unspecified numbers of male offspring were tested for locomotor activity in a residential maze, 80 minutes a day for 4 days. In 42-day old rats, statistically significant maze hyperactivity was observed in the last 20 minutes of the testing period. In adult rats, on the first (but not subsequent) days of maze testing, diurnal activity was significantly increased. The study authors concluded that these results suggest that developmental exposure to mirex may increase the length of time animals need to acclimate to a new environment, but that these data should be interpreted with caution. In another study, Shannon (1976) reported suggestive evidence of delayed or inhibited development of motor skills in prairie voles exposed to mirex during pre- and post-natal development, but the data are inadequately reported for evaluation.

4.3.2. Reproductive Toxicity

No studies were located regarding the effects of mirex on reproduction in humans.

There are a few studies examining reproductive performance in rats exposed to mirex, but no adequately designed and reported multiple generation studies of reproductive performance in animals exposed to mirex are available.

Khera et al. (1976) treated groups of 20 proven-fertile male Wistar rats with 0, 1.5, 3.0, or 6.0 mg/kg mirex by gavage for 10 consecutive days, followed by 14 sequential mating trials of 5 days each with two untreated virgin females per male. Following mating, females were sacrificed 13-15 days later; the numbers of viable embryos, deciduomas, and corpora lutea were determined. Treatment with 6.0 mg/kg caused a statistically significant decrease in weight gain. A statistically significant decrease in number of pregnancies was seen in the first mating trial of males dosed with 6.0 mg/kg, but not in the subsequent 14 trials. For acute mirex toxicity to male reproduction, this study identifies NOAELs of 1.5 and 3.0 mg/kg-day and a LOAEL of 6.0 mg/kg based on a reversible decrease in fertility in rats (Table 6).

Gaines and Kimbrough (1970) exposed groups of 10 male and 10 female Sherman rats to 0 or 25 ppm mirex (0 or 2.3 mg/kg-day) in the diet for 45 or 102 days before mating with non-exposed rats for 7 days. Females continued to receive their respective pre-mating diets during gestation and lactation. Exposed groups showed no significant decrease in the number of successful matings or number of litters delivered, except that the group of females exposed before mating for 102 days delivered only 6 litters compared with 9 litters in the control group of mated nonexposed males and females and 8-10 litters in the other groups. The average number of live pups per litter was statistically significantly decreased in the group of females exposed for 45 days before mating, but this effect was not observed in the group of females exposed for 102 days before mating. No effects on the number of successful matings, number of litter delivered or number of live pups per litter were observed in a group of 10 females who were exposed to 5 ppm mirex (0.4 mg/kg-day) for 102 days prior to mating (Table 6).

In the NTP study (1990), groups of 52 male F344/N rats were treated with 0, 0.007, 0.075, 0.75, 1.95, or 3.85 mg/kg-day in the diet for 104 weeks. No exposure-related adverse changes were observed in reproductive tissues examined histologically: the mammary gland, penis, preputial gland, prostate, seminal vesicle, testis, epididymis and scrotum.

Yarbrough et al. (1981) administered 0, 0.5, 5.0, 50, or 75 ppm (0, 0.05, 0.5, 5, or 7.5 mg/kg-day[12]) mirex in the diet to groups of 10 male Sprague Dawley rats for 28 days. Decreased

[12] Using a U.S. EPA (1988) reference body weight of 0.18 kg and a daily food consumption rate of 0.018 kg for male F344/N rat, doses of 0, 0.05, 0.5, 5, or 7.5 mg/kg-day were calculated. Sample calculation: 0.5 mg/kg food x 0.018 kg food/day ÷ 0.18 kg body weight = 0.05 mg/kg-day.

sperm counts were observed at 0.5, 5 and 50 (but not 75) ppm mirex and the incidence of severe degenerative testicular lesions was significantly increased at 50 and 75 ppm (Yarbrough et al., 1981).

In a reproductive and pharmacokinetic study, Smrek et al. (1977, 1978) treated groups of 5 male and 5 female goats with 0 or 1 mg mirex/kg body weight (mixed in sugar) for 61 weeks. Females received 10 mg/kg for an additional 4 weeks. Goats were bred at the onset of treatment and again 32 weeks after the start of exposure. An additional group of 5 females received 1 mg/kg for 18 weeks (starting after delivery of offspring from the first breeding), followed by 10 mg/kg for 4 weeks. The authors reported that "goats did not show any sign of toxicity." Mirex did not affect adult body weight, number of kids born, or kid body weight.

Chu et al. (1981b) fed groups of 15 male and 20 female Sprague Dawley rats with 0, 5, 10, 20, or 40 ppm mirex (0, 0.3, 0.7, 1.4, or 2.8 mg/kg-day for males and 0, 0.4, 0.8, 1.6, or 3.2mg/kg-day for females [13]) in the diet for 91 days prior to mating, and 15 days during mating. Females were mated to males of the same treatment group, and females continued to receive their pre-mating diet during gestation and lactation. A dose-dependent decrease in the number of females showing sperm in vaginal smears following mating was observed and was statistically significant at all dose levels of mirex (Chu et al., 1981b). The study authors considered the decrease in sperm to be evidence of testicular damage. In the 40-ppm group, no sperm was detected in the vaginal smears of females and no successful mating occurred; fertility index was affected only at this highest dose. Statistically significant decreases in mean litter size were also observed at all mirex doses tested. The average number of pups per litter were 10.2, 7.0, 5.8, and 7.1 for the 0-, 5-, 10-, and 20-ppm groups, respectively. Offspring survival at 21 days was significantly reduced for the 20-ppm group. Gestational survival index and 4-day survival were not altered by treatment (Chu et al., 1981b).

Shannon (1976) tested the effects of mirex on prairie voles (*Microtus ochrogaster*). Groups of 16 prairie vole pairs (1 male and 1 female) were exposed to 0, 1, 5, 10, 15, or 25 ppm mirex (estimated to be 0, 0.1, 0.6, 1.2, 2, or 3 mg/kg-day[14]) in the diet for 90 days, followed by a 4-month observation period. A dose-dependent increase in adult mortality was observed that was statistically significant at and above 0.6 mg/kg, and no animals survived in the highest dose-group. Pairs produced five or fewer litters during the experiment. Mirex treatment did not

[13]Chu et al. (1981b) treated male and female Sprague Dawley rats with 0, 5, 10, 20 or 40 ppm mirex. Based on U.S. EPA (1988) chronic reference values for Sprague Dawley rats for body weights (0.523 and 0.338 kg) and daily food intakes (0.036 and 0.027 kg/day), doses were calculated to be 0, 0.3, 0.7, 1.4, or 2.8 mg/kg-day for males and 0, 0.4, 0.8, 1.6, or 3.2 mg/kg-day for females. Sample calculation: 5 mg mirex/kg food x 0.036 kg food/day ÷ 0.523 kg body weight = 0.3 mg/kg-day.

[14]Shannon (1976) did not report body weight or food intake data. Based on body weight data for prairie voles reported in three sources (National Research Council, 1995; University of Kansas, 2001; Texas Parks and Wildlife, 1994), an adult body weight of 40 g was assumed. Using an U.S. EPA (1988) allometric equation for food intake, a daily food intake of 0.00508 kg/day was calculated. Sample calculation: 1 mg mirex/kg food x 0.00508 kg food/day ÷ 0.040 kg body weight = 0.1 mg/kg-day.

significantly affect the number of offspring per litter born or surviving to 4 days. A statistically significant reduction in pup survival to 21 days was seen at 0.6 mg/kg and above; no pups in the 2 mg/kg group survived. Data for other reproductive endpoints, including fertility, could not be assessed because of reporting deficiencies and the apparent failure to consider mortality as a confounding factor.

Shannon (1976) exposed groups of 10 pairs (1 male and 1 female) of prairie voles to 0, 0.1, 0.5, 0.7, 1.0, or 5.0 ppm mirex (estimated to be 0, 0.01, 0.06, 0.09, 0.13, or 0.6 mg/kg-day for both males and females[15]) for 5 months. Treatment did not adversely affect adult survival, the number of pups produced per litter, or pup survival. The control, 0.01, and 0.06 mg/kg offspring were used in a multigenerational study: pups were continued on their respective parental diet at weaning and were mated at 60 days. Behavioral development (of motor skills) was investigated, but reporting inconsistencies and deficiencies prevent adequate evaluation of these data and other reproductive data.

Treatment of pregnant rats with 10 mg/kg/day mirex for 5 (but not 1 or 10) days caused reduced blood flow to the ovaries, uterus and fetuses, with concurrent decreased ovarian and uterine weights (Buelke-Sam et al., 1983); because the effects were not consistent over time the significance of these observations is uncertain.

Fuller and Draper (1975) induced ovulation in immature 28-day-old female Long Evans rats with pregnant mare serum (PMS), and then administered 0.4 to 50 mg/animal mirex by subcutaneous injection. Mirex reduced the numbers of ova released 40-80% compared to control animals. Administration of human chorionic gonadotropin reversed the inhibitory effects of mirex; the authors suggest this is evidence that mirex does not act directly upon the ovary. A time-course found that mirex was an effective inhibitor of ovulation when introduced prior to, but not after, the lutenizing hormone surge. The authors speculated that mirex may influence ovulation through a neural mode of action that controls lutenizing hormone release.

Typical estrogenic effects were not produced in immature female Sprague Dawley rats given subcutaneous doses of up to 100 mg mirex/kg on postnatal day 22 or 1 mg/animal on postnatal days 2 and 3 (Gellert, 1978). Groups of 5 immature virgin 22-day-old Sprague Dawley rats were given single subcutaneous injections of 0, 1, 10, or 100 mg mirex/kg. As a positive control,1 µg/kg 17β-estradiol was administered to five animals. Unlike estradiol which significantly increased uterine weight within 24 hours, mirex had no effect on uterine weight. In a subsequent experiment, twin groups of 16 and 21 newborn rats were subcutaneously injected with sesame oil (control) or 1 mg/dose of mirex 2 and 3 days after birth. Pups were weaned at 21 days and sacrificed at 7 or 8 months. Mirex exposure had no effect on vaginal opening, estrous cycles, or ovulation compared with controls, but caused a 10% increase in body weight and 24% increase in uterine weight without influencing weights of the ovary, adrenal gland or

[15]Sample calculation: 0.1 mg mirex/kg food x 0.00508 kg food/day ÷ 0.040 kg body weight = 0.001 mg/kg-day. See previous footnote for sources of values for food intake and body weight.

the anterior pituitary. In contrast, similar exposure to 0.2 or 1 mg chlordecone ("Kepone", in which two chlorine atoms have been substituted by a ketone oxygen) produced definite estrogenic effects including shortened time to vaginal opening, increased incidences of persistent vaginal estrus and ovaries devoid of corpora lutea. Corroborating results were reported by Hammond et al. (1979) showing that subcutaneous doses of 50 mg mirex/kg for 3 days to 21-day-old female did not affect uterine weight, whereas similar exposure to 50 mg chlordecone/kg or 50 μmg estradiol-17β/kg increased uterine weight by 3- to 4-fold compared with controls.

4.4. OTHER RELEVANT STUDIES

4.4.1. Other Carcinogenicity Studies

Carcinogenic responses to subcutaneously administered mirex have been observed in two strains of mice (NCI, 1968). Groups of 18 males and 18 female mice of B6C3F1 and B6AKF1 mice received single subcutaneous injections of 1000 mg/kg mirex in 0.5% gelatin at 28 days of age. Surviving animals were sacrificed at 18 months. The term hepatoma was used to describe all liver tumors, except in cases of unmistakable pulmonary metastases, when the term hepatic carcinoma was used. Several tumor types, including those arising in the liver, thymus, spleen, abdominal lymph nodes, and Peyer's Patches, were included in the group of reticulum cell carcinoma. Statistically significant differences in tumor incidence between control and mirex-treated mice for total number, reticulum cell sarcoma, and hepatoma (by Chi-square methods) were reported. Because results were not evaluated by gender and strain, these results were verified by an evaluation of the raw data by Syracuse Research Corporation using the Fisher Exact Test (Table 7). Statistically significant elevations, compared with control incidences from multiple concurrent experiments, were observed for reticulum cell sarcoma in B6C3F1 males and B6AKF1 females and for hepatomas in B6AKF1 males. Pooling across sexes and strains, incidences for reticulum sarcomas or hepatomas in exposed mice were significantly elevated compared with controls (Table 7).

Moser et al. (1992) observed that dermal exposure to 25, 50, or 100 nmol mirex (in 200 μL acetone) per application 3 times/week for 34 weeks or 200 nmol for 20 weeks promoted time- and dose-dependent skin tumor formation in female CD-1 mice exposed to single initiating dermal doses of 200 nmol 7,12-dimethylbenz[a]anthracene (DMBA) in 200 μL acetone. Without initiation, dermal exposure to 100 or 200 nmol mirex failed to induce skin tumor formation, epidermal hyperplasia, epidermal protein kinase C activity, or ornithine decarboxylase activity. After 34 weeks of promotion with 25, 50, or 100 nmol mirex, 27%, 85% and 100% of mice developed skin tumors, respectively; the respective average numbers of tumors/mouse were 0.7, 7, and 12. No skin tumors occurred in mice initiated with DMBA and promoted with acetone for 34 weeks. In parallel studies of a number of biochemical and morphological events, mirex, at concentrations that produced a strong tumor promotion activity, did not induce ornithine decarboxylase or produce a strong cell proliferation response in mouse skin; these responses

Table 7. Survival and Tumor Incidence in Mice That Received 1000 mg Mirex/kg Body Weight by Subcutaneous Injection on Post-natal Day 28
Source: NCI, 1968

	B6C3F1 Males	B6C3F1 Females	B6AKF1 Males	B6AKF1 Females	Total
Mirex-exposed mice					
# starting	18	18	18	18	72
# surviving to 18 months	16	17	17	15	65
# necropsied	18	17	17	18	70
# reticulum cell sarcomas	6/18 *	0/17	1/17	3/18 *	10/70 *
# hepatomas	2/18	0/17	4/17 *	1/18	7/70 *
Pooled control mice					
# starting	150	160	167	162	639
# surviving to 18 months	130	153	153	153	589
# necropsied	145	141	136	161	583
# reticulum cell sarcomas	8/145	1/141	0/136	55/161	14/583
#hepatomas	0/145	9/141	0/136	1/161	10/583

*Statistically significantly different ($p<0.05$) from control (both untreated and vehicle-treated) mice pooled from multiple concurrent experiments (Fisher Exact test performed by Syracuse Research Corporation).

were observed with the strong phorbol ester mouse skin tumor promoter,12-*O*-tetradecanoyl-phorbol-13-acetate (Moser et al., 1992).

Moser et al. (1993) provided evidence that female mice are more susceptible to skin tumor promotion by mirex than male mice, and that ovarian hormones may enhance tumor promotion by mirex. Skin tumors formed in 96% of female mice (n=30) exposed to initiating doses of DMBA followed by dermal exposure to 200 nmol mirex, three times per week for 20 weeks; an average of 14.7 tumors per mouse was observed. In contrast, in a group of 30 male mice similarly exposed, 67% showed skin tumors at 20 weeks with an average of 4.7 tumors/mouse. No skin tumors were found in groups of mice initiated with DMBA and promoted with acetone or initiated with DMBA and promoted with 200 nmol mirex. More than 90% of skin tumors in male and female mice showed a mutated Ha-*ras* gene. To investigate the effects of ovarian hormones on tumor promotion, Moser et al. (1993) initiated female CD-1 mice with a single dermal application of 200 nmol DMBA; 2 weeks later either sham-surgery (controls) or ovariectomies were performed. After an additional 2 weeks, starting when mice were 12 weeks old, all mice were exposed to 200 nmol mirex 3 times per week for 20 weeks. A statistically significant decrease in tumor formation was seen in ovariectomized mice compared to ovary-intact mice (1.8 versus 6.0 tumors per mouse, respectively). The results suggest that ovarian hormones play a role in the tumor-promoting effect of mirex in female mice. The ability of mirex to promote skin tumors in female CD-1 mice following initiation has also been reported by Kim and Smart (1995) and Kim et al. (1997).

4.4.2. Genotoxicity Studies

No evidence of mirex genotoxicity has been identified. Stevens et al. (1979) reported that they were unable to demonstrate mirex binding to DNA or RNA. The NTP (1990) found that mirex was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535 or TA1537, with or without exogenous metabolic activation. Mirex did not induce chromosomal aberrations or sister chromatid exchanges in Chinese hamster ovary cells with or without metabolic activation. Other studies confirm that mirex did not induce reverse mutations in multiple strains of *Salmonella typhimurium* (Hallett et al., 1978; Mortelmans et al., 1986; Probst et al., 1981; Schoeny et al., 1979) with or without hepatic microsomal activation. Mirex did not induce mutations in the WP2 and WP2 uvrA- strains of *Escherichia coli* (Probst et al., 1981) with or without hepatic microsomal activation. Mirex did not significantly induce unscheduled DNA synthesis in rat, mouse, or hamster hepatocytes *in vitro* (Probst et al., 1981; Williams, 1980; Maslansky and Williams, 1981; Telang et al., 1981). Mirex did not induce gene mutations in the HGPRT locus of human fibroblasts *in vitro* (Tong et al., 1981).

Animal studies have not identified mirex genotoxicity. No dominant lethal effects, measured by viable embryos or deciduomas in pregnant females mated to male Wistar rats treated with 0, 1.5, 3.0, or 6.0 mg/kg mirex by gavage 10 consecutive days and were then mated for 14 consecutive days, were observed (Khera et al., 1976). Mitra et al. (1990) observed that at doses of mirex that induced hepatic ornithine-decarboxylase activity (up to 240 mg/kg) in female Sprague Dawley rats, DNA damage was not induced, as measured by alkaline elution.

Abraham et al. (1983) observed that dietary administration of 100 ppm mirex to male Sprague Dawley rats for 13 months disturbed the distribution of diploid versus tetraploid nuclei in livers; the effects were most significant in hepatocellular carcinomas.

Mirex is structurally similar to chlordecone (i.e., kepone) in which two chlorine atoms have been substituted by a ketone oxygen. Chordecone induced sister chromatid exchanges in CHO Chinese hamster ovary cells (NTP, 1990), and has been classified by IARC (1987) as *possibly carcinogenic to humans* (Group 2B), on the basis of insufficient human data and the induction of liver tumors (hepatocellular carcinomas) in Osborne Mendel rats and B6C3F1 mice (NCI, 1976).

4.4.3. Mechanistic Studies

The mechanism by which mirex causes non-neoplastic and neoplastic lesions in the liver is not yet completely understood. As discussed in the previous section, mirex has not been genotoxic in numerous short-term *in vitro* and a few *in vivo* tests, leading to the hypothesis that tumorigenic responses to mirex in the liver do not directly involve a genotoxic mechanism and may involve proliferation of cells initiated spontaneously, or by some other agent, to become tumors. Support for this hypothesis is provided by results in the two-stage mouse skin tumor bioassay showing that mirex does not initiate mouse skin tumors, but is a potent promoter of skin tumors initiated by DMBA (Kim and Smart, 1995; Kim et al., 1997; Moser et al., 1992, 1993).

Molecular details of how mirex may promote tumor formation, presumably through promotion of cell proliferation, are unknown, but results from several lines of investigation provide some suggestions of interactions with endocrine systems in mirex-induced tumor promotion and promotion of hepatocellular growth. In the two-stage mouse skin bioassay, ovariectomized mice showed a lesser skin tumor promotion response to mirex than sham-operated mice with intact ovaries (Moser et al., 1993). Exposure of Sprague Dawley rats to single high oral doses of mirex (100 mg/kg) induced rapid liver growth (about 70% increase in relative liver weight) without changing levels of serum enzymes indicative of liver damage or affecting some functional indices such as sulfobromophthalein clearance (Robinson and Yarbrough, 1978). Numerous studies of this phenomenon by Yarbrough and colleagues have led to the hypothesis that mirex-induced liver growth is composed of hypertrophic (enhanced growth of existing cells) and hyperplastic (enhanced cell division) components and that interactions with endocrine systems may be involved (see Yarbrough et al., 1984 for review). For example, adrenalectomized male rats showed a liver growth response to 100 mg/kg mirex that was only about 50% of that in rats with intact adrenal glands. In mirex-exposed adrenalectomized rats given corticosterone supplements, the liver growth response to mirex was restored, but was not preceded with a peak in hepatocellular DNA synthesis as noted in rats with intact adrenal glands (Yarbrough et al., 1984). Yarbrough et al. (1984) suggested that, in adrenalectomized rats, mirex induced liver growth predominately by a hyperplastic mode and that corticosterone supplement shifted the growth response to a hypertrophic response. In another study with thyroidectomized rats, the magnitude of liver growth in response to mirex was similar to that in rats with intact thyroids, but liver growth was not preceded by a peak in hepatocellular DNA synthesis,

indicating a shift to a hypertrophic mode of response (Yarbrough et al., 1984). Supplementation of thyroidectomized rats with thyroxine (T4) and mirex caused a further stimulation in liver growth over that in rats with intact thyroids and restored the preceding peak in hepatocellular DNA synthesis. Further details regarding the possible connection of endocrine systems to the modes of action whereby chronic oral exposure to mirex produces hepatocelluar cytomegaly and necrosis and liver tumors are unknown.

Another hypothesis related to mirex-induced hepatic responses involves mirex induction of ornithine decarboxylase, the first enzyme in the biosynthetic pathway for polyamines that play roles in regulation of various cell functions and metabolism. Oral exposure of rats to two doses of 120 mg mirex/kg, 21 and 4 hours prior to sacrifice, or to single doses of 180 mg/kg induced hepatic ornithine decarboxylase activity by 70- or 55-fold, respectively (Mitra et al., 1990). In contrast, mirex did not induce epidermal ornithine decarboxylase at a dermal dose level that strongly promoted mouse skin tumors after DMBA initiation, whereas tumor promoting dose levels of the phorbol ester,12-*O*-tetradecanoyl-phorbol-13-acetate, did induce ornithine decarboxylase (Kim et al., 1995; Kim and Smart, 1997; Moser et al., 1992, 1993).

Another hypothesis proposes that mirex is preferentially cytotoxic to tetraploid and octaploid hepatocytes and that this is a key phenomenon in the induction of mirex hepatotoxicity and carcinogenicity (Abraham et al., 1983). In a group of 10 male Sprague-Dawley rats exposed to 100 ppm mirex in the diet for 13 months, livers exhibited hypertrophy with necrosis, nodules, or carcinomas (incidences of these lesions were not reported). Control livers showed greater numbers of tetraploid nuclei than diploid nuclei (the reported ratio was 244:147, tetraploid:diploid), whereas, in exposed livers, decreasing numbers of tetraploid nuclei were observed in tissues with increasing severity of effect. The change in ploidy pattern was dramatic in liver tissue adjacent to carcinomas and in carcinomas; numbers of diploid nuclei were 3- to 4-fold greater than numbers of tetraploid nuclei. Abraham et al. (1983) noted that hepatocytes in primates and humans are predominately diploid (>99%) and that exposure of rhesus monkeys to mirex in the diet for 3 years did not produce histopathological changes in liver sections. Although Abraham et al. (1983) reported that exposure was to 5 or 20 ppm in the diet in this monkey study, the published report of this study (Fulfs et al., 1977), of which Abraham was a co-author, noted that the monkeys were given gavage doses of 0.25 or 1 mg/kg/day (6 days/week) for 3 years. Abraham et al. (1983) speculated that mirex may be less hepatotoxic to primates and humans than to rodents due to the absence of hepatocyte polyploid nuclei. Subsequent studies to further test this hypothesis are not available.

Other investigations have shown that acute or repeated oral exposure of Sprague Dawley rats to mirex at concentrations of 100 ppm in the diet can impair hepatobiliary function without elevating levels of serum enzymes indicative of liver cell damage (see Mehendale, 1981 for review). In these studies, it was shown that clearance of phenophthalein glucuronide was impaired by mirex exposure; clearance of other markers, such as sulfobromophthalein, were less sensitive to impairment by mirex. Other studies have shown that mirex inhibits uptake of endogenous organic anions [estradiol-17β(β-D-glucuronide), taurocholate, and L-alanine] by isolated rat hepatocytes (Teo and Vore, 1990) and significantly decreased bile acid concentration

and bile acid secretory rate in rats exposed to 50 mg/kg for 3 days compared with controls (Teo and Vore, 1991). Mirex impairment of hepatobiliary excretion functions has been hypothesized to involve inhibition of ATPase activities involved in membrane transport (Curtis and Mehendale, 1981). The possible role that this effect of mirex may have in the development of non-neoplastic and neoplastic hepatic lesions from chronic exposure is unknown.

The mechanisms or modes of action by which mirex causes cataracts in offspring of exposed female rats are not understood, except that exposure during lactation has been determined to be more important than exposure during gestation (Chernoff et al., 1979b; Gaines and Kimbrough, 1970; see Section 4.3.1). Modes of action by which mirex, at higher exposure levels during gestation, produces other developmental effects (such as increased fetal mortality, decreased survival to weaning, and increased fetal edema) are also not understood.

The mechanisms or modes of action by which repeated exposure to high doses of mirex impairs reproductive functions in male rats or female rats are also poorly understood. Decreased sperm counts have been reported in Sprague-Dawley rats exposed to doses as low as 0.5 mg/kg/day (5 ppm in the diet) with severe testicular degeneration occurring at doses of 5 or 7.5 mg/kg/day (50 or 75 ppm in diet) (Yarbrough et al., 1981), but further mechanistic details are unstudied. Decreased female fertility has been reported in a group of female rats exposed to 2.3 mg/kg/day (25 ppm in diet) for 102 days before mating with non-exposed males (Gaines and Kimbrough, 1970), but the mode of action is unexplained. Subcutaneous exposure to mirex did not produce typical estrogenic effects in immature female rats (Gellert, 1978) and did not directly inhibit ovulation in rats induced by pregnant mare serum (Fuller and Draper, 1975).

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION (IF KNOWN) — ORAL AND INHALATION

4.5.1. Oral Exposure

Mirex has been detected in some monitoring studies of human adipose tissue and human breast milk samples (Burse et al., 1989; Guttes et al., 1998; Kutz et al., 1974, 1985; Mes et al., 1993; Newsome et al., 1995), but no studies were located that investigated possible associations between mirex exposure and non-cancer health effects in humans.

Non-neoplastic toxic responses have been evaluated in dogs (Larson et al., 1979), rats (Chu et al., 1981a; Fulfs et al., 1977; Larson et al., 1979; NTP, 1990; Ulland et al., 1977; Yarbrough et al., 1981), mice (Fulfs et al., 1977), and monkeys (Fulfs et al., 1977) orally exposed to mirex for subchronic or chronic periods.

The liver appears to be one of the most sensitive targets of repeated oral exposure to mirex. Statistically significant non-neoplastic liver effects observed in animals chronically exposed to mirex include fatty metamorphosis, cytomegaly, and necrosis in F344/N rats exposed for 2 years to dietary doses of 0.7 mg/kg/day and greater, but not to doses of 0.007 or 0.08 mg/kg/day (NTP, 1990; PWG, 1992; see Table 4); hepatic cytomegaly, vacuolization, fatty

metamorphosis, and necrosis in CD rats exposed for up to 18 months to survival-reducing doses of 7 mg/kg/day and greater (Ulland et al., 1977); and hepatic cellular hypertrophy, necrosis, and proliferation of smooth endoplasmic reticulum in CD-1 mice exposed for 18 months to doses of 0.9 mg/kg/day, but not to 0.2 mg/kg/day (Fulfs et al., 1977). Only "occasional" focal lymphocytic infiltrations of the liver without other liver lesions were observed in monkeys exposed to 0.25 or 1 mg/kg for up to 36 months (Fulfs et al., 1977), and no gross or histopathologic changes were observed in Sprague Dawley rats exposed for 12 months to 2 mg/kg/day mirex (Fulfs et al., 1977). Several studies of subchronically exposed animals provide supporting evidence that the liver is a sensitive target of mirex toxicity. Liver effects from subchronic exposure, generally observed at higher exposure levels than those producing liver lesions from chronic exposure, have been observed in rats (Chu et al., 1981a; Larson et al., 1979; Yarbrough et al., 1981) and dogs (Larson et al., 1979).

Non-neoplastic lesions in several other organs have been observed in animals repeatedly exposed to mirex, but these lesions appear either to occur at higher dose levels than those affecting the liver, to be less consistent across studies or gender, or to be less persistent than mirex-induced liver lesions. For example, thickening of the follicular epithelium, loss of colloid, and collapse of the follicles were observed in the thyroid of Sprague Dawley rats exposed to doses of 0.7 or 6.5 mg/kg/day for 28 days, but these lesions regressed in a 48-week post-exposure period (Chu et al., 1981a). In contrast, liver lesions in rats exposed to 6.5 mg/kg/day persisted through the 48-week period (Chu et al., 1981a). Increased incidences of similar non-neoplastic thyroid lesions were not observed in F344/N rats exposed for 2 years to dietary doses as high as 3.9 mg/kg/day in males or 7.7 mg/kg/day in females (NTP, 1990). In the NTP (1990) chronic rat bioassay, the lowest dose level producing toxic hepatitis in male and female F344 rats (0.7 mg/kg/day) also produced increased incidence of parathyroid hyperplasia and splenic fibrosis in males (but not females), and increased incidence of nephropathy in females (see Table 3). The nephropathy in this study, however, appears to be more strongly associated with the age of the rats in this study than with exposure. Incidences of nephropathy were high in non-exposed controls and were not significantly elevated (compared with controls) in females in the second study exposed to doses as high as 7.7 mg/kg/day. The NTP study authors (1990) concluded that the "parathyroid hyperplasia is likely a secondary physiological response to the nephropathy" and thus support the interpretation that the parathyroid hyperplasia in male rats was not strongly associated with exposure. No such interpretation of the increased incidence of splenic fibrosis was proposed, but the splenic response was only observed in male rats (liver lesions occurred in both male and female rats exposed to mirex) and has not been reported in other animal studies involving subchronic or chronic oral exposure to mirex. Increased incidences of epithelial hyperplasia of the renal pelvis were observed in male rats exposed to higher doses ($\geq$1.9 mg/kg/day) than the lowest doses producing toxic hepatitis (see Table 3).

Another sensitive effect associated with oral exposure to mirex is cataract development in offspring of exposed female rats. Cataracts in offspring have been reported in studies with female rats orally exposed before mating, during gestation, and/or during lactation to dose levels as low as 0.4 mg/kg/day, but the effect has been more consistently observed at higher dose levels (see Table 7). Exposure during lactation appears to be a critical period for affecting this

endpoint. An early study (Gaines and Kimbrough, 1970) showed that exposure of female Sherman rats to doses of approximately 2.3 mg/kg/day for 45 or 102 days before mating and continuing through gestation and lactation produced decreased numbers of live offspring and offspring surviving to weaning and increased incidences of offspring with cataracts (Gaines and Kimbrough, 1970). The same dose level administered to the rat dams only during lactation also produced cataracts in the offspring. Exposure of a group of females to 0.4 mg/kg/day prior to mating and during gestation and lactation did not affect these endpoints in this study, but increased incidence of cataracts and decreased survival to weaning were observed in a group of offspring of non-exposed females who were nursed by foster dams exposed to 0.4 mg/kg/day (5 ppm in diet) before mating and during gestation (Gaines and Kimbrough, 1970). In another study, in which male and female Sprague Dawley rats were exposed to mirex in the diet for 13 weeks before mating and during mating, followed by exposure of the dams during pregnancy and lactation, statistically significant increased incidence of cataracts in female offspring (but not male offspring) were observed in the group exposed to 0.4 mg/kg/day (5 ppm in diet) (Chu et al., 1981b). Cataracts were detected in male offspring of parents exposed to 1.5 mg/kg/day (20 ppm in diet).

Studies of dose-response relationships for cataracts in offspring established 1 mg/kg/day as a no-effect level and 2.5 mg/kg/day as the lowest effect level when lactating Sherman or Long Evans rat dams were given gavage doses on post natal days 1 through 4 (Chernoff et al., 1979a). Dose levels of 10 and 15 mg/kg were identified as the no-effect level and lowest effect level, respectively, for cataracts in offspring when Long Evans rat dams were given gavage doses only on post natal day 1 (Chernoff et al., 1979a). Mirex-induced cataracts in rat offspring have been observed in several other studies in which rat dams were exposed to dose levels $\geq$5 mg/kg/day during lactation (Rogers and Grabowski, 1984; Scotti et al., 1981) or $\geq$6 mg/kg/day during gestation (Rogers and Grabowski, 1983; Rogers et al., 1984). In addition, increased incidence of offspring with cataracts were reported in studies in which lactating female CD-1 mice were given gavage doses $\geq$3 mg/kg/day on post-natal days 1-4 (Chernoff et al., 1979a).

Other developmental effects including fetal edema, external abnormalities, and reduced fetal survival have been observed in other studies of pregnant rats exposed during gestation to doses of 3 mg/kg/day and greater. In studies with CD albino rats exposed to gavage doses on gestation days 7-16, signs of maternal toxicity (increased relative liver weight and decreased weight gain) and developmental toxicity (edematous live fetuses and decreased sternal ossification) were observed at doses $\geq$7 mg/kg/day, but not at 5 mg/kg/day (Chernoff et al., 1979a). At dose levels $\geq$19 mg/kg/day, more severe developmental effects (e.g., increased fetal mortality and decreased birth weight) were observed (Chernoff et al., 1979a). Other gestational exposure studies identified increased incidence of CD rat fetuses with external abnormalities (edema, ectopic gonads, and hydrocephaly) at 6 mg/kg/day and higher (Kavlock et al., 1982); and, in Wistar rat fetuses, increased incidences of fetal mortality and external abnormalities at 6 and 12.5 mg/kg/day, increased resorptions at 3 mg/kg/day, and no developmental effects at 1.5 mg/kg/day (Khera et al., 1976). Altered electrocardiographic patterns have also been observed in rat offspring of dams exposed during gestation to doses of 5-6 mg/kg/day (Grabowski, 1983a,b; Grabowski and Payne, 1980, 1983a,b).

No adequate multiple-generation reproductive toxicity study has been conducted for mirex, but results from single-generation rat studies indicate that exposure to mirex can affect male and female reproductive ability. Impaired male fertility has been observed in Wistar rats exposed to gavage doses of 6 mg/kg/day for 10 days (but not in rats exposed to 3 mg/kg/day) before mating with non-exposed females (Khera et al., 1976) and in male Sprague Dawley rats exposed to 2.8 mg/kg-day for 13 weeks prior to mating and during mating to similarly exposed females (Chu et al., 1981b). In the latter study, no decreases were observed in the percentage of pregnant females at lower exposure levels up to 1.4 mg/kg-day in the diet, and no sperm was detected in vaginal smears of females at 2.8 mg/kg-day, but decreased average number of pups per litter occurred in groups exposed to 0.4, 0.8, or 1.5 mg/kg/day (Chu et al., 1981b). Decreased sperm counts have been reported in male Sprague Dawley rats exposed for 28 days to doses as low as 0.5 mg/kg/day (5 ppm in diet), with severe testicular degeneration occurring at doses of 5 or 7.5 mg/kg/day (50 or 75 ppm in diet) (Yarbrough et al., 1981). Male fertility was not affected in Sherman rats exposed to 2.3 mg/kg/day (25 ppm in diet) for 45 or 102 days before mating with non-exposed female rats, but a group of female Sherman rats exposed for 102 days to 25 ppm in the diet before mating with non-exposed males delivered fewer number of litters than a group of non-exposed male and female Sherman rats (Gaines and Kimbrough, 1970). The mode of action of this effect of mirex on female reproductive ability in Sherman rats is unexplained. Subcutaneous mirex did not display typical estrogenic effects in immature female Sprague Dawley rats (Gellert, 1978) and did not appear to directly act on the ovary in inhibiting ova release in Long Evans rats induced by pregnant mare serum (Fuller and Draper, 1975).

4.5.2. Inhalation Exposure

No studies were located examining potential health effects in humans or animals following repeated inhalation exposure to mirex.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION — SYNTHESIS OF HUMAN, ANIMAL, AND OTHER SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN CARCINOGENICITY, AND LIKELY MODE OF ACTION

Studies designed to investigate possible associations between mirex exposure and cancer in humans are limited to a case-control study of postmenopausal women with breast cancer that found no statistically significant association between detectable mirex in serum and the occurrence of breast cancer (Moysich et al., 1998). Thus, the available human data are inadequate for assessing the potential carcinogenicity of mirex in humans.

Carcinogenicity studies of animals chronically exposed to mirex are restricted to dietary studies of two strains of rats, F344/N (NTP, 1990) and CD rats (Ulland et al., 1977) and dietary studies in two strains of mice, B6C3F1 and B6AKF1 (Innes et al., 1969). The liver histopathology slides from the NTP bioassay were re-evaluated by a workgroup convened by PATHCO, Inc. (PWG, 1992). Significantly increased incidences of liver tumors were found in F344/N rats of both sexes, in male CD rats, and in B6C3F1 and B6AKF1 mice of both sexes.

The liver tumors were reported to be mostly benign in F344/N rats and an equal mix of benign and malignant tumors in CD rats. Innes et al. (1969) reported that metastases of liver tumors were rare, but did not report the occurrences.

In addition, statistically significant increased incidences of tumors at several other sites were observed, but these observations were not as consistent across studies as the tumorigenic response in the liver. Increased incidences of adrenal pheochromocytomas (mostly benign) were observed in groups of male F344/N rats exposed to 25 and 50 ppm in the diet, in one group of female F344/N rats exposed to 50 ppm, but not in another group of female F344/N rats exposed to 50 ppm (NTP, 1990). Increased incidences of mononuclear cell leukemia were found in male F344/N rats exposed to 25 ppm, but not in males exposed to 50 ppm, and in F344/N females exposed to 25 and 50 ppm in a first study, but only in females exposed to 100 ppm in a second study (NTP, 1990). Significantly increased incidence of tumors at these sites were not found in the studies of CD rats (Ulland et al., 1977). In B6C3F1 and B6AKF1 mice, no lymphomas were induced by oral administration of mirex, but examination for neoplastic lesions in other organs was not comprehensive in this study (Innes et al., 1969). In the only other animal studies related to the potential carcinogenicity of mirex, subcutaneous injection of large (1000 mg/kg) single doses produced increased incidences of reticulum cell sarcomas in B6C3F1 mice (Innes et al., 1969), and dermally applied mirex promoted skin tumors in mice given initiating dermal doses of 7,12-dimethylbenz[a]anthracene (Kim and Smart, 1995; Moser et al., 1992, 1993). Overall, the database provides adequate evidence of carcinogenic hepatic responses to lifetime oral exposure to mirex in rats and mice, and less consistent evidence of carcinogenic responses at other sites.

Results from short-term genotoxicity testing indicate that mirex is not genotoxic, and thereby suggest that mirex may produce carcinogenic responses via a non-genotoxic mode of action. Mirex did not produce reverse mutations in *Salmonella typhimurium* or in *Escherichia coli* with or without metabolic activation (Hallett et al., 1978; Mortelmans et al., 1986; Probst et al., 1981; Schoeny et al., 1979), forward mutations at the HGPRT locus in human fibroblast cells (Tong et al., 1981), unscheduled DNA synthesis in rat, mouse, or hamster hepatocytes (Maslansky and Williams, 1981; Probst et al., 1981; Williams, 1980), or sister chromatid exchanges or chromosomal aberrations in Chinese hamster ovary cells (NTP, 1990). Mirex did not induce dominant lethal mutations in offspring of male rats exposed to oral doses as high as 6 mg/kg/day for 10 days (Khera et al., 1976) and did not produce hepatic DNA damage in mice exposed to oral doses >80 mg/kg/day for 5 days (Umegaki et al., 1993) or in rats exposed to two oral doses of 120 mg/kg (Mitra et al., 1990).

Following U.S. EPA (1986a) *Guidelines for Carcinogen Risk Assessment*, mirex is placed in Cancer Group B2- *Probable Human Carcinogen,* based on inadequate human evidence and adequate evidence of carcinogenicity in animals (increased incidence of liver tumors in two strains of each of two species, rats and mice). Following U.S. EPA (1999a) Draft Revised Guidelines for Carcinogen Risk Assessment, the hazard descriptor, *Likely to be Carcinogenic to Humans*, is appropriate for mirex based on consistent findings of hepatic carcinogenic responses, and less consistent findings of tumors in other tissues, in several studies of rats and mice

chronically exposed to mirex in the diet. The human relevance of the animal evidence of carcinogenicity is assumed in the absence of adequate human data or mechanistic data to indicate that the mode of carcinogenic action in animals is not relevant to humans.

The modes of action whereby mirex induces liver tumors in animals are not yet completely understood, but available data suggest that a nongenotoxic mode involving mirex-induced hepatic cell proliferation is plausible. Short-term genotoxicity testing, both *in vitro* and *in vivo*, has produced no evidence of genotoxic actions from mirex, and metabolic studies indicate that animals are not able to metabolize mirex and produce potentially reactive metabolic intermediates that may be genotoxic. Mirex is a promoter, but not an initiator, of skin tumors in the 2-stage mouse skin model, providing indirect support for an hypothesis that mirex may cause liver tumors via promotion of previously initiated cells. Short-term and repeated oral exposure to mirex are known to cause hepatic cytomegaly, vacuolization, fatty metamorphosis, and necrosis, which may be precursor events to the eventual development of liver tumors, but this hypothesis is not fully established.

The NTP (1990) rat study provides useful data for describing dose-response relationships for liver tumors and non-neoplastic liver lesions (see Table 4). Five or six dietary concentrations plus a control were included in this study (0, 0.1, 1, 10, 25, or 50 ppm in males and 0, 0.1, 1, 10, 25, or 50 ppm in females in one study, and 0, 50, and 100 ppm in another study). Incidences for hepatocellular adenomas and carcinomas (as re-evaluated by PWG, 1992) were significantly elevated at the 25- and 50-ppm level in males and the 50- and 100-ppm levels in females, but were not elevated at the 0.1, 1, or 10-ppm levels (Table 4). This observation is consistent with the existence of a threshold for liver cancer above the 10-ppm dietary exposure level, but is not definitive evidence given the limited number of animals (n = 50/sex) included in this type of bioassay. The apparent threshold for non-neoplastic liver lesions was above the 1-ppm level; statistically significant elevated incidences of hepatotoxic effects were observed at dietary concentrations of 10 ppm and greater. Most (but not all) rats that had liver tumors in this study were diagnosed with non-neoplastic liver lesions such as cytomegaly, fatty metamorphosis, vacuolization, or necrosis. Following U.S. EPA (1999a) guidance, a linear approach to dose-response assessment should be taken for agents such as mirex that are not DNA reactive and whose mode of action is not established. It is acknowledged, however, that the evidence for a nongenotoxic mode of action involving non-neoplastic liver cell changes as precursor events is stronger than evidence for a genotoxic mode of action without a threshold.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

The extent to which children are more susceptible to the toxic effects of mirex is not known. No data is available regarding the adverse effects of mirex in human children. However, mirex has demonstrated developmental toxicity in rodents (discussed in Section 4.3.2), suggesting that infants and children may be more likely than adults to experience adverse effects following exposure to mirex.

4.7.2. Possible Gender Differences

The extent to which men and women differ in susceptibility to mirex toxicity is not known. No human data are available to suggest there are gender differences in the toxicity of mirex.

Results from animal studies indicate that the possibility of gender differences in susceptibility to mirex depends on the endpoint evaluated.

Several studies indicate that male rats may be more susceptible than female rats to mirex hepatotoxicity, but the results are not entirely consistent. The NTP (1990) observed statistically significant increases in mortality and the incidences of neoplastic liver lesions at lower doses in male than in female F344/N rats. Based on the PWG (1992) re-evaluation of the liver histopathology, the apparent gender difference in susceptibility to mirex carcinogenicity in the liver was still evident, but significantly increased incidences of non-neoplastic toxic hepatitis occurred at the same exposure levels in male and female groups (see Table 4). In addition, the female groups displayed higher incidences of toxic hepatitis than male groups at the same exposure level (Table 4). Gaines and Kimbrough (1970) concluded that male Sherman rats were slightly more sensitive to the hepatotoxic effects of mirex, based on light and electron microscopic analysis of livers after 166 days of dietary exposure. Khera et al. (1976) observed statistically significant reduced weight gain at a lower dietary dose of mirex in males compared to females.

Female mice may be more susceptible than males to the skin tumor promoting property of mirex. Moser et al. (1993) concluded that ovarian hormones may increase the susceptibility of female CD-1 mice to the dermal tumor-promoting activity of mirex. CD-1 mice were dermally exposed to 7,12-dimethylbenz[a]anthracene to initiate tumor formation, and then exposed to 200 nmol mirex for 20 weeks. In ovary-intact female mice, compared to ovariectomized mice, a statistically significant increase in the number of tumors per mouse was observed. Compared with male mice, female ovary-intact mice exhibited a higher number of mice bearing tumors, more tumors per mouse, a shorter time to first tumor formation and a shorter tumor latency.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect—With Rationale and Justification

As discussed in Section 4.5.1., results from rodent studies (predominantly rat studies) indicate that sensitive targets of repeated oral exposure to mirex include the liver, ocular development in offspring, and offspring survival during gestation or lactation. Non-neoplastic liver lesions including fatty metamorphosis, cytomegaly, and necrosis have been observed in F344/N rats exposed for 2 years to dietary doses as low as 0.7 mg/kg/day (10 ppm in diet) (NTP, 1990; PWG, 1992). Non-neoplastic lesions in other tissues (such as epithelial hyperplasia in the renal pelvis, thyroid lesions, or splenic fibrosis) have been observed in rats subchronically or chronically exposed to orally administered mirex, but these effects appear either to occur at higher dose levels than those affecting the liver, to be less consistent across studies or gender, or to be less persistent than mirex-induced liver lesions (see Section 4.5.1). Increased incidence of cataracts have been observed in offspring of female rats exposed to doses as low as 0.4 mg/kg/day (5 ppm in diet) prior to mating and during gestation and lactation, but this effect has been observed more consistently across studies and offspring gender at dose levels >1-2 mg/kg/day (see Table 7 in Section 4.5.1). Likewise, there are two reports that exposure to dose levels of 0.4 mg/kg/day produced decreased survival of pups to weaning (Gaines and Kimbrough, 1970) and decreased number of pups per litter (Chu et al., 1981b), but these endpoints were affected more consistently across studies at dose levels >1-2 mg/kg/day.

Results from single-generation reproduction studies in rats indicate that although repeated oral exposure to low doses of mirex decreased sperm counts, effects on male or female fertility occur with repeated exposure to dose levels greater than 2-3 mg/kg/day. Oral exposure of male rats to mirex doses as low as 0.5 mg/kg/day (5 ppm in diet) decreased sperm counts (Yarbrough et al., 1981), but effects on male fertility were only observed in one study that subchronically exposed male and female rats to high levels of mirex in the diet (40 ppm mirex; estimated dose of about 3 mg/kg/day) and not at lower levels (Chu et al., 1981b; Gaines and Kimbrough, 1970). A possible effect on female rat fertility (i.e., decreased number of litters) has been reported in a group of female rats exposed to 25 ppm in the diet (estimated dose of 2.3 mg/kg/day) for 102 days before mating with non-exposed males, but this effect was not observed at lower exposure levels (1 or 5 ppm in diet).

The NTP (1990) rat bioassay (with liver histopathology as re-evaluated by the PWG [1992]) is selected as the principal study for RfD derivation because it provides the best available characterization of dose-response relationships for one of the sensitive targets of repeated oral exposure to mirex identified in animal studies. The available animal data indicate that an RfD based on liver effects in rats will be protective for other potential effects from chronic exposure to mirex, including developmental and reproductive effects.

Current understanding of modes of action by which mirex adversely affects the liver, ocular development in offspring, offspring survival during gestation or lactation, or male or female reproductive performance is unclear and inadequate to disregard the possible relevance of these effects to humans exposed to mirex. A hypothesis has been presented that humans and other primates may be less susceptible to the hepatotoxicity of mirex than rats and mice (Abraham et al., 1983), but support for this hypothesis is not strong. A study of rhesus monkeys exposed to gavage doses of 0.25 or 1 mg/kg/day (6 days/week) for 3 years (reported by Abraham et al., 1983 and Fulfs et al., 1977) found only "occasional" focal lymphocytic infiltrations of the liver without other liver lesions, but the limited number of animals in the study, the short duration of exposure (as a percentage of a reference lifetime of 18 years for rhesus monkeys), and the lack of other corroborative primate data preclude drawing definitive conclusions regarding the relative susceptibility of rodents and primates to the hepatotoxicity of chronic exposure to mirex.

5.1.2. Methods of Analysis—Including Models (PBPK, BMD, etc.)

Using the array of mathematical models in the EPA Benchmark Dose Software, models were fit to the incidence data for toxic hepatitis in male or female F344 rats in the NTP (1990) bioassay (Appendix B). The best fits as indicated by graphical examination and goodness of fit statistics for both sets of data were with the log-logistic model. Figures 1 and 2 in Appendix B plot observed and predicted incidences of toxic hepatitis as functions of human equivalent doses based on the male and female rat responses, respectively. The models predict ED10 values (human equivalent doses associated with 10% incidence of toxic hepatitis) of 0.28 and 0.13 mg/kg/day based on the male and female rat data, respectively. These predicted values are for extra risk (i.e. incidence - background incidence/ 1- background incidence; $P_{(d)} - P_{(0)}/ 1-P_{(0)}$). The points of departure for the benchmark dose approach to RfD derivation (i.e., the lower 95% confidence limit on the ED10 = LED10) are 0.20 and 0.10 mg/kg/day based on male and female rat data, respectively. Given that these LED10 values are similar, an average LED10 of 0.15 mg/kg/day is taken as the point of departure for derivation of the RfD for mirex.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors and Modifying Factors

The point of departure from the benchmark dose modeling of toxic hepatitis (LED 10 for toxic hepatitis = 0.15 mg/kg/day) is divided by a total uncertainty factor of 300 (10 for extrapolating from rats to humans, 10 to account for human variability, and 3 to account for data base deficiencies including the lack of 2-generation reproductive toxicity studies) to derive a chronic oral RfD for mirex of 5×10^{-4} mg/kg/day (0.5 µg/kg/day or 0.0005 mg/kg/day).

This RfD is similar in value to one derived in 1992 (2×10^{-4} mg/kg/day) based on the original NTP (1990) liver histopathology, a NOAEL/LOAEL approach that used a rat NOAEL of 0.07 mg/kg/day (without dosimetric adjustment) for liver and thyroid lesions, and a total uncertainty factor of 300 (10 for extrapolating from rats to humans, 10 for human variability, and 3 for database deficiencies).

Confidence in the principal study is high. It included sufficient numbers of animals of both sexes for statistical analysis, adequate numbers of exposure levels to provide good descriptions of dose-response relationships for relevant endpoints, and was adequately designed, conducted, and reported. Confidence in the data base is medium. Several subchronic and chronic oral exposure studies are available in rats and mice identifying the liver as a sensitive target of mirex toxicity and, as discussed in Section 4.5.1. Analysis of the data base indicates that the RfD based on liver effects will be protective of other identified sensitive effects from mirex exposure including splenic fibrosis from chronic exposure, developmental effects including cataracts and reduced pre-weaning offspring survival, and impaired male or female reproductive performance. Confidence in the data base could be increased with data from 2-generation reproductive toxicity studies and comparative mechanistic studies providing more information regarding possible differences between primates and rodents in susceptibility to mirex toxicity. Resultant confidence in the RfD is medium to high.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

No inhalation RfC is derived due to the lack of appropriate inhalation exposure toxicity data for humans or animals and the lack of rat and human PBPK models for mirex that would facilitate extrapolating across exposure routes. In addition, the oral route is the most relevant route for exposure to mirex in the general environment.

5.3. CANCER ASSESSMENT

As discussed in Section 4.6, mirex is likely to be carcinogenic to humans based on consistent findings of hepatic carcinogenic responses, and less consistent carcinogenic responses at other tissue sites, in several studies of rats and mice chronically exposed to mirex in the diet. The animal evidence for mirex carcinogenicity is assumed to be relevant to humans in the absence of adequate human data or mechanistic data to indicate that the mechanisms of mirex carcinogenic action in rodents are not relevant to humans.

The mechanisms by which mirex induces liver tumors in animals are not completely understood. Mirex is nongenotoxic in multiple short-term *in vitro* and *in vivo* genotoxicity tests and is a promoter, but not an initiator, of skin tumors in the two-stage mouse skin model. Short-term or repeated oral exposure of rodents to mirex causes hepatic cytomegaly, vacuolization, fatty metamorphosis and necrosis, which may be precursor events to the eventual development of liver tumors, but this hypothesis is not fully established. Consistent with this hypothesis is the correlation found between non-neoplastic liver lesions and hepatocellular adenomas in mirex-exposed rats in the NTP (1990) bioassay, but not all rats with adenomas were diagnosed with non-neoplastic liver lesions (as diagnosed by the PWG [1992] histopathological examination). For example, among the six male rats with adenomas in the group exposed to 1.9 mg/kg/day, four were diagnosed toxic hepatitis or eosinophillic foci, but two were not. In the group exposed to 3.9 mg/kg/day, the diagnosis was eight with, and two without, non-neoplastic liver lesions among the 10 male rats with liver adenomas.

Following U.S. EPA (1999a) guidance, a linear approach to cancer dose-response assessment should be used for agents like mirex, whose mode of action is not fully understood. It is acknowledged, however, that the evidence for a nongenotoxic mode of action involving non-neoplastic liver cell changes as precursor events for mirex-induced cancer is stronger than evidence for a genotoxic mode of action without a threshold. Incidence data for male rats in the NTP (1990) bioassay (with liver histopathology re-evaluated by the PWG [1992]) were used in this analysis as male rats appeared to be more susceptible to mirex-induced liver tumors than female rats. As discussed previously, the NTP bioassay provides the best available data to describe dose-response relationships for neoplastic and non-neoplastic liver responses.

One linear approach involved fitting a model with an exponential polynomial function of dose and a Weibull function of time-to-tumor to the incidence data for combined adenomas and carcinomas in male rats and associated doses converted to human equivalent doses (this conversion is discussed in Section 5.1.2). A licensed computer program, *Multi-Weib* (K.S. Crump and Company, Ruston, LA), was used to fit the model to the data. A three-degree polynomial form of the dose function provided the best fit of the data. The lower 95% confidence limit of the dose associated with a 10% extra risk (LED10) for tumors was 0.193 mg/kg/day (calculated for a full lifetime, 105 weeks). Using linear extrapolation from the LED10 to the origin, predicted doses associated with extra risks of 10^{-4}, 10^{-5}, and 10^{-6} are 0.0002, 0.00002, and 0.000002 mg/kg/day, respectively. Using the upper 95% confidence limit on the slope in the low-dose region (i.e., the q1* or oral slope factor = 0.53 per mg/kg/day), predicted doses associated with extra risks of 10^{-4}, 10^{-5}, and 10^{-6} are the same: 0.0002, 0.00002, and 0.000002 mg/kg/day, respectively.

Linear approaches without the time-to-tumor analysis were also applied to the male rat tumor incidence data (Table 4) using the array of discontinuous mathematical models in the EPA Benchmark Dose Software. For liver adenomas and carcinomas combined, a probit model provided the best fit among the models examined. The ED10 and LED10 values predicted by the model were 0.6 and 0.5 mg/kg/day. Extrapolating from the LED10, predicted doses associated with extra risks of 10^{-4}, 10^{-5}, and 10^{-6} are 0.0005, 0.00005, and 0.000005 mg/kg/day.

The model that included time-to-tumor as an explanatory variable was selected as the linear approach of choice for these data, because there was statistically significantly reduced survival of male rats in the two highest dose groups in the NTP (1990) bioassay. The modeling provides a means of adjusting for less-than-lifetime exposure for the rats with tumors that died early, and estimating risk for liver tumors with full life-time exposure.

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Human data regarding mirex toxicity are limited, but results from testing in animals indicate multiple targets of toxicity with the liver, the developing fetus and neonate, and the male reproductive organs being the most sensitive targets identified to date. Chronic oral exposure to mirex is anticipated to increase risks in humans for 1) liver cytomegaly and necrosis based on observations in rats exposed for life to mirex at dose levels as low as 0.7 mg/kg/day (10 ppm in the diet), 2) developmental effects including impaired ocular development (cataracts) and decreased post-natal survival based on observations in rats exposed before mating and during gestation and lactation to dose levels as low as 0.4 to 1.5 mg/kg/day (5-20 ppm in the diet), and 3) decreased sperm counts and male fertility based on observations of decreased sperm counts in male rats exposed for 28 days to oral doses as low as 0.5 mg/kg/day (5 ppm in diet) and decreased fertility in male rats exposed for 13 weeks to doses of 2.8 mg/kg/day (40 ppm in diet) or for 10 days to gavage doses of 10 mg/kg/day (but not 6 mg/kg/day).

In addition, mirex is likely to be carcinogenic to humans based on consistent findings of increased incidences of liver tumors in several studies of rats and mice chronically exposed to mirex in the diet and less consistent findings (across and within studies) of carcinogenic responses in other tissues or organs in rats. The mechanisms by which mirex induces liver tumors in animals are not completely understood. Mirex has not been genotoxic in multiple short-term tests and is a promoter, but not an initiator, of skin tumors in the two-stage mouse skin model. Short-term or repeated oral exposure of rodents to mirex causes hepatic cytomegaly, vacuolization, fatty metamorphosis and necrosis, which may be precursor events to the eventual development of liver tumors, but this hypothesis is not fully established. Supporting this hypothesis is the correlation found between non-neoplastic liver lesions and hepatocellular adenomas in mirex-exposed rats, but not all rats with adenomas were diagnosed with non-neoplastic liver lesions.

6.2. DOSE RESPONSE

The array of results from animal studies indicates three similarly sensitive targets of mirex chronic noncancer toxicity (i.e., the liver, the developing fetus and neonate, and the male reproductive organs). The NTP (1990) rat bioassay (with liver histopathology re-evaluated by the PWG [1992]) is selected as the principal study for RfD derivation because it provides the best characterization of dose-response relationships for these sensitive targets. It is anticipated that an RfD based on liver effects will be protective for other potential effects from chronic exposure to mirex, including developmental and reproductive effects.

An RfD for mirex of 0.0005 mg/kg/day is derived using a point of departure from the benchmark modeling of toxic hepatitis incidence rat data and human equivalent doses (LED10 = 0.15 mg/kg/day) divided by a total uncertainty factor of 300 (10 for extrapolating from rats to

humans, 10 to account for human variability, and 3 to account for data base deficiencies including the lack of two-generation reproductive toxicity studies). The value of this RfD is similar to the RfD derived in 1992 (0.0002 mg/kg/day) based on the original NTP liver histopathology, a NOAEL/LOAEL approach and an uncertainty factor of 300 (10 for interspecies extrapolation without dosimetric adjustment, 10 for human variability, and 3 for data base deficiencies). Confidence in the RfD is medium to high based on high confidence in the principal study and medium confidence in the data base.

U.S. EPA (1999a) Draft Revised Guidelines for Carcinogen Risk Assessment states that for chemicals like mirex, where there is an absence of sufficient tumor mode of action information, a linear approach for carcinogenic risk assessment should be used. Several linear extrapolation approaches involving mathematical modeling of liver male rat tumor incidences and human equivalent doses (from the NTP, 1990 bioassay with re-evaluation of liver histopathology by PWG [1992]) were taken and arrived at similar estimates of doses associated with extra risk levels of 10^{-4}, 10^{-5}, and 10^{-6}: 0.0002-0.0005, 0.00002-0.00005, and 0.000002-0.000005 mg/kg/day, respectively (0.2-0.5, 0.02-0.05, 0.0002-0.005 μg/kg/day). The approach that included time-to-tumor as an explanatory variable was selected as the linear approach of choice for these data, because there was statistically significantly reduced survival of male rats in the two highest dose groups in the NTP (1990) bioassay. With this approach, predicted doses associated with extra risks of 10^{-4}, 10^{-5}, and 10^{-6} are: 0.0002, 0.00002, and 0.000002 mg/kg/day, respectively (0.2, 0.02, and 0.002 μg/kg/day).

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APPENDIX B: BENCHMARK DOSE MODELING RESULTS

All dichotomous models in the Benchmark Dose Software (version 1.3.2) were fit to the data for the incidence of toxic hepatitis in F344/N male and female rats fed Mirex in the diet for 2 years (Table 1). As assessed by the chi-square goodness-of-fit statistic, the log-logistic, gamma, 5-degree polynomial multistage, quantal linear, and Weibull models for the male rats, and the log-logistic model for the female rats adequately fit the data for the incidence of toxic hepatitis. The log-logistic models provided the best fit as assessed by Aikake's Information Criteria (AIC) for both the male and female data (Table 2).

Table 1. Incidence of toxic hepatitis in F344/N male and female rats fed Mirex in the diet for 2 years

	Rat dose mg/kg-day					
	0	0.007	0.08	0.7	1.9	3.9
Male	1/52	2/52	5/52	11/52	28/52	29/52
Female	1/52	1/52	3/52	25/52	34/52	35/52

Table 2. Dose response modeling of incidence data for toxic hepatitis in male and female F344/N rats fed mirex in the diet for 2 years

MODEL	ED_{10}	LED_{10}	x2 p-value	AIC
Male rats				
Log-logistic[b]	0.28	0.20	0.40	264.63
Gamma[a]	0.40	0.32	0.13	267.57
Multi-stage[c]	0.40	0.32	0.13	267.57
Quantal linear	0.40	0.32	0.13	267.57
Weibull[a]	0.40	0.32	0.13	267.57
Log-probit[b]	0.68	0.55	0.02	271.90
Logistic	0.99	0.84	0.00	283.99
Probit	0.93	0.79	0.00	282.06
Quantal quadratic	1.19	1.04	0.00	287.92
Female rats				
Log-logistic[b]	0.13	0.10	0.23	256.82
Gamma[a]	0.24	0.20	0.00	269.03
Logistic	0.69	0.59	0.00	305.53
Multi-stage[c]	0.24	0.20	0.00	269.03
Probit	0.67	0.58	0.00	303.88
Log-probit[b]	0.38	0.32	0.00	273.91
Quantal linear	0.24	0.20	0.00	269.03
Quantal quadratic	0.97	0.85	0.00	317.03
Weibull[a]	0.24	0.20	0.00	269.03

a = Restrict power >=1

b = Slope restricted to >1

c = Restrict betas >=0; Degree of polynomial = 5

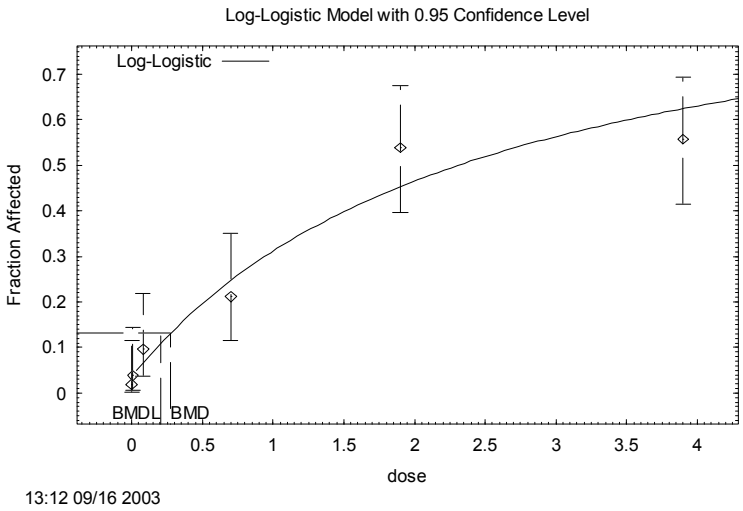

Figure 1: Dose response modeling of incidence data for toxic hepatitis in male F344/N rats fed mirex in the diet for 2 years. BMD = ED_{10}; BMDL = LED_{10}; Dose unit = mg/kg-day

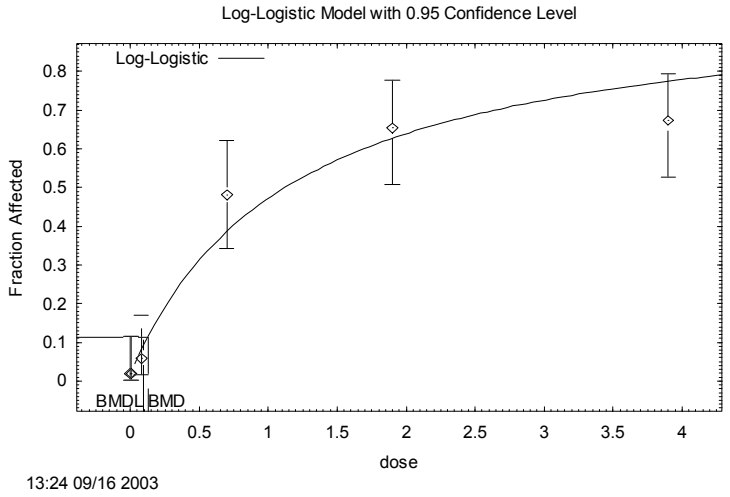

Figure 2: Dose response modeling of incidence data for toxic hepatitis in female F344/N rats fed mirex in the diet for 2 years. BMD = ED_{10}; BMDL = LED_{10}; Dose unit = mg/kg-day