



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.

U.S. Environmental Protection Agency
Washington DC

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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 (extrapulmonary or systemic effects). It is generally expressed in units of mg/m³.

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 µg/m³ 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*

1 (U.S. EPA, 2000a); Benchmark Dose Technical Guidance Document (U.S. EPA, 2000b);
2 *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S.
3 EPA, 2000c) and a memorandum from EPA Administrator, Carol Browner, dated March 21,
4 1995, Subject: Guidance on Risk Characterization.

5
6 Literature search strategies employed for these compounds were based on the CASRN
7 and at least one common name. At a minimum, the following databases were searched: RTECS,
8 HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE,
9 CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information
10 submitted by the public to the IRIS Submission Desk was also considered in the development of
11 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, crystalline 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 483-487°C, and decomposition occurs between 485 and 525°C (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 x 10 ⁻⁷ mm Hg at 25°C
Water solubility:	insoluble, practically insoluble, 0.60 mg/L, 0.2 mg/L at 24°C
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 [¹⁴C]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 [¹⁴C]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 [¹⁴C]-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/kg¹) unlabeled dietary mirex. In pre-fed animals, total

¹Gibson 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.

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

3
4 Groups of male or female Sprague Dawley rats were administered a single oral dose of
5 approximately 0.15 to 0.3 mg/kg [¹⁴C]-mirex in soybean oil (Chambers and Yarbrough, 1979;
6 Chambers et al., 1982). Fecal excretion during the first two days of treatment was 28.85% for
7 males and 21.2% for females; fecal excretion during the next 12 days was 2.6% for males and
8 3.3% for females. Gastrointestinal absorption was therefore determined to be approximately
9 71-79%.

10
11 Groups of 12 female Sprague Dawley rats and groups of 50 Japanese quail were fed diets
12 containing 0, 0.3, 3.0 or 30 ppm [¹⁴C]mirex for various periods of time (Ivie et al., 1974).
13 Percentages of elimination were reportedly similar among different dose levels. Rats eliminated
14 24.2% of mirex within 24 hours and an additional 5.9% during the next 6 days, indicating that
15 approximately 75.2% was absorbed gastrointestinally. Quail eliminated 15.8% within 24 hours
16 and an additional 5.0% during the next 6 days, indicating that approximately 84.2% was
17 absorbed gastrointestinally.

18
19 One female rhesus monkey received a single 1 mg/kg dose of [¹⁴C]mirex in ethanol
20 orally, in a gelatin capsule (Wiener et al., 1976). Approximately 25% of the radiocarbon dose
21 appeared in the feces in the first 48 hours, indicating that gastrointestinal absorption was
22 approximately 75%.

23
24 Doses of 0, 0.25 or 1.0 mg/kg of mirex were administered orally to male and female
25 rhesus monkeys for 510 days (Pittman et al., 1976). At an unspecified time near study
26 termination, a 24-hour fecal sample was analyzed. Recovery was approximately 10% suggesting
27 that gastrointestinal absorption was 90%.

28
29 Rozman et al. (1984) administered [¹⁴C]mirex to 3 lactating dairy goats intraruminally.
30 Fecal elimination was not significant on day 1, and on days 2, 3, and 4 was 13.89%, 6.41% and
31 4.87% of the total administered dose. Total fecal elimination over 14 days was 44.5%. Peak
32 elimination in milk was on day 3. Therefore, gastrointestinal absorption in sheep was
33 approximately 80%.

34
35 Absorption of mirex following milk ingestion is a source of mirex exposure. After giving
36 birth, unspecified numbers of CD rat dams received 0, 1, or 10 mg/kg [¹⁴C]mirex on postnatal
37 days 2-5; single pups were sacrificed on postnatal days 3, 5, 7, 9, 12, 15 and 17. Mirex was
38 detected in maternal milk, milk samples taken from pups' stomachs, in pup brain and liver tissue
39 (Kavlock et al., 1980).

40 **3.1.2. Other Routes of Absorption**

41
42
43 One study was identified demonstrating absorption of mirex by inhalation. Research
44 cigarettes, 85 mm, were impregnated with 100 ppm [¹⁴C]mirex in acetone; lightly anesthetized

1 female Sprague Dawley rats were exposed to eight 5-mL puff volumes of mainstream smoke at
2 15-second intervals (Atallah and Dorough, 1975; Dorough and Atallah, 1975). Within 4
3 minutes, 47% of the mirex was exhaled, 36% remained in the lungs, and 14.4% was present in
4 other tissues of the body.

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

13
14 Smrek et al. (1978) conducted pharmacokinetic experiments in groups of 5 male and 5
15 female goats. Authors report that mirex was detected in young fetuses harvested from mirex-
16 treated mothers, and conclude that mirex was absorbed transplacentally.

17
18 Although no data was found regarding dermal exposure of mirex leading to systemic
19 absorption, one laboratory has reported that mirex promotes formation of skin tumors in female
20 CD-1-mice initiated with 200 nm 7,12-dimethylbenz(a)anthracene (Moser et al., 1992, 1993;
21 Meyer et al., 1993, 1994; Kim and Smart, 1995; Kim et al., 1997). These observations appear to
22 indicate that mirex is locally absorbed following dermal exposure.

23 24 **3.2. DISTRIBUTION**

25
26 Mirex is highly stable and rapidly partitions into fat (ATSDR, 1995). Mirex has been
27 detected in human adipose tissues (Kutz et al., 1974, 1985; Burse et al., 1989). Testing of
28 adipose tissues found mirex concentrations of 0.16 to 5.94 ppm in men and 0.3 to 1.13 ppm in
29 women who lived in areas of high insecticidal usage of mirex (Kutz et al., 1974). In residents of
30 Memphis, Tennessee, living near a waste site, adipose mirex levels were as high as 3.72 ppm
31 (Burse et al., 1989); serum levels were predictive of adipose levels.

32
33 A 6 mg/kg dose of [¹⁴C]mirex in corn oil was administered to five male CD-1 rats by
34 gavage (Mehendale et al., 1972). After 7 days, all animals were sacrificed. Of the 93.88% total
35 recovery, 58.5% was present in feces, 0.69% in urine, 27.8% in fat, 1.75% in liver, 0.76% in
36 small intestine, 3.20% in muscle, 0.23% in large intestine, 0.06% in stomach, 0.03% in heart,
37 0.09% in kidney, 0.07% in brain, 0.12% in testes, and 0.08% in lung. On a percent of total
38 administered mirex per gram of tissue basis, mirex was most concentrated in fat at 1.54%,
39 kidneys at 0.25%, and liver at 0.15%; all other tissues were 0.10% or less.

40
41 A single 0.2 mg/kg dose of [¹⁴C]-mirex was administered by gavage to an unspecified
42 number of mature female Sprague Dawley rats (Gibson et al., 1972). Tissue distribution was
43 measured in four tissues, in descending order of mirex concentration, fat, liver, kidney, muscle
44 and brain. Animals were sacrificed and measured 1, 2, 7, 14, and 28 days after mirex

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

3
4 A single dose of approximately 1 mg/kg [¹⁴C]mirex was given intravenously to 2 female
5 rhesus monkeys and orally to 1 female rhesus monkey (Kennedy et al., 1975; Wiener et al.,
6 1976). In injected animals, plasma mirex levels were initially high and decreased rapidly within
7 hours and were not accurately detectable after 9 days; in the orally dosed animal, radiolabel was
8 not detected in plasma prior to 2 hours and reached a maximum at 5 hours. Route of
9 administration did not otherwise significantly affect tissue distribution or excretion rates. The
10 highest concentration was in fat, approximately 80% of the retained radiolabel. In decreasing
11 order of magnitude, mirex was found in the adrenal, peripheral nerve, thyroid, and skin. Low
12 levels were detected in all 38 tissues and the 7 types of fat tested.

13
14 Groups of male or female Sprague Dawley rats received a single oral dose of
15 approximately 0.15 to 0.3 mg/kg [¹⁴C]mirex (Chambers et al., 1982). Mirex accumulated in fat
16 at high levels, in nervous tissues at low to moderate levels, and in the testes and the
17 gastrointestinal tract at low levels. In females, mirex concentrations in the ovaries were more
18 than 5-fold higher than in other organs at 1 week after dosing but were similar to other organs at
19 2 weeks after dosing; a similar response was seen with 2,8-dihydromirex.

20
21 Groups of 12 female Sprague Dawley rats and groups of 50 new-hatched Japanese quail
22 were fed diets containing 0, 0.3, 3.0 or 30 ppm [¹⁴C]mirex for up to 16 months (Ivie et al., 1974),
23 pairs of animals were sacrificed periodically to measure tissue concentrations. In both rats and
24 quail, mirex accumulated rapidly in adipose tissue and was detectable in all tissues measured; no
25 plateau of residue accumulation was observed in any tissue. Average tissue distributions of
26 mirex in rats were: brain 1.0%, muscle 1.0%, liver 3.0%, kidney 8.0%, fat 1.0%, skin 0.5%,
27 feces 8.0%, other 0.3%. After cessation of dosing, mirex was more persistent in fat and skin
28 than in other tissues. In quail, average mirex tissue distributions were: muscle 1.0%, liver 0.3%,
29 fat 0.3%, skin 0.8%, feces 6.8%, egg yolk 1.0%, other 0.5%. Brain and kidney concentrations
30 were not measured in quail. After cessation of dosing, mirex was more persistent in fat, skin,
31 and feathers than in other tissues.

32
33 Studies have investigated the distribution of mirex in livestock. In male and female goats
34 receiving mirex orally for 61 weeks, a steady-state was reached in plasma but not adipose tissue
35 after 5 months of exposure (Smrek et al., 1977). Mirex was detected in the fat, liver, and brain
36 of goats chronically dosed with mirex (Smrek et al., 1978). Mirex accumulated in the fat of
37 Pitman-Moore minipigs fed 50 mg/day mirex for 7 days (calculated by ATSDR, 1995 as 3-4.5
38 mg/kg/day) (Morgan et al., 1979). Samples of back-fat were taken at the end of dosing and 9
39 days after dosing, mirex levels were 39.8 ppm and 41.5 ppm, respectively (Morgan et al., 1979);
40 the increase over time suggests a slow redistribution of mirex. Other porcine tissues were also
41 measured 9 days after dosing; mirex levels were 0.34 ppm in plasma, 0.12 ppm in red blood
42 cells, 1.24 ppm in liver, 0.44 ppm in kidneys, and 0.62 ppm in the brain (Morgan et al., 1979).

1 Several studies have specifically investigated the distribution of mirex into the nervous
2 system. Following oral dosing of male rats, mirex was detected in the brain within 30 minutes
3 to 2 hours; at 4 days mirex was present in the cerebral cortex, cerebellum, brain stem and spinal
4 cord, as well as the liver, kidneys, testes, and omental fat (Brown and Yarbrough, 1988). In
5 chronic dosing experiments with 0, 89 or 890 µg/kg/day mirex, a dose-dependent accumulation
6 was observed in the rat brain (Thorne et al., 1978). In mice, mirex accumulated in the brain; at
7 48 hours mirex concentrations were higher in the medulla, pons and striatum compared to the
8 cortex, midbrain and cerebellum but no differences within the brain were observed 6, 12, or 48
9 hours after dosing (Fujimori et al., 1982). Khera et al. (1976) detected 3.5-fold higher mirex
10 concentrations in fetal brain tissue compared to adult tissue.

11
12 Studies with pregnant animals have demonstrated that mirex accumulates in maternal and
13 fetal tissues. Pregnant rats received oral doses of 0, 1.5, 3.0, 6.0, or 12.5 mg/kg mirex on days
14 6-15 of gestation (Khera et al., 1976). In adult females, mirex concentrations were highest in
15 adipose tissue. Mirex was detected in maternal and fetal tissues at all dose levels, except the
16 control; fetal brain levels of mirex were higher than maternal brain levels, but lower
17 concentrations of mirex were present in other fetal tissues compared to their mothers (Khera et
18 al., 1976). Another study observed that tissue levels were 4- to 5-fold higher in mothers than in
19 fetuses, with mirex concentrating in the liver, brain, heart and kidneys (3:2:2:1 ratio) in fetuses
20 (Kavlock et al., 1980). Smrek et al. (1978) detected mirex in fetal goat tissues following chronic
21 maternal exposure.

22
23 Chernoff et al. (1979b) administered 0 or 25 ppm [¹⁴C]mirex in the diet to groups of at
24 least 17 CD rat dams on gestational day 4 onward, and pups were switched to mothers of
25 different groups at birth. Control pups were not exposed pre- or postnatally to mirex. Pups in
26 the prenatal group were born to mothers who received 25 ppm mirex from day 4 of gestation
27 onward; they were switched after birth to control-treated mothers for lactation. Pups in the
28 postnatal group were not exposed prenatally, but were switched after birth to mothers who had
29 been and continued to receive 25 ppm mirex in the diet; they were also weaned onto feed
30 containing 25 ppm mirex. Pups in the perinatal group were exposed both pre- and postnatally to
31 mirex, as described above. In newborn mice exposed prenatally to 25 ppm mirex, 2 ppm was
32 detected in the brain and 10 ppm in the liver. In the postnatal group, 1.9 ppm mirex was detected
33 in the liver at lactational day 9 and trace amounts of mirex were detected in other tissues; after
34 day 31, trace levels of mirex (<1 ppm) were only detected in fat. For the postnatal and perinatal
35 pups, 35-80 ppm mirex was detected in the milk of dams exposed during lactation to 25 ppm
36 mirex. Mirex distribution in prenatal and postnatal pups was similar and concentrations peaked
37 at day 21, when brain, kidney and liver concentrations were approximately 10, 20 and 160 ppm.
38 Small amounts of mirex were detected in the eyes. After weaning (day 21), mirex tissue
39 concentrations decreased; the authors attribute this both to elimination and dilution resulting
40 from rapid growth.

41
42 One study investigating mirex distribution following inhalation was identified. Research
43 cigarettes were impregnated with [¹⁴C] mirex and lightly anesthetized female Sprague Dawley
44 rats were exposed to mainstream smoke (Atallah and Dorough, 1975; Dorough and Atallah,

1 1975). Within 4 minutes, 47% of the mirex was exhaled, 36% remained in the lungs, 11.1% was
2 present in the blood, and 1.3% was recovered from the heart tissue.

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

12 13 **3.3. METABOLISM**

14
15 No studies were located regarding the metabolism of mirex in humans. Experiments
16 indicate that animals are not capable of metabolizing mirex, but that gut bacteria may be capable
17 (Stein et al., 1976; Stein and Pittman, 1977). In experiments using radiolabelled mirex to assay
18 for metabolism, no metabolism of mirex was detected in rhesus monkeys (Wiener et al., 1976;
19 Pittman et al., 1976), rats (Gibson et al., 1972; Ivie et al., 1974), Japanese quail (Ivie et al.,
20 1974), cattle (Dorough and Ivie, 1974), mosquito fish (Ivie et al., 1974) or fathead minnow
21 (Huckins et al., 1982). The potential for conversion of mirex to chlordecone in Pitman-Moore
22 minipigs was assayed; no evidence of metabolism was found (Morgan et al., 1979).

23
24 A study of mirex in adult female rhesus monkeys has been reported in four papers
25 (Kennedy et al., 1975; Stein et al., 1976; Wiener et al., 1976; Stein and Pittman, 1977). Two
26 monkeys were given a single intravenous injection of 1 mg/kg [¹⁴C]mirex. Chromatographic
27 analyses of identified a nonpolar metabolite as either 10-monohydromirex or 9-monohydromirex
28 in feces but not in fat tissues; authors concluded that gut bacteria may have metabolized mirex;
29 the metabolite was less than 3% of the total administered dose.

30
31 Andrade and Wheeler (1975) exposed sewer sludge microorganisms to mirex under
32 anaerobic conditions; using gas chromatography and mass spectrometry, a metabolite was
33 isolated and putatively identified as 10-monohydromirex or 9-monohydromirex.

34
35 Mehendale et al. (1972) conducted *in vitro* metabolism experiments with liver
36 preparations in the presence of cofactors from rats, mice, and rabbits and plant root extracts. No
37 evidence of dechlorination of mirex was detected.

38 39 **3.4. ELIMINATION AND EXCRETION**

40
41 Fecal elimination of mirex is high during the first 48 hours after oral dosing and is
42 attributed to elimination of unabsorbed mirex (reviewed ATSDR, 1995). Fecal elimination of
43 absorbed mirex is slow and is the primary route of excretion; 1% or less of mirex is eliminated in

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

3
4 A 6 mg/kg dose of [¹⁴C]mirex in corn oil was administered to five male CD-1 rats by
5 gavage (Mehendale et al., 1972). Within 48 hours, 55% of the dose was excreted in feces;
6 authors consider this to represent the portion of mirex that passed unabsorbed through the
7 alimentary tract. Over a period of 7 days, 58.5% of the total administered dose was excreted in
8 feces and 0.69% in urine. The authors estimate the first half-life of mirex is 38 hours and the
9 second half-life (of absorbed mirex) is in excess of 100 days (Mendendale et al., 1972).

10
11 A single 0.2 mg/kg dose of [¹⁴C]-mirex was administered by gavage to an unspecified
12 number of mature female Sprague Dawley rats (Gibson et al., 1972). Of the total dose
13 eliminated in feces, 6.74% was eliminated in the first day, 8.52% in the second day, 1.28% in the
14 third day, 0.82% in the fourth day, and 0.67% on days 5-7. Total fecal elimination in 7 days was
15 18.03%. Between 0.4-0.5% of the total dose of mirex was eliminated in urine each day, 0.26%
16 over 7 days. Absorption was decreased slightly by pre-feeding with a single dose of 250 ppm
17 (20 mg/kg¹) unlabeled dietary mirex; 12.84% was excreted in feces on the first day, 7.18% on
18 the second day, and 4.94% on days 3-7 (24.96% total for days 1-7).

19
20 In female rhesus monkeys, 25% of orally administered mirex was eliminated unabsorbed;
21 subsequently, 7% of the total dose was eliminated in feces over 386 days (Wiener et al., 1976).

22
23 In groups of 5 male and 5 female adult common goats dosed orally with 0 or 1 mg/kg-day
24 mirex for 61 weeks (Smrek et al., 1978), the half-life of mirex after cessation of dosing was less
25 than 52 weeks in both males and females.

26
27 Ivie et al. (1974) reported the fecal elimination of mirex from groups of 12 female
28 Sprague Dawley rats and groups of 50 Japanese quail were fed diets containing 30 ppm
29 [¹⁴C]mirex (Ivie et al., 1974) on days 1 and 7, and after 1, 6, 12 and 16 months. In rats, the
30 average percentage of consumed mirex eliminated in feces was 27.1% (24.2% on day 1, 30.1%
31 on day 7, and 26.3-28.2% thereafter). In quail, the average percentage of consumed mirex
32 eliminated in feces was 24.0% (15.8% on day 1, 20.8% on day 7, and 25.4-27.9% thereafter). In
33 rats treated for 6 months with mirex and monitored for up to 10 months later, less than half of the
34 administered dose was excreted. Therefore the half-life of mirex in rats was greater than 10
35 months. In female quail, egg laying resulted in dramatic decreases in tissue mirex concentrations
36 compared to male quail. In female quail, a 75% reduction in residual mirex was observed within
37 3 months after cessation of dosing; in male quail, 40% of residual mirex was eliminated within
38 10 months. Ivie et al. (1974) also treated mosquito fish with a diet containing 50 ppm [¹⁴C]mirex
39 for 15 or 56 days. After cessation of treatment, 50% of mirex was eliminated in 30 days; after
40 200 days, 25% of the original body burden remained.

41
42 Rozman et al. (1984) administered [¹⁴C]mirex to three lactating dairy goats
43 intraruminally. Fecal elimination was not significant on day 1, and on days 2, 3, and 4 was
44 13.89%, 6.41% and 4.87%, respectively, of the total administered dose. Total fecal elimination

1 over 14 days was 44.5%. Urinary elimination averaged 0.029% per day, 0.41% over 14 days.
2 Elimination in milk peaked on day 3; of the total administered dose of mirex, 14.6% was
3 eliminated in milk in 14 days. After the 14 days of observation, 50 g/kg feed containing light
4 paraffin mineral oil was added to the diet of 2 of the goats for 16 days, followed by basal diet for
5 16 days, diet plus mineral oil for 14 days, and then basal diet for 14 days. The third goat
6 received 50 g/kg feed of hexadecane on days 32-46 (14 days). Goats ate all feed provided; no
7 laxative effects were observed. Mineral oil increased the rate of fecal excretion of mirex 2-fold,
8 but did not significantly alter milk or blood levels of mirex. The authors conclude that light
9 paraffin mineral oil enhances the removal of body stores of mirex from lactating goats.

10
11 Mirex is eliminated into milk and eggs. Mirex was detected in 3 of 14 human milk
12 samples tested (Mes et al., 1978). Following oral dosing, mirex has been detected in the milk of
13 a cow (Dorough and Ivie, 1974), rats (Kavlock et al., 1980), and goats (Smrek et al., 1977).
14 Mirex has been detected in eggs of Japanese quail (Ivie et al., 1974).

1
2
3 **4. HAZARD IDENTIFICATION**

4 **4.1. STUDIES IN HUMANS — EPIDEMIOLOGY, CASE REPORTS, CLINICAL**
5 **CONTROLS**

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

10
11 Moysich et al. (1998) performed a case-control study assessing a wide variety of
12 potential factors in 154 primary postmenopausal breast cancer cases and 192 postmenopausal
13 community controls. They reported that among parous women who had never breast-fed, the
14 detection of mirex in serum was associated with increased breast cancer risk, odds ratio 2.42
15 (95% confidence intervals 0.98-4.32). For this subgroup, increased risk was also associated with
16 detection of polychlorinated biphenyls. In the total population sample, detectable tissue mirex
17 was not associated with increased incidence of breast cancer, odds ratio 1.37 (95% confidence
18 intervals 0.78-2.39).

19
20 **4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN**
21 **ANIMALS — ORAL AND INHALATION**

22
23 **4.2.1. Subchronic Toxicity**

24
25 No studies were located in which health effects were evaluated in animals following
26 inhalation exposure to mirex.

27
28 Subchronic oral exposure to mirex has been associated with increased liver weight or
29 liver lesions in Charles River or Sprague Dawley rats at doses as low as 0.3 mg/kg/day (Chu et
30 al., 1981a; Larson et al., 1979; Yarbrough et al., 1981), decreased sperm counts in male rats at
31 doses as low as 0.05 mg/kg/day and testicular degeneration at higher doses of 5 or 10 mg/kg/day
32 (Yarbrough et al., 1981); and thyroid lesions (such as follicular epithelial thickening and
33 follicular collapse) in Sprague Dawley rats at doses as low as 0.7 mg/kg/day (Chu et al., 1981a;
34 Yarbrough et al., 1981). Similar lesions were not found in a study of beagle dogs provided
35 dietary doses as high as 4 mg/kg/day for 13 weeks (Larson et al., 1979).

36
37 Larson et al. (1979) administered 0, 4, 20 or 100 ppm mirex (reported purity 98%) (0,
38 0.2, 0.8, or 4 mg/kg/day for males and females²) in the diet to groups of 2 male and 2 female

²Larson 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.

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

12
13 The same report (Larson et al., 1979) describes the dietary administration of 0, 5, 20, 80,
14 320 or 1280 ppm mirex (reported purity 98%) to groups of 10 male and 10 female Charles River
15 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
16 mg/kg-day for females³). During weeks 4 and 13, food consumption was measured, and
17 unspecified urinalysis and hematology were performed on 4 male or 5 female rats, respectively,
18 per group. Histopathology was performed for the adrenal gland, bone marrow, brain, cecum,
19 gonad, heart, kidney, liver, lung, pancreas, pituitary, small and large intestine, spleen, stomach,
20 urinary bladder and thyroid. One of 10 female rats in the 26 mg/kg dose-group died; all female
21 rats and 5 of 10 male rats in the highest dose-group died. Statistically significant decreases in
22 average body weight were observed in females at 94 mg/kg-day and males at both 22 and 93
23 mg/kg-day. Dose-dependent decreases in food consumption were observed in both sexes, and
24 were statistically significant at the lowest doses tested. Mirex caused a dose-responsive increase
25 in relative and absolute liver weight, statistically significant in female rats at all doses except 6.5
26 mg/kg and in male rats at 1.2 mg/kg and above. Liver vacuolation and swelling were observed
27 in rats receiving at least 1.2 mg/kg mirex; the incidence was not reported. No liver necrosis or
28 inflammation was reported, and no abnormalities were detected in other organs. This study does
29 not identify a NOAEL, and identifies a LOAEL of 0.3 mg/kg-day (5 ppm in the diet) based on
30 altered liver weight in female rats with changes in liver histopathology noted at higher doses.

31
32 Yarbrough et al. (1981) exposed groups of 10 male Sprague Dawley rats to 0, 0.5, 5.0, 50
33 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
34 consumption, and urinary and fecal output were determined weekly. At study termination,

³Larson 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).

⁴Yarbrough 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.

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

29
30 Chu et al. (1981a) treated groups of 40 male Sprague-Dawley rats with 0, 5, or 50 ppm
31 mirex (reported purity 98%) in the diet for 28 days (0, 0.7, or 7 mg/kg-day⁵). Ten animals per
32 group were sacrificed immediately after the 28-day exposure. At 12, 24, and 48 weeks post-
33 exposure, 10 animals per group were sacrificed. Endpoints evaluated were hematologic analysis,
34 serum chemistry, measurements of testicular sorbitol dehydrogenase and hepatic mixed function
35 oxidase activity, serum T3 and T4 levels, and histopathology of the adrenal gland, bone marrow,
36 brain, esophagus, heart, kidney, liver, lung, trachea and bronchi, lymph node (mesenteric and
37 mediastinal), pancreas, parathyroid, pituitary, skeletal muscle, small and large intestine, spleen,
38 stomach, testis and epididymis, thoracic aorta, thymus and thyroid. No animals died following

⁵Chu 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.

1 28 days of exposure, or during the first 24 weeks of recovery. One rat in the 7 mg/kg mirex
2 group died before 48 weeks but the cause was not determined. Treatment with 7 mg/kg mirex
3 induced statistically significant decreases in food-intake and body weight and an increase in
4 relative and absolute liver weight observed immediately after and 12 weeks after treatment; these
5 effects had reversed by 24 and 48 weeks recovery. Liver congestion was seen in rats treated
6 with 7 mg/kg mirex immediately following treatment, and liver mottling was observed in mirex-
7 treated rats at all timepoints. The incidence of liver and thyroid lesions are presented in Table 1.
8 In the livers of rats treated with mirex, pericentral fatty vacuolation, “cytoplasmic vacuolation,
9 reduction in aggregated basophilia, nuclear anisokaryosis and hyperchromicity of hepatocytes”
10 were observed. Livers of rats dosed with 0.7 mg/kg mirex appeared to have recovered by 48
11 weeks, but lesions still were apparent at 48 weeks in rats exposed to 7 mg/kg/day (see Table 1).
12 Mirex induced thyroid lesions including “a reduction in colloid density, angular collapse of
13 follicles and an increase in epithelial height with nuclear vesiculation.” Thyroid lesions from
14 treatment with 0.7 or 7 mg/kg mirex appeared to have repaired within 12 weeks (Table 1).
15 Elevated incidence of testicular lesions (aspermia or inhibited spermatogenesis) was also
16 observed in mirex-treated rats, but the elevation was not statistically significant. Treatment with
17 mirex induced statistically significant changes in serum biochemistry: immediately after the 28-
18 day exposure, 0.7 or 7 mg/kg mirex increased serum calcium, uric acid, alkaline phosphatase,
19 and inorganic phosphorus and decreased glutamic oxalacetic transaminase, total protein,
20 bilirubin and glucose. Twelve-weeks post exposure, inorganic phosphorous levels remained
21 elevated and glutamic oxalacetic transaminase activity remained lower in rats treated with 0.7 or
22 7 mg/kg mirex; in the 7 mg/kg group, lactic dehydrogenase was decreased and sorbitol
23 dehydrogenase activity was increased. After 24 weeks post-exposure, lactic dehydrogenase was
24 significantly lower in rats treated with 0.7 or 7 mg/kg mirex, and aminopyrine –demethylase
25 activity was elevated in the 50 ppm mirex group. All parameters had returned to normal by 48
26 weeks post-exposure. No other consistent and statistically significant adverse exposure-related
27 effects were found. The lowest exposure level, 0.7 mg/kg-day (5 ppm in diet), was an adverse
28 effect level for reversible liver and thyroid lesions in rats. The liver lesions were more persistent
29 than the thyroid lesions, and showed no evidence of recovery at the higher dose level of 7
30 mg/kg/day.

31 32 **4.2.2. Chronic Toxicity and Cancer Bioassay**

33
34 There are three adequate studies that have investigated the toxicity or carcinogenicity of
35 mirex in rats and mice orally exposed for chronic periods (Innes et al., 1969; NTP, 1990; Ulland
36 et al., 1977). Two other chronic duration oral exposure studies have been published that focus
37 on liver responses to mirex (Abraham et al., 1983; Fulfs et al., 1977), but they are of restricted
38 usefulness because they are only focused on liver endpoints and incidence data for liver lesions
39 were not adequately reported.
40

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

^aThyroid lesions consisted of follicular epithelial thickening, colloid loss, and follicular collapse.

^bLiver lesions consisted of fatty infiltration, cytoplasmic vacuolation, anisokaryosis, and hepatocyte necrosis.

^cSignificantly different from controls, $p < 0.05$ (Fisher Exact test performed by Syracuse Research Corporation).

1 NTP (1990) fed groups of 52 male and 52 female F344/N rats (initial body weight 120
2 and 100 g, respectively) 0, 0.1, 1, 10, 25, or 50 ppm mirex (reported purity >96%) in the diet for
3 104 weeks. A second study treated groups of 52 female F344/N rats with 0, 50, or 100 ppm
4 mirex in the diet for 104 weeks, because early toxicity was not observed. Based on food
5 consumption and body weight data, the NTP (1990) estimated these doses to be 0, 0.007, 0.07,
6 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.
7 Doses were estimated at 0, 3.9, and 7.7 mg/kg-day for female rats in the second study. Average
8 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
9 study. The following toxicological parameters were assessed: clinical signs, body weight,
10 survival, gross pathology and histologic examination of adrenal gland, bone marrow, brain,
11 esophagus, heart, kidney, liver, lung and bronchi, mammary gland, lymph nodes (submandibular
12 and/or mesenteric), pancreas, parathyroid gland, pituitary gland, prostate/testis or ovary/uterus,
13 salivary glands, skin, small and large intestine, spleen, stomach, thymus, thyroid gland, trachea
14 and urinary bladder.

15
16 No clinical signs of toxicity were reported for male or female treated rats. Statistically
17 significant reductions in survival were seen in male rats treated with 1.9 or 3.9 mg/kg mirex
18 (19/52 and 15/52, respectively, versus 44/52 for controls). The statistical significance of
19 reported body weight changes were not provided. Male rats in the 1.9 and 3.9 mg/kg-day dose
20 groups gained less weight than controls during the first 70 weeks of exposure and lost weight
21 between 70 and 104 weeks of exposure; body weights after 104 weeks of exposure were 11%
22 (1.9 mg/kg-day) and 18% (3.9 mg/kg-day) less than controls. In the first study, female rats in
23 the 3.9 mg/kg-day group gained less weight than controls; body weights after 104 weeks of
24 exposure were 8% less than controls. In the second study, females in the 3.9 and 7.7 mg/kg-day
25 groups gained less weight than controls; body weights after 104 weeks of exposure were 8% (3.9
26 mg/kg-day) and 18% (7.7 mg/kg-day) less than controls.

27
28 NTP (1990) concluded that this study provided *clear evidence of carcinogenic activity*
29 for male and female F344/N rats, based on “marked increased incidences of benign neoplastic
30 nodules of the liver, as well as by increased incidences of pheochromocytomas of the adrenal
31 glands and transitional cell papillomas of the kidney in males and by increased incidences of
32 mononuclear cell leukemia in females.” The dose-responsive increases in the incidences of
33 hepatic neoplastic nodules were statistically significant ($p < 0.05$) in mirex-treated male rats at
34 and above 0.7 mg/kg and in female rats of the second study at 3.9 and 7.7 mg/kg mirex. No
35 statistically significant increase in the incidences of hepatic carcinomas were observed in the
36 exposed groups compared with controls. The combined incidences for benign
37 pheochromocytomas or malignant pheochromocytomas were statistically significantly elevated
38 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
39 study, but not in females exposed to 3.9 or 7.7 mg/kg/day in the second study (see Table 2). A
40 statistically significant trend was found for increased incidence of transitional cell papillomas of
41 the kidney in males rats, but the incidence for these neoplasms was not significantly elevated in
42 the highest dose groups compared with controls (Table 2). Incidences of mononuclear cell
43 leukemia were significantly elevated in male rats exposed to 1.9 mg/kg/day (but not at 3.9
44 mg/kg/day), in female rats exposed to 1.9 and 3.9 mg/kg/day in the first study, and in female rats

1 exposed to 7.7 mg/kg/day in the second study (Table 2). Combined analysis of the female
2 groups found statistically significant elevations of incidence of mononuclear cell leukemia at 0.7
3 mg/kg and above.
4

5 The combined incidence of thyroid follicular cell adenomas or carcinomas was
6 statistically significantly increased in male rats exposed to 3.9 mg/kg/day, but NTP (1990)
7 concluded that an “association between follicular cell tumors and exposure to mirex is unlikely”,
8 because increased incidence of these tumors were not observed in males exposed to 1.9
9 mg/kg/day or in any exposed groups of female rats. In addition, no statistically significant
10 exposure-related increases were observed in incidences of non-neoplastic thyroid lesions,
11 although Chu et al. (1981a) reported that 28-day exposure to dietary concentrations as low as 5
12 ppm produced increased incidence of reversible thyroid lesions in Sprague Dawley rats
13 (follicular epithelial thickening, colloid loss, and follicular collapse).
14

15 Non-neoplastic liver effects were dose-dependent and were reportedly dramatic:
16 statistically significant increases in hepatocytomegaly were seen in male rats at 0.007 mg/kg (but
17 not at 0.08 mg/kg) and in male and female rats at and above 0.7 mg/kg; abundant eosinophilic
18 cytoplasm and vacuolated cytoplasm were observed. Other statistically significant increases
19 were: hepatic fatty metamorphosis, at and above 0.7 mg/kg in both sexes; hepatocyte necrosis,
20 in male rats treated with 1.9 and 3.9 mg/kg mirex, in female rats of the first study treated with
21 0.7 and 3.9 mg/kg, and in female rats of the second study treated with 3.9 mg/kg; and angiectasis
22 (dilated sinusoids) in male rats treated with 0.7 mg/kg or more. Thus, 0.007 and 0.08 mg/kg/day
23 (0.1 and 1 ppm in diet) were no-effect levels for non-neoplastic liver responses in this study.
24

25 Other statistically significant elevated incidences of non-neoplastic lesions in the exposed
26 groups compared with controls were (see Table 3 for incidence data):
27

- 28 1) parathyroid hyperplasia in male rats exposed to doses of 0.7 mg/kg/day and
29 higher;
- 30
- 31 2) nephropathy in female rats exposed to 0.08, 0.7, 1.9 and 3.9 mg/kg/day in the first
32 study (but not in females exposed to 3.9 and 7.7 mg/kg/day in the second study);
33
- 34 3) epithelial hyperplasia of the renal pelvis in male rats exposed to doses of 1.9
35 mg/kg/day and higher, and
36
- 37 4) splenic fibrosis in male rats exposed to 0.7 mg/kg/day or higher.
38

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

4 **Source: NTP, 1990**

5

6 Dietary Concentration	0 ppm	0.1 ppm	1 ppm	10 ppm	25 ppm	50 ppm	100 ppm
7 Dose (mg/kg/day)	0	0.007	0.08	0.7	1.9	3.9	7.7
8 Lesion	(# Affected Rats/ # Examined Rats)						
9 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
10 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
11 F + FII Combined	14/104	8/52	11/52	14/52 ^b	18/52 ^b	27/104 ^b	14/52 ^b
12 Kidney transitional cell papilloma	0/51 M	0/51 M	0/52 M	0/52 M	1/51 M	3/52 M	

13

14 ^aStatistically significant (p < 0.05) difference from controls by Incidental Tumor Tests performed by NTP.

15 ^bStatistically significant (p < 0.05) for combined controls (14/104) by Fisher Exact.

16

17

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

1 NTP (1990) concluded that nephropathy occurred at similar incidences in control and
2 exposed groups of male and female rats, but that the severity was increased at doses of 1.9
3 mg/kg/day and greater. Average severity scores (2 = mild; 3 = moderate, and 4 = marked) for
4 control through high-dose male rats were 3.1, 3.0, 3.0, 3.7, 3.5, and 3.9. For females, respective
5 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
6 second study.

7
8 The liver histopathological slides prepared during the original NTP (1990) study were re-
9 evaluated by a group of pathologists convened by Pathco, Inc. (the "Pathology Working Group";
10 PWG, 1992) using more current criteria and terminology than those used in the original
11 pathology report. Neoplastic nodules were reclassified as hepatocellular adenomas, eosinophilic
12 foci of cellular alteration, or regenerative hyperplasia. The term toxic hepatitis was used to
13 describe non-neoplastic lesions based on the occurrence of centrilobular hepatocytomegaly,
14 centrilobular fatty change, apoptosis (individual cell necrosis), centrilobular necrosis, and bile
15 duct proliferation. A U.S. EPA-sponsored review by Garman (U.S. EPA, 1999b) of the PWG
16 (1992) report concluded that the re-evaluation was appropriate and that the re-evaluated
17 incidence data for rat liver tumors were valid for risk assessment. The Office of Emergency and
18 Remedial Response recommended that the PWG incidence data be used for risk assessment at
19 Superfund sites (U.S. EPA, 1999a). Table 4 highlights the incidence of liver lesions for male
20 and female rats based on the re-evaluation by the PWG (1992).

21
22 The re-evaluation found statistically significant increases in the incidence of
23 hepatocellular adenomas in male rats treated with 1.9 and 3.9 mg/kg/day, and in female rats of
24 the second study exposed to doses of 3.9 and 7.7 mg/kg/day (see Table 4). Thus, the re-
25 evaluation indicated 1.9 mg/kg/day as the lowest dose level inducing a significant tumor
26 response, whereas the NTP (1990) original pathological examination showed statistically
27 significant increased incidence of "neoplastic nodules" at 0.7 mg/kg/day. The incidence of
28 hepatocellular carcinoma was not affected by mirex-treatment (regardless of criteria or
29 terminology used in the pathological exam). Statistically significant increases in the incidences
30 of foci of cellular alteration and toxic hepatitis occurred in both male rats and female rats
31 exposed to 0.7 mg/kg mirex and higher; angiectasis and cystic degeneration were more frequent
32 in male rats exposed to at least 0.7 mg/kg mirex. Regenerative hyperplasia reportedly only
33 occurred in the presence of toxic hepatitis or leukemia.

34
35 The PWG (1992) concluded that "increased incidences of hepatocellular adenomas in
36 both sexes were limited to doses of mirex which also induced hepatotoxicity and eosinophilic
37 foci" (see Table 4), that a correlation between the latter two lesions was observed at doses up to
38 1.9 mg/kg mirex, and that "hepatotoxicity was also observed at doses lower than those with
39 increased incidences of tumors." Despite the strong association between non-neoplastic liver
40 lesions and the hepatocellular adenomas, not all rats with hepatocellular adenomas were
41 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 21/52 F ^a 27/52 FII ^a	26/52 FII ^a
Toxic hepatitis	1/52 M 1/52 F 1/52 FII	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 0/52 FII	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 5/52 FII ^{a, b}	6/52 FII ^{a, b}

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

^bStatistically 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.

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

9 Summarizing the findings from the NTP (1990) rat bioassay (with liver histopathology
10 re-evaluated by the PWG [1992]), exposure for 2 years to dietary dose levels of 0.007 or 0.08
11 mg/kg/day produced no consistent statistically significant increased incidence of neoplastic or
12 non-neoplastic lesions in male or female F344/N rats (Tables 2, 3 and 4). In females in the first
13 study, 0.08 mg/kg-day dosage induced a statistically significantly ($p < 0.05$) increased incidence
14 of nephropathy, but in females exposed to higher doses (3.9 and 7.7 mg/kg-day) in the second
15 study, no statistically significant increases were found when compared with controls. At 0.7
16 mg/kg/day (and higher), statistically significant increased incidences of non-neoplastic lesions
17 were found in several organs: toxic hepatitis consisting of centrilobular hepatocytomegaly, fatty
18 change, apoptosis, necrosis, and bile duct proliferation (in both sexes), parathyroid hyperplasia
19 in male rats only, nephropathy in female rats (only in the first study), and splenic fibrosis in
20 male rats only (Tables 3 and 4). Exposure to 1.9 mg/kg/day produced statistically significant
21 incidences of the following tumor types: liver adenomas in male rats, pheochromocytomas or
22 malignant pheochromocytomas in male rats, and mononuclear cell leukemia in male and female
23 rats (Table 2 and 4). At 3.9 mg/kg/day significantly elevated incidences included liver adenomas
24 in male and female rats (second study) (Table 4), liver adenomas or carcinomas in male and
25 female rats (Table 4), benign or malignant pheochromocytomas in male rats (Table 2), and
26 mononuclear leukemia in female rats (first study) (Table 2). In females exposed to 7.7
27 mg/kg/day in the second study, elevated incidences occurred for mononuclear cell leukemia,
28 liver adenomas, and liver adenomas and carcinomas combined (Tables 2, 3, and 4). Other
29 statistically significant neoplastic responses were observed in several groups of exposed rats, but
30 they were not as strongly associated with mirex exposure as liver neoplasms in both sexes,
31 pheochromocytomas in male rats, or mononuclear cell leukemia in female rats, and are not
32 interpreted as exposure-related. These included a statistically significant trend for increasing
33 incidence of transitional cell papillomas of the renal pelvis in male rats with increasing exposure
34 level, but the highest incidence for this neoplasm (3/52) was found in the highest dose group and
35 was not significantly different from the control incidence by a number of statistical tests.
36

37 The Innes et al. (1969) study (also reported as IARC, 1979) reports the effects of lifetime
38 feeding of mirex or 119 other test compounds to mice, starting at 7 days of age. Two strains
39 were used: (C57BL/6 x C3H/Anf)F1 (strain B6C3F1) and (C57BL/6 x AKR)F1 (strain
40 B6AKF1). Four untreated-control groups and one gelatin-control group of each sex and strain
41 (15-18/group) were included in the study. Innes et al. (1969) administered nothing, gelatin, or
42 10 mg/kg mirex in gelatin to groups of 18 male and 18 female mice of the two strains by gavage
43 from 7-28 days of age; then mice were fed diets containing 0 or 26 ppm mirex for the remainder
44 of their lifetimes. Assuming an average chronic reference body weight of 0.024 kg and food

1 intake of 0.0063 kg/day for male and female B6C3F1 mice (U.S. EPA, 1988), an estimated daily
2 dietary dose of 7 mg/kg/day is calculated. This was considered a maximum tolerated dose.
3 Mirex-treatment decreased survival: control animals survived to 78-89 weeks; all treated males
4 had died by 59 weeks on study, and all the females had died by 70 weeks. Whether the animals
5 died spontaneously or were terminated is unclear from the report, but it could be inferred that
6 mirex was severely toxic to the mice. Control animals were sacrificed between 78 and 89 weeks.
7 At sacrifice, the thoracic and abdominal cavities were given a “thorough examination” with
8 histological examination of major organs and of all grossly visible lesions. The incidence data
9 for non-neoplastic lesions were not discussed in the report. Statistical analysis of tumor
10 incidence data (for liver tumors, pulmonary tumors, and lymphomas) were performed using
11 separate and combined control group data. The term “hepatoma” was used for all liver tumors
12 regardless of metastasis; the authors reported that metastases were rare.
13

14 In mirex-treated animals, the increases in incidence of hepatomas in mirex-treated
15 animals were statistically significant: B6C3F1 males: controls 8/79 (10%), treated 6/18 (33%);
16 B6C3F1 females: controls 0/87 (0%), treated 8/16 (50%); B6AKF1 males: controls 5/90 (6%),
17 treated 5/15 (33%); B6AKF1 females: controls 1/82 (1.2%), treated 10/16 (62.5%). The tumor
18 incidences in all treated groups were significantly elevated relative to the pooled controls. No
19 pulmonary tumors or lymphomas were observed in treated mice. In this study, 10 other
20 compounds of the 120 total tested also induced statistically significant elevations in the
21 incidences of tumor formation.
22

23 Groups of 26 male and 26 female Charles River CD rats were fed 40 or 80 ppm mirex in
24 the diet (99% pure) for 10 weeks (Ulland et al., 1977). These doses were the predicted
25 maximum tolerated dose and half maximum tolerated dose. Because no significant adverse
26 effects were observed, the doses were increased to 50 or 100 ppm, respectively. Rats were dosed
27 for a total of 18 months. After cessation of treatment, animals were observed for 6 months. The
28 time weighted doses were calculated to be 4 or 7 mg/kg for males and 4 or 8 mg/kg for females⁶.
29 Groups of 20 male and 20 female rats were maintained as controls. All rats were necropsied and
30 subjected to histopathologic examination of the adrenal glands, cerebrum, cerebellum,
31 esophagus, heart, kidneys, liver (2-3 sections), lungs, pancreas, parathyroid gland, pituitary
32 glands, spinal cord, small and large intestine, spleen, stomach, thymus, thyroid, urinary bladder,
33 and either testes or ovaries and uterus. Food and water consumption data were not reported.
34 Treatment had no effect on body weight. Survival was significantly reduced in both groups of
35 treated males and in high-dose females. Neoplastic and non-neoplastic hepatic lesions occurred
36 at elevated incidences in exposed rats compared with controls (see Table 5).
37

⁶Ulland 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.

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

21
22 Fulfs et al. (1977) orally administered mirex to mice for 19 months, rats for 8 or 12
23 months, and monkeys for up to 26 months and examined histological, ultrastructural and
24 biochemical endpoints in the livers; potential toxic or carcinogenic responses in other tissues
25 were not examined. The study provides limited evidence that mice livers may be more sensitive
26 than rat or monkey livers, but it is of restricted usefulness for risk assessment due to its limited
27 focus on the liver and inadequate reporting of incidence data.

28
29 Groups of 100 male and 100 female CD-1 mice received 1, 5, 15 or 30 ppm mirex (0.2,
30 0.9, 3, or 5 mg/kg-day⁷) in the diet for at least 19 months with paired control groups of 100 male
31 and female mice (400 and 400, total) (Fulfs et al., 1977). Groups of 6 were sacrificed at 2, 4, 6,
32 9, 10, 15, and 18 months. Body weights were reported to be not affected by treatment, but were
33 not otherwise reported. Due to poor survival in the 5 mg/kg group, these animals were removed
34 from the study. Survival was not otherwise reported. Increased relative liver weights were seen
35 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
36 (females only) at 18 months. Livers from exposed mice were reportedly enlarged and

⁷Fulfs 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.

1 **Table 5. Incidence of Hepatic Lesions in Charles River CD Rats Exposed Chronically to 0,**
 2 **50, or 100 PPM Mirex in the Diet**
 3 **Source: Ulland et al., 1977**
 4

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

16 ^aValue in parentheses indicate the number of animals also having neoplastic nodule and/or carcinoma

17 ^bValue in parentheses indicate the number of animals also having carcinoma

18 ^cSignificantly different from controls, $p < 0.05$, Fisher Exact Test performed by Syracuse Research Corporation

19 ^dDifferent from controls, $p = 0.053$, Fisher Exact Test performed by Syracuse Research Corporation

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

9
10 Sprague Dawley rats were fed 5 ppm mirex for 12 months or 30 ppm mirex for 8 months
11 (estimated doses of 0.3 or 2 mg/kg⁸). Gender, use of controls, and numbers per group were not
12 specified. It was reported that no differences were observed between control versus treated
13 animals for body weight, liver weight, gross pathology, histopathology, or biochemistry (Fulfs et
14 al., 1977).

15
16 Groups of 2 male and 2 female rhesus monkeys were treated with 0, 0.25, or 1 mg/kg
17 mirex by gavage, 6 days per week, for up to 26 months. Baseline liver biopsies were taken from
18 all 12 monkeys before administration, from high-dose animals at 16, 19, and 26 months, and
19 from low-dose animals at 36 months. Livers from exposed monkeys were reported to appear
20 normal, except for the occurrence of “occasional focal lymphocytic infiltration” (Fulfs et al.,
21 1977).

22
23 Groups of 10 male Sprague Dawley rats were fed 0 or 100 ppm mirex (0 or 8 mg/kg-
24 day⁹) in the diet for 13 months (Abraham et al., 1983). Body weight was measured at study
25 initiation and termination. An unspecified number of animals were injected with tritiated
26 thymidine 1 hour prior to sacrifice; livers were examined for normal, abnormal and adjacent
27 areas; from which samples were taken for analysis of nuclei, histology, and autoradiography.
28 Livers of control-treated rats were reportedly normal; livers of mirex-treated rats exhibited
29 centrilobular and pericentral hepatocyte hypertrophy and necrosis. Hypertrophic nodules,
30 evolved nodules with trabeculi and acini, and advanced carcinomas were observed; however,
31 incidence and statistical significance of these findings were not reported. Mirex treatment
32 caused statistically significant increases in DNA synthesis (measured by thymidine-uptake) in
33 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.

1 **4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES — ORAL AND INHALATION**
2

3 No studies were located regarding the effects of mirex on reproduction or development in
4 humans. Results from animal experiments indicate that mirex adversely affects reproductive
5 performance in both male and female animals and is a developmental toxicant.
6

7 **4.3.1. Developmental Toxicity**
8

9 No studies were located regarding developmental effects in humans associated with
10 exposure to mirex.
11

12 Developmental effects including cataracts and reduced survival have been observed in
13 the offspring of female rodents exposed during gestation or lactation to orally administered
14 mirex.
15

16 Mirex-induced cataracts and reduced survival (Table 6) to weaning were found in rat
17 offspring in an early 2-generation reproductive toxicity study (Gaines and Kimbrough, 1970).
18 Groups of 10 male and 10 female Sherman rats were exposed to 0 or 25 ppm in the diet for 45 or
19 102 days before they were mated for 7 days to rats without pre-exposure. Females continued to
20 receive their respective pre-mating diet through gestation and lactation. Reported estimates of
21 daily doses ranged from 1.3-3.1 mg/kg-day for males and 1.8-2.8 mg/kg-day for exposed males
22 and females, respectively; the average of the midpoint of these ranges is 2.3 mg/kg-day.
23 Offspring were examined grossly at birth, during lactation, and at weaning for mortality and
24 development of abnormalities, including cataracts. No effects on litter number, number of live
25 pups at birth, pup survival to weaning, or percentage of offspring with cataracts were found in
26 groups in which male rats were exposed for 45 or 102 days. In groups in which female rats were
27 exposed to 2.3 mg/kg/day for 45 or 102 days before mating and during gestation and lactation,
28 pup survival to weaning was statistically significantly reduced to 53% and 61%,
29 respectively, compared with 89% in offspring of non-exposed males and females. Respective
30 percentages of offspring with cataracts in these groups were 33.3% and 46.2%, compared with
31 no cataracts in offspring of non-exposed males and females. No effects on offspring endpoints
32 were found in another group of 10 females exposed to 5 ppm mirex for 102 days prior to mating
33 and during gestation and lactation. Daily doses were reported to range from 0.31-0.49 mg/kg-
34 day; the midpoint of this range is 0.4 mg/kg-day. To determine if *in utero* or lactational
35 exposure to mirex was responsible for cataract formation, one group of 10 female rats received 5
36 ppm mirex in the diet for 49 days before mating and then throughout gestation and lactation.
37 Three groups of 10 female rats were bred without pre-treatment. At birth, offspring of mirex-
38 treated dams were switched with one of the non-exposed groups, and the offspring of the
39 remaining two non-exposed groups were switched with each other. In offspring treated *in utero*
40 with mirex, cataract incidence was 1.6%. In offspring receiving mirex only in milk, cataract
41 incidence was 37.5%; no offspring of the control-control switched groups exhibited cataract
42 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
1	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
2	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
3 4	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
5 6	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
7 8	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
9 10	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

1 Khera et al. (1976) treated groups of 18-20 mated female Wistar rats with 0, 1.5, 3, 6, or
2 12.5 mg/kg mirex in corn oil by gavage on days 6-15 of gestation, and were euthanized on day
3 22. Fetuses were examined for weight, viability, and external malformations; one third of each
4 litter was studied for visceral changes and two thirds for skeletal anomalies. Treatment with
5 12.5 mg/kg caused statistically significant dose-dependent increases in maternal death during
6 gestation and incidence of pregnancy failure, and decreased maternal weight gain. At 12.5
7 mg/kg, a statistically significant decrease in fetal weight was observed. In animals treated with
8 6 or 12.5 mg/kg, statistically significant decreases in the number of live fetuses per pregnancy
9 and in the total number of visceral anomalies were detected; visceral anomalies included
10 subcutaneous edema, cleft palate, scoliosis, runts, short tail, and heart defects (Table 6).
11

12 Chernoff et al. (1979a) administered 0, 5, 7, 9.5, 19 or 38 mg/kg mirex by gastric gavage
13 to groups of 38, 30, 10, 37, 32 or 10 pregnant CD albino rats, respectively, on days 7-16 of
14 gestation and sacrificed animals on day 21. Maternal deaths occurred in exposed groups but the
15 incidences were not statistically significant or dose-dependent. Maternal weight gain decreased
16 in a dose-dependent fashion and was statistically significant at and above 9.5 mg/kg. Relative
17 maternal liver weight increased in a dose-dependent fashion and was statistically significant at
18 and above 7 mg/kg. No fetuses survived exposure to 38 mg/kg. Statistically significantly
19 increased fetal mortality and decreased birth weight were seen in offspring of the 19-mg/kg dose
20 group (65.1% and 3.46 g for treated versus 4.0% and 3.84 g for controls, respectively). The
21 average number of implants was not affected by treatment. Dose-dependent increased incidence
22 of edematous live fetuses and decreased sternal ossification occurred and were statistically
23 significant at and above 7 mg/kg. Severe edema was frequent in dead fetuses. The incidence of
24 other somatic anomalies, a category including enlarged cerebral ventricles and undescended
25 testes was significantly elevated in offspring of the 9.5- and 19-mg/kg groups.
26

27 In a study of cataract formation in postnatally exposed offspring, doses of 0, 1.0, 2.5, 5 or
28 10 mg/kg mirex (Table 6) were administered to Long-Evans rat and Sherman rat dams on
29 postnatal days 1-4; each group consisted of at least 10 litters (Chernoff et al., 1979a). In Long-
30 Evans rat offspring, statistically significant decreases in offspring body weight were seen at 2.5
31 mg and above; data were not provided for Sherman rats. Statistically significant decreases in
32 viability were seen in litters of both strains of rats that received 10 mg/kg mirex, on the first day
33 of measurement post-dosing and afterward. No ocular lesions were observed at 1.0 mg/kg
34 mirex; statistically significant dose-dependent changes were observed at 2.5 mg/kg and above in
35 both strains. Cataracts and “an outlining of the lens” were observed when the neonates’ eyes
36 opened, on days 12-14. Lens outlining was persistent through weaning and was difficult to
37 discern in mature animals. In a separate experiment, Long-Evans dams received single oral
38 doses of 0, 5, 10 or 15 mg/kg on postnatal day 1 (day of birth); the number of litters were 8, 14,
39 12 and 20, respectively. No cataracts were detected in the 5-mg/kg group. Increased incidences
40 were observed at 10 and 15 mg/kg, but the increase was statistically significant only at 15 mg/kg.
41

42 Chernoff et al. (1979a) also observed mirex-induced developmental cataract formation in
43 CD-1 mice. On postnatal days 1-4, groups of at least 10 nursing mothers were given 0, 1.5, 3.0,
44 6.0 or 9.0 mg/kg by gavage; animals were observed weekly until postnatal day 37. Statistically

1 significant decreases in pup survival were seen in the 6.0- and 9.0-mg/kg dose groups.
2 Statistically significant decreases in body weight were seen in all treated litters; this effect was
3 transient below 6.0 mg/kg, but animals treated with 9.0 mg/kg did not recover by 37 days. No
4 mirex-induced ocular lesions were seen at 1.5 mg/kg; statistically significant increases in
5 cataract incidence were observed at 3.0 mg/kg and higher.
6

7 Chernoff et al. (1979b) examined the influence of mirex exposure during gestation,
8 during gestation and lactation, and during lactation alone, on survival and development of
9 cataracts in offspring of groups of 17 CD rat dams administered 25 ppm mirex in the diet (2.8
10 mg/kg-day¹⁰). Control pups were not exposed pre- or post-natally to mirex. Pups in the prenatal
11 group were born to mothers who received 25 ppm mirex from day 4 of gestation onward; they
12 were switched after birth to control-treated mothers for lactation. Pups in the postnatal group
13 were not exposed prenatally, but were switched after birth to mothers who had been and
14 continued to receive 25 ppm mirex in the diet; they were also weaned onto feed containing 25
15 ppm mirex. Pups in the perinatal group were exposed both pre- and post-natally to mirex. A
16 statistically significant increase was seen in the incidence of stillborn pups exposed prenatally
17 (3% of controls, 14% of treated). Postnatal survival was significantly decreased in both the
18 prenatally and perinatally exposed animals beginning on lactation day 8 (94% control, 95%
19 postnatal, 74% prenatal, 77% perinatal). Postnatal mirex exposure decreased neonatal body
20 weight gain; these changes were statistically significant from day 8 to 46 in both the postnatal
21 and perinatal groups. Incidences of cataracts and outlined lenses in pups were, respectively:
22 control 0% and 0%; prenatal 5.8% and 0%; postnatal 23.8% and 18.8%, perinatal 18.1% and
23 12.6%.
24

25 Chu et al. (1981b) treated groups of 15 male and 20 female Sprague Dawley rats with 0,
26 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
27 mg/kg-day for females¹¹) in the diet for 91 days prior to mating, and 15 days during mating.
28 Average doses for males and females were 0, 0.4, 0.8, 1.5, or 3.0 mg/kg/day. Females were
29 mated to males of the same treatment group, and females continued to receive treatment during
30 gestation and lactation. When compared to controls, statistically significant decreases in mean
31 litter size were observed at all mirex doses tested. Offspring survival at 21 days was
32 significantly reduced for the 20 ppm group. Fertility index, gestational survival index and 4-day
33 survival were not altered by treatment (Chu et al., 1981b). Statistically significant increases in
34 the incidence of cataracts (Table 6) were seen in female fetuses treated with 0.4 or 1.6 mg/kg-
35 day (insufficient numbers of animals examined prevent evaluation of other treatment groups).

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.

1 Additional studies have verified the developmental effects of mirex in vehicle delivered
2 to pregnant rats during gestation by gavage. Grabowski and Payne (1980) exposed pregnant
3 Long-Evans rats to 0, 5, 6, 7, or 10 mg/kg mirex by gavage on gestation days 6-15 (Table 6). At
4 7 and 10 mg/kg, maternal death was elevated and the increased incidence of fetal death was
5 statistically significant. Microphthalmos (abnormally small eye size) and edema were observed
6 in fetuses from all exposed groups. In fetuses with edema compared to those without, the
7 incidence of increased heart rate, and first- and second-degree heart block were statistically
8 significantly increased. Scotti et al. (1981) observed lens swelling in offspring after treating
9 lactating Sherman rats with 5 mg/kg-day on post-natal days 1-5 (Table 6). In fetuses of pregnant
10 Long-Evans rats treated orally with 6 mg/kg-day on gestation days 8-15, the following
11 statistically significant effects were found: increased incidences of cataracts and associated
12 ocular changes (Rogers and Grabowski, 1983); increased plasma glucose and hematocrit (Rogers
13 et al., 1984); and increased incidence of edema with associated altered plasma protein levels,
14 colloid osmotic pressure, and electrocardiograms (Grabowski, 1981; Grabowski and Payne,
15 1983a,b). Other studies support these findings (Grabowski and Payne, 1983a,b; Rogers and
16 Grabowski, 1984).

17
18 Kavlock et al. (1982) treated six pregnant CD rats orally (vehicle was not reported) with
19 0, 6, or 12 mg/kg/day mirex on gestational days 7-16, and collected fetuses on day 21. The study
20 report contained no information regarding possible maternal toxicity. Mirex treatment caused
21 fetal edema and decreased body weight. The numbers of live fetuses and fetuses with other
22 gross abnormalities were not affected by exposure. Treatment with either 6 or 12 mg/kg day
23 (Table 6) caused statistically significant decreases in fetal: brain weight; liver weight and total
24 glycogen; kidney total protein and alkaline phosphatase. Treatment with 12 mg/kg day also
25 caused statistically significant decreases in fetal: brain total DNA, and protein; lung weight;
26 liver weight and total glycogen; kidney weight, total protein, and alkaline phosphatase activity;
27 and kidney weight. Mirex treatment did not affect lung biochemistry.

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

41 42 **4.3.2. Reproductive Toxicity**

43
44 No studies were located regarding the effects of mirex on reproduction in humans.

1 There are a few studies examining reproductive performance in rats exposed to mirex,
2 but no adequately designed and reported multiple generation studies of reproductive
3 performance in animals exposed to mirex are available.
4

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

15 Gaines and Kimbrough (1970) exposed groups of 10 male and 10 female Sherman rats to
16 0 or 25 ppm mirex (0 or 2.3 mg/kg-day) in the diet for 45 or 102 days before mating with non-
17 exposed rats for 7 days. Females continued to receive their respective pre-mating diets during
18 gestation and lactation. Exposed groups showed no significant decrease in the number of
19 successful matings or number of litters delivered, except that the group of females exposed
20 before mating for 102 days delivered only 6 litters compared with 9 litters in the control group of
21 mated nonexposed males and females and 8-10 litters in the other groups. The average number
22 of live pups per litter was statistically significantly decreased in the group of females exposed for
23 45 days before mating, but this effect was not observed in the group of females exposed for 102
24 days before mating. No effects on the number of successful matings, number of litter delivered
25 or number of live pups per litter were observed in a group of 10 females who were exposed to 5
26 ppm mirex (0.4 mg/kg-day) for 102 days prior to mating (Table 6).
27

28 In the NTP study (1990), groups of 52 male F344/N rats were treated with 0, 0.007,
29 0.075, 0.75, 1.95, or 3.85 mg/kg-day in the diet for 104 weeks. No exposure-related adverse
30 changes were observed in reproductive tissues examined histologically: the mammary gland,
31 penis, preputial gland, prostate, seminal vesicle, testis, epididymis and scrotum.
32

33 Yarbrough et al. (1981) administered 0, 0.5, 5.0, 50, or 75 ppm (0, 0.05, 0.5, 5, or 7.5
34 mg/kg-day¹²) 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.

1 sperm counts were observed at 0.5, 5 and 50 (but not 75) ppm mirex and the incidence of severe
2 degenerative testicular lesions was significantly increased at 50 and 75 ppm (Yarbrough et al.,
3 1981).

4
5 In a reproductive and pharmacokinetic study, Smrek et al. (1977, 1978) treated groups of
6 5 male and 5 female goats with 0 or 1 mg mirex/kg body weight (mixed in sugar) for 61 weeks.
7 Females received 10 mg/kg for an additional 4 weeks. Goats were bred at the onset of treatment
8 and again 32 weeks after the start of exposure. An additional group of 5 females received 1
9 mg/kg for 18 weeks (starting after delivery of offspring from the first breeding), followed by 10
10 mg/kg for 4 weeks. The authors reported that “goats did not show any sign of toxicity.” Mirex
11 did not affect adult body weight, number of kids born, or kid body weight.

12
13 Chu et al. (1981b) fed groups of 15 male and 20 female Sprague Dawley rats with 0, 5,
14 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
15 3.2mg/kg-day for females¹³) in the diet for 91 days prior to mating, and 15 days during mating.
16 Females were mated to males of the same treatment group, and females continued to receive
17 their pre-mating diet during gestation and lactation. A dose-dependent decrease in the number of
18 females showing sperm in vaginal smears following mating was observed and was statistically
19 significant at all dose levels of mirex (Chu et al., 1981b). The study authors considered the
20 decrease in sperm to be evidence of testicular damage. In the 40-ppm group, no sperm was
21 detected in the vaginal smears of females and no successful mating occurred; fertility index was
22 affected only at this highest dose. Statistically significant decreases in mean litter size were also
23 observed at all mirex doses tested. The average number of pups per litter were 10.2, 7.0, 5.8, and
24 7.1 for the 0-, 5-, 10-, and 20-ppm groups, respectively. Offspring survival at 21 days was
25 significantly reduced for the 20-ppm group. Gestational survival index and 4-day survival were
26 not altered by treatment (Chu et al., 1981b).

27
28 Shannon (1976) tested the effects of mirex on prairie voles (*Microtus ochrogaster*).
29 Groups of 16 prairie vole pairs (1 male and 1 female) were exposed to 0, 1, 5, 10, 15, or 25 ppm
30 mirex (estimated to be 0, 0.1, 0.6, 1.2, 2, or 3 mg/kg-day¹⁴) in the diet for 90 days, followed by a
31 4-month observation period. A dose-dependent increase in adult mortality was observed that
32 was statistically significant at and above 0.6 mg/kg, and no animals survived in the highest dose-
33 group. Pairs produced five or fewer litters during the experiment. Mirex treatment did not

¹³Chu 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.

¹⁴Shannon (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.

1 significantly affect the number of offspring per litter born or surviving to 4 days. A statistically
2 significant reduction in pup survival to 21 days was seen at 0.6 mg/kg and above; no pups in the
3 2 mg/kg group survived. Data for other reproductive endpoints, including fertility, could not be
4 assessed because of reporting deficiencies and the apparent failure to consider mortality as a
5 confounding factor.

6
7 Shannon (1976) exposed groups of 10 pairs (1 male and 1 female) of prairie voles to 0,
8 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
9 for both males and females¹⁵) for 5 months. Treatment did not adversely affect adult survival,
10 the number of pups produced per litter, or pup survival. The control, 0.01, and 0.06 mg/kg
11 offspring were used in a multigenerational study: pups were continued on their respective
12 parental diet at weaning and were mated at 60 days. Behavioral development (of motor skills)
13 was investigated, but reporting inconsistencies and deficiencies prevent adequate evaluation of
14 these data and other reproductive data.

15
16 Treatment of pregnant rats with 10 mg/kg/day mirex for 5 (but not 1 or 10) days caused
17 reduced blood flow to the ovaries, uterus and fetuses, with concurrent decreased ovarian and
18 uterine weights (Buelke-Sam et al., 1983); because the effects were not consistent over time the
19 significance of these observations is uncertain.

20
21 Fuller and Draper (1975) induced ovulation in immature 28-day-old female Long Evans
22 rats with pregnant mare serum (PMS), and then administered 0.4 to 50 mg/animal mirex by
23 subcutaneous injection. Mirex reduced the numbers of ova released 40-80% compared to control
24 animals. Administration of human chorionic gonadotropin reversed the inhibitory effects of
25 mirex; the authors suggest this is evidence that mirex does not act directly upon the ovary. A
26 time-course found that mirex was an effective inhibitor of ovulation when introduced prior to,
27 but not after, the lutenizing hormone surge. The authors speculated that mirex may influence
28 ovulation through a neural mode of action that controls lutenizing hormone release.

29
30 Typical estrogenic effects were not produced in immature female Sprague Dawley rats
31 given subcutaneous doses of up to 100 mg mirex/kg on postnatal day 22 or 1 mg/animal on
32 postnatal days 2 and 3 (Gellert, 1978). Groups of 5 immature virgin 22-day-old Sprague Dawley
33 rats were given single subcutaneous injections of 0, 1, 10, or 100 mg mirex/kg. As a positive
34 control, 1 µg/kg 17β-estradiol was administered to five animals. Unlike estradiol which
35 significantly increased uterine weight within 24 hours, mirex had no effect on uterine weight. In
36 a subsequent experiment, twin groups of 16 and 21 newborn rats were subcutaneously injected
37 with sesame oil (control) or 1 mg/dose of mirex 2 and 3 days after birth. Pups were weaned at
38 21 days and sacrificed at 7 or 8 months. Mirex exposure had no effect on vaginal opening,
39 estrous cycles, or ovulation compared with controls, but caused a 10% increase in body weight
40 and 24% increase in uterine weight without influencing weights of the ovary, adrenal gland or

¹⁵Sample 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.

1 the anterior pituitary. In contrast, similar exposure to 0.2 or 1 mg chlordecone (“Kepone”, in
2 which two chlorine atoms have been substituted by a ketone oxygen) produced definite
3 estrogenic effects including shortened time to vaginal opening, increased incidences of persistent
4 vaginal estrus and ovaries devoid of corpora lutea. Corroborating results were reported by
5 Hammond et al. (1979) showing that subcutaneous doses of 50 mg mirex/kg for 3 days to 21-
6 day-old female did not affect uterine weight, whereas similar exposure to 50 mg chlordecone/kg
7 or 50 µmg estradiol-17β/kg increased uterine weight by 3- to 4-fold compared with controls.
8

9 **4.4. OTHER RELEVANT STUDIES**

10 **4.4.1. Other Carcinogenicity Studies**

11
12
13 Carcinogenic responses to subcutaneously administered mirex have been observed in
14 two strains of mice (NCI, 1968). Groups of 18 males and 18 female mice of B6C3F1 and
15 B6AKF1 mice received single subcutaneous injections of 1000 mg/kg mirex in 0.5% gelatin at
16 28 days of age. Surviving animals were sacrificed at 18 months. The term hepatoma was used
17 to describe all liver tumors, except in cases of unmistakable pulmonary metastases, when the
18 term hepatic carcinoma was used. Several tumor types, including those arising in the liver,
19 thymus, spleen, abdominal lymph nodes, and Peyer’s Patches, were included in the group of
20 reticulum cell carcinoma. Statistically significant differences in tumor incidence between
21 control and mirex-treated mice for total number, reticulum cell sarcoma, and hepatoma (by Chi-
22 square methods) were reported. Because results were not evaluated by gender and strain, these
23 results were verified by an evaluation of the raw data by Syracuse Research Corporation using
24 the Fisher Exact Test (Table 7). Statistically significant elevations, compared with control
25 incidences from multiple concurrent experiments, were observed for reticulum cell sarcoma in
26 B6C3F1 males and B6AKF1 females and for hepatomas in B6AKF1 males. Pooling across
27 sexes and strains, incidences for reticulum sarcomas or hepatomas in exposed mice were
28 significantly elevated compared with controls (Table 7).
29

30 Moser et al. (1992) observed that dermal exposure to 25, 50, or 100 nmol mirex (in 200
31 µL acetone) per application 3 times/week for 34 weeks or 200 nmol for 20 weeks promoted time-
32 and dose-dependent skin tumor formation in female CD-1 mice exposed to single initiating
33 dermal doses of 200 nmol 7,12-dimethylbenz[a]anthracene (DMBA) in 200 µL acetone. Without
34 initiation, dermal exposure to 100 or 200 nmol mirex failed to induce skin tumor formation,
35 epidermal hyperplasia, epidermal protein kinase C activity, or ornithine decarboxylase activity.
36 After 34 weeks of promotion with 25, 50, or 100 nmol mirex, 27%, 85% and 100% of mice
37 developed skin tumors, respectively; the respective average numbers of tumors/mouse were 0.7,
38 7, and 12. No skin tumors occurred in mice initiated with DMBA and promoted with acetone for
39 34 weeks. In parallel studies of a number of biochemical and morphological events, mirex, at
40 concentrations that produced a strong tumor promotion activity, did not induce ornithine
41 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).

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

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

22 23 **4.4.2. Genotoxicity Studies**

24
25 No evidence of mirex genotoxicity has been identified. Stevens et al. (1979) reported
26 that they were unable to demonstrate mirex binding to DNA or RNA. The NTP (1990) found
27 that mirex was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535 or
28 TA1537, with or without exogenous metabolic activation. Mirex did not induce chromosomal
29 aberrations or sister chromatid exchanges in Chinese hamster ovary cells with or without
30 metabolic activation. Other studies confirm that mirex did not induce reverse mutations in
31 multiple strains of *Salmonella typhimurium* (Hallett et al., 1978; Mortelmans et al., 1986; Probst
32 et al., 1981; Schoeny et al., 1979) with or without hepatic microsomal activation. Mirex did not
33 induce mutations in the WP2 and WP2 *uvrA*- strains of *Escherichia coli* (Probst et al., 1981)
34 with or without hepatic microsomal activation. Mirex did not significantly induce unscheduled
35 DNA synthesis in rat, mouse, or hamster hepatocytes *in vitro* (Probst et al., 1981; Williams,
36 1980; Maslansky and Williams, 1981; Telang et al., 1981). Mirex did not induce gene mutations
37 in the HGPRT locus of human fibroblasts *in vitro* (Tong et al., 1981).

38
39 Animal studies have not identified mirex genotoxicity. No dominant lethal effects,
40 measured by viable embryos or deciduomas in pregnant females mated to male Wistar rats
41 treated with 0, 1.5, 3.0, or 6.0 mg/kg mirex by gavage 10 consecutive days and were then mated
42 for 14 consecutive days, were observed (Khera et al., 1976). Mitra et al. (1990) observed that at
43 doses of mirex that induced hepatic ornithine-decarboxylase activity (up to 240 mg/kg) in
44 female Sprague Dawley rats, DNA damage was not induced, as measured by alkaline elution.

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

4
5 Mirex is structurally similar to chlordecone (i.e., kepone) in which two chlorine atoms
6 have been substituted by a ketone oxygen. Chlordecone induced sister chromatid exchanges in
7 CHO Chinese hamster ovary cells (NTP, 1990), and has been classified by IARC (1987) as
8 *possibly carcinogenic to humans* (Group 2B), on the basis of insufficient human data and the
9 induction of liver tumors (hepatocellular carcinomas) in Osborne Mendel rats and B6C3F1 mice
10 (NCI, 1976).

11 12 **4.4.3. Mechanistic Studies**

13
14 The mechanism by which mirex causes non-neoplastic and neoplastic lesions in the liver
15 is not yet completely understood. As discussed in the previous section, mirex has not been
16 genotoxic in numerous short-term *in vitro* and a few *in vivo* tests, leading to the hypothesis that
17 tumorigenic responses to mirex in the liver do not directly involve a genotoxic mechanism and
18 may involve proliferation of cells initiated spontaneously, or by some other agent, to become
19 tumors. Support for this hypothesis is provided by results in the two-stage mouse skin tumor
20 bioassay showing that mirex does not initiate mouse skin tumors, but is a potent promoter of skin
21 tumors initiated by DMBA (Kim and Smart, 1995; Kim et al., 1997; Moser et al., 1992, 1993).

22
23 Molecular details of how mirex may promote tumor formation, presumably through
24 promotion of cell proliferation, are unknown, but results from several lines of investigation
25 provide some suggestions of interactions with endocrine systems in mirex-induced tumor
26 promotion and promotion of hepatocellular growth. In the two-stage mouse skin bioassay,
27 ovariectomized mice showed a lesser skin tumor promotion response to mirex than sham-
28 operated mice with intact ovaries (Moser et al., 1993). Exposure of Sprague Dawley rats to
29 single high oral doses of mirex (100 mg/kg) induced rapid liver growth (about 70% increase in
30 relative liver weight) without changing levels of serum enzymes indicative of liver damage or
31 affecting some functional indices such as sulfobromophthalein clearance (Robinson and
32 Yarbrough, 1978). Numerous studies of this phenomenon by Yarbrough and colleagues have led
33 to the hypothesis that mirex-induced liver growth is composed of hypertrophic (enhanced growth
34 of existing cells) and hyperplastic (enhanced cell division) components and that interactions with
35 endocrine systems may be involved (see Yarbrough et al., 1984 for review). For example,
36 adrenalectomized male rats showed a liver growth response to 100 mg/kg mirex that was only
37 about 50% of that in rats with intact adrenal glands. In mirex-exposed adrenalectomized rats
38 given corticosterone supplements, the liver growth response to mirex was restored, but was not
39 preceded with a peak in hepatocellular DNA synthesis as noted in rats with intact adrenal glands
40 (Yarbrough et al., 1984). Yarbrough et al. (1984) suggested that, in adrenalectomized rats, mirex
41 induced liver growth predominately by a hyperplastic mode and that corticosterone supplement
42 shifted the growth response to a hypertrophic response. In another study with thyroidectomized
43 rats, the magnitude of liver growth in response to mirex was similar to that in rats with intact
44 thyroids, but liver growth was not preceded by a peak in hepatocellular DNA synthesis,

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

7
8 Another hypothesis related to mirex-induced hepatic responses involves mirex induction
9 of ornithine decarboxylase, the first enzyme in the biosynthetic pathway for polyamines that play
10 roles in regulation of various cell functions and metabolism. Oral exposure of rats to two doses
11 of 120 mg mirex/kg, 21 and 4 hours prior to sacrifice, or to single doses of 180 mg/kg induced
12 hepatic ornithine decarboxylase activity by 70- or 55-fold, respectively (Mitra et al., 1990). In
13 contrast, mirex did not induce epidermal ornithine decarboxylase at a dermal dose level that
14 strongly promoted mouse skin tumors after DMBA initiation, whereas tumor promoting dose
15 levels of the phorbol ester, 12-*O*-tetradecanoyl-phorbol-13-acetate, did induce ornithine
16 decarboxylase (Kim et al., 1995; Kim and Smart, 1997; Moser et al., 1992, 1993).

17
18 Another hypothesis proposes that mirex is preferentially cytotoxic to tetraploid and
19 octaploid hepatocytes and that this is a key phenomenon in the induction of mirex hepatotoxicity
20 and carcinogenicity (Abraham et al., 1983). In a group of 10 male Sprague-Dawley rats exposed
21 to 100 ppm mirex in the diet for 13 months, livers exhibited hypertrophy with necrosis, nodules,
22 or carcinomas (incidences of these lesions were not reported). Control livers showed greater
23 numbers of tetraploid nuclei than diploid nuclei (the reported ratio was 244:147,
24 tetraploid:diploid), whereas, in exposed livers, decreasing numbers of tetraploid nuclei were
25 observed in tissues with increasing severity of effect. The change in ploidy pattern was dramatic
26 in liver tissue adjacent to carcinomas and in carcinomas; numbers of diploid nuclei were 3- to
27 4-fold greater than numbers of tetraploid nuclei. Abraham et al. (1983) noted that hepatocytes in
28 primates and humans are predominately diploid (>99%) and that exposure of rhesus monkeys to
29 mirex in the diet for 3 years did not produce histopathological changes in liver sections.
30 Although Abraham et al. (1983) reported that exposure was to 5 or 20 ppm in the diet in this
31 monkey study, the published report of this study (Fulfs et al., 1977), of which Abraham was a
32 co-author, noted that the monkeys were given gavage doses of 0.25 or 1 mg/kg/day (6
33 days/week) for 3 years. Abraham et al. (1983) speculated that mirex may be less hepatotoxic to
34 primates and humans than to rodents due to the absence of hepatocyte polyploid nuclei.
35 Subsequent studies to further test this hypothesis are not available.

36
37 Other investigations have shown that acute or repeated oral exposure of Sprague Dawley
38 rats to mirex at concentrations of 100 ppm in the diet can impair hepatobiliary function without
39 elevating levels of serum enzymes indicative of liver cell damage (see Mehendale, 1981 for
40 review). In these studies, it was shown that clearance of phenolphthalein glucuronide was
41 impaired by mirex exposure; clearance of other markers, such as sulfobromophthalein, were less
42 sensitive to impairment by mirex. Other studies have shown that mirex inhibits uptake of
43 endogenous organic anions [estradiol-17 β (β -D-glucuronide), taurocholate, and L-alanine] by
44 isolated rat hepatocytes (Teo and Vore, 1990) and significantly decreased bile acid concentration

1 and bile acid secretory rate in rats exposed to 50 mg/kg for 3 days compared with controls (Teo
2 and Vore, 1991). Mirex impairment of hepatobiliary excretion functions has been hypothesized
3 to involve inhibition of ATPase activities involved in membrane transport (Curtis and
4 Mehendale, 1981). The possible role that this effect of mirex may have in the development of
5 non-neoplastic and neoplastic hepatic lesions from chronic exposure is unknown.
6

7 The mechanisms or modes of action by which mirex causes cataracts in offspring of
8 exposed female rats are not understood, except that exposure during lactation has been
9 determined to be more important than exposure during gestation (Chernoff et al., 1979b; Gaines
10 and Kimbrough, 1970; see Section 4.3.1). Modes of action by which mirex, at higher exposure
11 levels during gestation, produces other developmental effects (such as increased fetal mortality,
12 decreased survival to weaning, and increased fetal edema) are also not understood.
13

14 The mechanisms or modes of action by which repeated exposure to high doses of mirex
15 impairs reproductive functions in male rats or female rats are also poorly understood. Decreased
16 sperm counts have been reported in Sprague-Dawley rats exposed to doses as low as 0.5
17 mg/kg/day (5 ppm in the diet) with severe testicular degeneration occurring at doses of 5 or 7.5
18 mg/kg/day (50 or 75 ppm in diet) (Yarbrough et al., 1981), but further mechanistic details are
19 unstudied. Decreased female fertility has been reported in a group of female rats exposed to 2.3
20 mg/kg/day (25 ppm in diet) for 102 days before mating with non-exposed males (Gaines and
21 Kimbrough, 1970), but the mode of action is unexplained. Subcutaneous exposure to mirex did
22 not produce typical estrogenic effects in immature female rats (Gellert, 1978) and did not
23 directly inhibit ovulation in rats induced by pregnant mare serum (Fuller and Draper, 1975).
24

25 **4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND** 26 **MODE OF ACTION (IF KNOWN) — ORAL AND INHALATION**

27 **4.5.1. Oral Exposure**

28 Mirex has been detected in some monitoring studies of human adipose tissue and human
29 breast milk samples (Burse et al., 1989; Guttes et al., 1998; Kutz et al., 1974, 1985; Mes et al.,
30 1993; Newsome et al., 1995), but no studies were located that investigated possible associations
31 between mirex exposure and non-cancer health effects in humans.
32

33 Non-neoplastic toxic responses have been evaluated in dogs (Larson et al., 1979), rats
34 (Chu et al., 1981a; Fulfs et al., 1977; Larson et al., 1979; NTP, 1990; Ulland et al., 1977;
35 Yarbrough et al., 1981), mice (Fulfs et al., 1977), and monkeys (Fulfs et al., 1977) orally
36 exposed to mirex for subchronic or chronic periods.
37

38 The liver appears to be one of the most sensitive targets of repeated oral exposure to
39 mirex. Statistically significant non-neoplastic liver effects observed in animals chronically
40 exposed to mirex include fatty metamorphosis, cytomegaly, and necrosis in F344/N rats exposed
41 for 2 years to dietary doses of 0.7 mg/kg/day and greater, but not to doses of 0.007 or 0.08
42 mg/kg/day (NTP, 1990; PWG, 1992; see Table 4); hepatic cytomegaly, vacuolization, fatty
43
44

1 metamorphosis, and necrosis in CD rats exposed for up to 18 months to survival-reducing doses
2 of 7 mg/kg/day and greater (Ulland et al., 1977); and hepatic cellular hypertrophy, necrosis, and
3 proliferation of smooth endoplasmic reticulum in CD-1 mice exposed for 18 months to doses of
4 0.9 mg/kg/day, but not to 0.2 mg/kg/day (Fulfs et al., 1977). Only “occasional” focal
5 lymphocytic infiltrations of the liver without other liver lesions were observed in monkeys
6 exposed to 0.25 or 1 mg/kg for up to 36 months (Fulfs et al., 1977), and no gross or
7 histopathologic changes were observed in Sprague Dawley rats exposed for 12 months to 2
8 mg/kg/day mirex (Fulfs et al., 1977). Several studies of subchronically exposed animals provide
9 supporting evidence that the liver is a sensitive target of mirex toxicity. Liver effects from
10 subchronic exposure, generally observed at higher exposure levels than those producing liver
11 lesions from chronic exposure, have been observed in rats (Chu et al., 1981a; Larson et al., 1979;
12 Yarbrough et al., 1981) and dogs (Larson et al., 1979).

13
14 Non-neoplastic lesions in several other organs have been observed in animals repeatedly
15 exposed to mirex, but these lesions appear either to occur at higher dose levels than those
16 affecting the liver, to be less consistent across studies or gender, or to be less persistent than
17 mirex-induced liver lesions. For example, thickening of the follicular epithelium, loss of colloid,
18 and collapse of the follicles were observed in the thyroid of Sprague Dawley rats exposed to
19 doses of 0.7 or 6.5 mg/kg/day for 28 days, but these lesions regressed in a 48-week post-
20 exposure period (Chu et al., 1981a). In contrast, liver lesions in rats exposed to 6.5 mg/kg/day
21 persisted through the 48-week period (Chu et al., 1981a). Increased incidences of similar non-
22 neoplastic thyroid lesions were not observed in F344/N rats exposed for 2 years to dietary doses
23 as high as 3.9 mg/kg/day in males or 7.7 mg/kg/day in females (NTP, 1990). In the NTP (1990)
24 chronic rat bioassay, the lowest dose level producing toxic hepatitis in male and female F344 rats
25 (0.7 mg/kg/day) also produced increased incidence of parathyroid hyperplasia and splenic
26 fibrosis in males (but not females), and increased incidence of nephropathy in females (see Table
27 3). The nephropathy in this study, however, appears to be more strongly associated with the age
28 of the rats in this study than with exposure. Incidences of nephropathy were high in non-
29 exposed controls and were not significantly elevated (compared with controls) in females in the
30 second study exposed to doses as high as 7.7 mg/kg/day. The NTP study authors (1990)
31 concluded that the “parathyroid hyperplasia is likely a secondary physiological response to the
32 nephropathy” and thus support the interpretation that the parathyroid hyperplasia in male rats
33 was not strongly associated with exposure. No such interpretation of the increased incidence of
34 splenic fibrosis was proposed, but the splenic response was only observed in male rats (liver
35 lesions occurred in both male and female rats exposed to mirex) and has not been reported in
36 other animal studies involving subchronic or chronic oral exposure to mirex. Increased
37 incidences of epithelial hyperplasia of the renal pelvis were observed in male rats exposed to
38 higher doses (≥ 1.9 mg/kg/day) than the lowest doses producing toxic hepatitis (see Table 3).

39
40 Another sensitive effect associated with oral exposure to mirex is cataract development in
41 offspring of exposed female rats. Cataracts in offspring have been reported in studies with
42 female rats orally exposed before mating, during gestation, and/or during lactation to dose levels
43 as low as 0.4 mg/kg/day, but the effect has been more consistently observed at higher dose
44 levels (see Table 7). Exposure during lactation appears to be a critical period for affecting this

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

17
18 Studies of dose-response relationships for cataracts in offspring established 1 mg/kg/day
19 as a no-effect level and 2.5 mg/kg/day as the lowest effect level when lactating Sherman or Long
20 Evans rat dams were given gavage doses on post natal days 1 through 4 (Chernoff et al., 1979a).
21 Dose levels of 10 and 15 mg/kg were identified as the no-effect level and lowest effect level,
22 respectively, for cataracts in offspring when Long Evans rat dams were given gavage doses only
23 on post natal day 1 (Chernoff et al., 1979a). Mirex-induced cataracts in rat offspring have been
24 observed in several other studies in which rat dams were exposed to dose levels ≥ 5 mg/kg/day
25 during lactation (Rogers and Grabowski, 1984; Scotti et al., 1981) or ≥ 6 mg/kg/day during
26 gestation (Rogers and Grabowski, 1983; Rogers et al., 1984). In addition, increased incidence of
27 offspring with cataracts were reported in studies in which lactating female CD-1 mice were
28 given gavage doses ≥ 3 mg/kg/day on post-natal days 1-4 (Chernoff et al., 1979a).

29
30 Other developmental effects including fetal edema, external abnormalities, and reduced
31 fetal survival have been observed in other studies of pregnant rats exposed during gestation to
32 doses of 3 mg/kg/day and greater. In studies with CD albino rats exposed to gavage doses on
33 gestation days 7-16, signs of maternal toxicity (increased relative liver weight and decreased
34 weight gain) and developmental toxicity (edematous live fetuses and decreased sternal
35 ossification) were observed at doses ≥ 7 mg/kg/day, but not at 5 mg/kg/day (Chernoff et al.,
36 1979a). At dose levels ≥ 19 mg/kg/day, more severe developmental effects (e.g., increased fetal
37 mortality and decreased birth weight) were observed (Chernoff et al., 1979a). Other gestational
38 exposure studies identified increased incidence of CD rat fetuses with external abnormalities
39 (edema, ectopic gonads, and hydrocephaly) at 6 mg/kg/day and higher (Kavlock et al., 1982);
40 and, in Wistar rat fetuses, increased incidences of fetal mortality and external abnormalities at 6
41 and 12.5 mg/kg/day, increased resorptions at 3 mg/kg/day, and no developmental effects at 1.5
42 mg/kg/day (Khera et al., 1976). Altered electrocardiographic patterns have also been observed
43 in rat offspring of dams exposed during gestation to doses of 5-6 mg/kg/day (Grabowski,
44 1983a,b; Grabowski and Payne, 1980, 1983a,b).

1 No adequate multiple-generation reproductive toxicity study has been conducted for
2 mirex, but results from single-generation rat studies indicate that exposure to mirex can affect
3 male and female reproductive ability. Impaired male fertility has been observed in Wistar rats
4 exposed to gavage doses of 6 mg/kg/day for 10 days (but not in rats exposed to 3 mg/kg/day)
5 before mating with non-exposed females (Khera et al., 1976) and in male Sprague Dawley rats
6 exposed to 2.8 mg/kg-day for 13 weeks prior to mating and during mating to similarly exposed
7 females (Chu et al., 1981b). In the latter study, no decreases were observed in the percentage of
8 pregnant females at lower exposure levels up to 1.4 mg/kg-day in the diet, and no sperm was
9 detected in vaginal smears of females at 2.8 mg/kg-day, but decreased average number of pups
10 per litter occurred in groups exposed to 0.4, 0.8, or 1.5 mg/kg/day (Chu et al., 1981b).
11 Decreased sperm counts have been reported in male Sprague Dawley rats exposed for 28 days to
12 doses as low as 0.5 mg/kg/day (5 ppm in diet), with severe testicular degeneration occurring at
13 doses of 5 or 7.5 mg/kg/day (50 or 75 ppm in diet) (Yarbrough et al., 1981). Male fertility was
14 not affected in Sherman rats exposed to 2.3 mg/kg/day (25 ppm in diet) for 45 or 102 days before
15 mating with non-exposed female rats, but a group of female Sherman rats exposed for 102 days
16 to 25 ppm in the diet before mating with non-exposed males delivered fewer number of litters
17 than a group of non-exposed male and female Sherman rats (Gaines and Kimbrough, 1970). The
18 mode of action of this effect of mirex on female reproductive ability in Sherman rats is
19 unexplained. Subcutaneous mirex did not display typical estrogenic effects in immature female
20 Sprague Dawley rats (Gellert, 1978) and did not appear to directly act on the ovary in inhibiting
21 ova release in Long Evans rats induced by pregnant mare serum (Fuller and Draper, 1975).
22

23 **4.5.2. Inhalation Exposure**

24
25 No studies were located examining potential health effects in humans or animals
26 following repeated inhalation exposure to mirex.
27

28 **4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER** 29 **CHARACTERIZATION — SYNTHESIS OF HUMAN, ANIMAL, AND OTHER** 30 **SUPPORTING EVIDENCE, CONCLUSIONS ABOUT HUMAN** 31 **CARCINOGENICITY, AND LIKELY MODE OF ACTION** 32

33 Studies designed to investigate possible associations between mirex exposure and cancer
34 in humans are limited to a case-control study of postmenopausal women with breast cancer that
35 found no statistically significant association between detectable mirex in serum and the
36 occurrence of breast cancer (Moysich et al., 1998). Thus, the available human data are
37 inadequate for assessing the potential carcinogenicity of mirex in humans.
38

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

1 The liver tumors were reported to be mostly benign in F344/N rats and an equal mix of benign
2 and malignant tumors in CD rats. Innes et al. (1969) reported that metastases of liver tumors
3 were rare, but did not report the occurrences.
4

5 In addition, statistically significant increased incidences of tumors at several other sites
6 were observed, but these observations were not as consistent across studies as the tumorigenic
7 response in the liver. Increased incidences of adrenal pheochromocytomas (mostly benign) were
8 observed in groups of male F344/N rats exposed to 25 and 50 ppm in the diet, in one group of
9 female F344/N rats exposed to 50 ppm, but not in another group of female F344/N rats exposed
10 to 50 ppm (NTP, 1990). Increased incidences of mononuclear cell leukemia were found in male
11 F344/N rats exposed to 25 ppm, but not in males exposed to 50 ppm, and in F344/N females
12 exposed to 25 and 50 ppm in a first study, but only in females exposed to 100 ppm in a second
13 study (NTP, 1990). Significantly increased incidence of tumors at these sites were not found in
14 the studies of CD rats (Ulland et al., 1977). In B6C3F1 and B6AKF1 mice, no lymphomas were
15 induced by oral administration of mirex, but examination for neoplastic lesions in other organs
16 was not comprehensive in this study (Innes et al., 1969). In the only other animal studies related
17 to the potential carcinogenicity of mirex, subcutaneous injection of large (1000 mg/kg) single
18 doses produced increased incidences of reticulum cell sarcomas in B6C3F1 mice (Innes et al.,
19 1969), and dermally applied mirex promoted skin tumors in mice given initiating dermal doses
20 of 7,12-dimethylbenz[a]anthracene (Kim and Smart, 1995; Moser et al., 1992, 1993). Overall,
21 the database provides adequate evidence of carcinogenic hepatic responses to lifetime oral
22 exposure to mirex in rats and mice, and less consistent evidence of carcinogenic responses at
23 other sites.
24

25 Results from short-term genotoxicity testing indicate that mirex is not genotoxic, and
26 thereby suggest that mirex may produce carcinogenic responses via a non-genotoxic mode of
27 action. Mirex did not produce reverse mutations in *Salmonella typhimurium* or in *Escherichia*
28 *coli* with or without metabolic activation (Hallett et al., 1978; Mortelmans et al., 1986; Probst et
29 al., 1981; Schoeny et al., 1979), forward mutations at the HGPRT locus in human fibroblast cells
30 (Tong et al., 1981), unscheduled DNA synthesis in rat, mouse, or hamster hepatocytes
31 (Maslansky and Williams, 1981; Probst et al., 1981; Williams, 1980), or sister chromatid
32 exchanges or chromosomal aberrations in Chinese hamster ovary cells (NTP, 1990). Mirex did
33 not induce dominant lethal mutations in offspring of male rats exposed to oral doses as high as 6
34 mg/kg/day for 10 days (Khera et al., 1976) and did not produce hepatic DNA damage in mice
35 exposed to oral doses >80 mg/kg/day for 5 days (Umegaki et al., 1993) or in rats exposed to two
36 oral doses of 120 mg/kg (Mitra et al., 1990).
37

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

1 chronically exposed to mirex in the diet. The human relevance of the animal evidence of
2 carcinogenicity is assumed in the absence of adequate human data or mechanistic data to indicate
3 that the mode of carcinogenic action in animals is not relevant to humans.
4

5 The modes of action whereby mirex induces liver tumors in animals are not yet
6 completely understood, but available data suggest that a nongenotoxic mode involving mirex-
7 induced hepatic cell proliferation is plausible. Short-term genotoxicity testing, both *in vitro* and
8 *in vivo*, has produced no evidence of genotoxic actions from mirex, and metabolic studies
9 indicate that animals are not able to metabolize mirex and produce potentially reactive metabolic
10 intermediates that may be genotoxic. Mirex is a promoter, but not an initiator, of skin tumors in
11 the 2-stage mouse skin model, providing indirect support for an hypothesis that mirex may cause
12 liver tumors via promotion of previously initiated cells. Short-term and repeated oral exposure
13 to mirex are known to cause hepatic cytomegaly, vacuolization, fatty metamorphosis, and
14 necrosis, which may be precursor events to the eventual development of liver tumors, but this
15 hypothesis is not fully established.
16

17 The NTP (1990) rat study provides useful data for describing dose-response relationships
18 for liver tumors and non-neoplastic liver lesions (see Table 4). Five or six dietary concentrations
19 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,
20 25, or 50 ppm in females in one study, and 0, 50, and 100 ppm in another study). Incidences for
21 hepatocellular adenomas and carcinomas (as re-evaluated by PWG, 1992) were significantly
22 elevated at the 25- and 50-ppm level in males and the 50- and 100-ppm levels in females, but
23 were not elevated at the 0.1, 1, or 10-ppm levels (Table 4). This observation is consistent with
24 the existence of a threshold for liver cancer above the 10-ppm dietary exposure level, but is not
25 definitive evidence given the limited number of animals (n = 50/sex) included in this type of
26 bioassay. The apparent threshold for non-neoplastic liver lesions was above the 1-ppm level;
27 statistically significant elevated incidences of hepatotoxic effects were observed at dietary
28 concentrations of 10 ppm and greater. Most (but not all) rats that had liver tumors in this study
29 were diagnosed with non-neoplastic liver lesions such as cytomegaly, fatty metamorphosis,
30 vacuolization, or necrosis. Following U.S. EPA (1999a) guidance, a linear approach to dose-
31 response assessment should be taken for agents such as mirex that are not DNA reactive and
32 whose mode of action is not established. It is acknowledged, however, that the evidence for a
33 nongenotoxic mode of action involving non-neoplastic liver cell changes as precursor events is
34 stronger than evidence for a genotoxic mode of action without a threshold.
35

36 **4.7. SUSCEPTIBLE POPULATIONS**

37 38 **4.7.1. Possible Childhood Susceptibility**

39 The extent to which children are more susceptible to the toxic effects of mirex is not
40 known. No data is available regarding the adverse effects of mirex in human children. However,
41 mirex has demonstrated developmental toxicity in rodents (discussed in Section 4.3.2),
42 suggesting that infants and children may be more likely than adults to experience adverse effects
43 following exposure to mirex.
44

1 **4.7.2. Possible Gender Differences**

2
3 The extent to which men and women differ in susceptibility to mirex toxicity is not
4 known. No human data are available to suggest there are gender differences in the toxicity of
5 mirex.

6
7 Results from animal studies indicate that the possibility of gender differences in
8 susceptibility to mirex depends on the endpoint evaluated.

9
10 Several studies indicate that male rats may be more susceptible than female rats to mirex
11 hepatotoxicity, but the results are not entirely consistent. The NTP (1990) observed statistically
12 significant increases in mortality and the incidences of neoplastic liver lesions at lower doses in
13 male than in female F344/N rats. Based on the PWG (1992) re-evaluation of the liver
14 histopathology, the apparent gender difference in susceptibility to mirex carcinogenicity in the
15 liver was still evident, but significantly increased incidences of non-neoplastic toxic hepatitis
16 occurred at the same exposure levels in male and female groups (see Table 4). In addition, the
17 female groups displayed higher incidences of toxic hepatitis than male groups at the same
18 exposure level (Table 4). Gaines and Kimbrough (1970) concluded that male Sherman rats were
19 slightly more sensitive to the hepatotoxic effects of mirex, based on light and electron
20 microscopic analysis of livers after 166 days of dietary exposure. Khera et al. (1976) observed
21 statistically significant reduced weight gain at a lower dietary dose of mirex in males compared
22 to females.

23
24 Female mice may be more susceptible than males to the skin tumor promoting property
25 of mirex. Moser et al. (1993) concluded that ovarian hormones may increase the susceptibility
26 of female CD-1 mice to the dermal tumor-promoting activity of mirex. CD-1 mice were
27 dermally exposed to 7,12-dimethylbenz[a]anthracene to initiate tumor formation, and then
28 exposed to 200 nmol mirex for 20 weeks. In ovary-intact female mice, compared to
29 ovariectomized mice, a statistically significant increase in the number of tumors per mouse was
30 observed. Compared with male mice, female ovary-intact mice exhibited a higher number of
31 mice bearing tumors, more tumors per mouse, a shorter time to first tumor formation and a
32 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.

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

14 **5.1.2. Methods of Analysis—Including Models (PBPK, BMD, etc.)**

15
16
17 Using the array of mathematical models in the EPA Benchmark Dose Software, models
18 were fit to the incidence data for toxic hepatitis in male or female F344 rats in the NTP (1990)
19 bioassay (Appendix B). The best fits as indicated by graphical examination and goodness of fit
20 statistics for both sets of data were with the log-logistic model. Figures 1 and 2 in Appendix B
21 plot observed and predicted incidences of toxic hepatitis as functions of human equivalent doses
22 based on the male and female rat responses, respectively. The models predict ED10 values
23 (human equivalent doses associated with 10% incidence of toxic hepatitis) of 0.28 and 0.13
24 mg/kg/day based on the male and female rat data, respectively. These predicted values are for
25 extra risk (i.e. incidence - background incidence/ 1- background incidence; $P_{(d)} - P_{(0)} / 1 - P_{(0)}$).
26 The points of departure for the benchmark dose approach to RfD derivation (i.e., the lower 95%
27 confidence limit on the ED10 = LED10) are 0.20 and 0.10 mg/kg/day based on male and female
28 rat data, respectively. Given that these LED10 values are similar, an average LED10 of 0.15
29 mg/kg/day is taken as the point of departure for derivation of the RfD for mirex.

30 31 **5.1.3. RfD Derivation—Including Application of Uncertainty Factors and Modifying** 32 **Factors**

33
34 The point of departure from the benchmark dose modeling of toxic hepatitis (LED 10 for
35 toxic hepatitis = 0.15 mg/kg/day) is divided by a total uncertainty factor of 300 (10 for
36 extrapolating from rats to humans, 10 to account for human variability, and 3 to account for data
37 base deficiencies including the lack of 2-generation reproductive toxicity studies) to derive a
38 chronic oral RfD for mirex of 5×10^{-4} mg/kg/day (0.5 µg/kg/day or 0.0005 mg/kg/day).

39
40 This RfD is similar in value to one derived in 1992 (2×10^{-4} mg/kg/day) based on the
41 original NTP (1990) liver histopathology, a NOAEL/LOAEL approach that used a rat NOAEL
42 of 0.07 mg/kg/day (without dosimetric adjustment) for liver and thyroid lesions, and a total
43 uncertainty factor of 300 (10 for extrapolating from rats to humans, 10 for human variability, and
44 3 for database deficiencies).

1 Confidence in the principal study is high. It included sufficient numbers of animals of
2 both sexes for statistical analysis, adequate numbers of exposure levels to provide good
3 descriptions of dose-response relationships for relevant endpoints, and was adequately designed,
4 conducted, and reported. Confidence in the data base is medium. Several subchronic and
5 chronic oral exposure studies are available in rats and mice identifying the liver as a sensitive
6 target of mirex toxicity and, as discussed in Section 4.5.1. Analysis of the data base indicates
7 that the RfD based on liver effects will be protective of other identified sensitive effects from
8 mirex exposure including splenic fibrosis from chronic exposure, developmental effects
9 including cataracts and reduced pre-weaning offspring survival, and impaired male or female
10 reproductive performance. Confidence in the data base could be increased with data from
11 2-generation reproductive toxicity studies and comparative mechanistic studies providing more
12 information regarding possible differences between primates and rodents in susceptibility to
13 mirex toxicity. Resultant confidence in the RfD is medium to high.

14 **5.2. INHALATION REFERENCE CONCENTRATION (RfC)**

15
16
17 No inhalation RfC is derived due to the lack of appropriate inhalation exposure toxicity
18 data for humans or animals and the lack of rat and human PBPK models for mirex that would
19 facilitate extrapolating across exposure routes. In addition, the oral route is the most relevant
20 route for exposure to mirex in the general environment.

21 **5.3. CANCER ASSESSMENT**

22
23
24 As discussed in Section 4.6, mirex is likely to be carcinogenic to humans based on
25 consistent findings of hepatic carcinogenic responses, and less consistent carcinogenic responses
26 at other tissue sites, in several studies of rats and mice chronically exposed to mirex in the diet.
27 The animal evidence for mirex carcinogenicity is assumed to be relevant to humans in the
28 absence of adequate human data or mechanistic data to indicate that the mechanisms of mirex
29 carcinogenic action in rodents are not relevant to humans.

30
31 The mechanisms by which mirex induces liver tumors in animals are not completely
32 understood. Mirex is nongenotoxic in multiple short-term *in vitro* and *in vivo* genotoxicity tests
33 and is a promoter, but not an initiator, of skin tumors in the two-stage mouse skin model. Short-
34 term or repeated oral exposure of rodents to mirex causes hepatic cytomegaly, vacuolization,
35 fatty metamorphosis and necrosis, which may be precursor events to the eventual development
36 of liver tumors, but this hypothesis is not fully established. Consistent with this hypothesis is the
37 correlation found between non-neoplastic liver lesions and hepatocellular adenomas in mirex-
38 exposed rats in the NTP (1990) bioassay, but not all rats with adenomas were diagnosed with
39 non-neoplastic liver lesions (as diagnosed by the PWG [1992] histopathological examination).
40 For example, among the six male rats with adenomas in the group exposed to 1.9 mg/kg/day,
41 four were diagnosed toxic hepatitis or eosinophilic foci, but two were not. In the group exposed
42 to 3.9 mg/kg/day, the diagnosis was eight with, and two without, non-neoplastic liver lesions
43 among the 10 male rats with liver adenomas.

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

10
11 One linear approach involved fitting a model with an exponential polynomial function of
12 dose and a Weibull function of time-to-tumor to the incidence data for combined adenomas and
13 carcinomas in male rats and associated doses converted to human equivalent doses (this
14 conversion is discussed in Section 5.1.2). A licensed computer program, *Multi-Weib* (K.S.
15 Crump and Company, Ruston, LA), was used to fit the model to the data. A three-degree
16 polynomial form of the dose function provided the best fit of the data. The lower 95%
17 confidence limit of the dose associated with a 10% extra risk (LED10) for tumors was 0.193
18 mg/kg/day (calculated for a full lifetime, 105 weeks). Using linear extrapolation from the
19 LED10 to the origin, predicted doses associated with extra risks of 10^{-4} , 10^{-5} , and 10^{-6} are 0.0002,
20 0.00002, and 0.000002 mg/kg/day, respectively. Using the upper 95% confidence limit on the
21 slope in the low-dose region (i.e., the $q1^*$ or oral slope factor = 0.53 per mg/kg/day), predicted
22 doses associated with extra risks of 10^{-4} , 10^{-5} , and 10^{-6} are the same: 0.0002, 0.00002, and
23 0.000002 mg/kg/day, respectively.

24
25 Linear approaches without the time-to-tumor analysis were also applied to the male rat
26 tumor incidence data (Table 4) using the array of discontinuous mathematical models in the EPA
27 Benchmark Dose Software. For liver adenomas and carcinomas combined, a probit model
28 provided the best fit among the models examined. The ED10 and LED10 values predicted by
29 the model were 0.6 and 0.5 mg/kg/day. Extrapolating from the LED10, predicted doses
30 associated with extra risks of 10^{-4} , 10^{-5} , and 10^{-6} are 0.0005, 0.00005, and 0.000005 mg/kg/day.

31
32 The model that included time-to-tumor as an explanatory variable was selected as the
33 linear approach of choice for these data, because there was statistically significantly reduced
34 survival of male rats in the two highest dose groups in the NTP (1990) bioassay. The modeling
35 provides a means of adjusting for less-than-lifetime exposure for the rats with tumors that died
36 early, and estimating risk for liver tumors with full life-time exposure.

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

8
9 U.S. EPA (1999a) Draft Revised Guidelines for Carcinogen Risk Assessment states that
10 for chemicals like mirex, where there is an absence of sufficient tumor mode of action
11 information, a linear approach for carcinogenic risk assessment should be used. Several linear
12 extrapolation approaches involving mathematical modeling of liver male rat tumor incidences
13 and human equivalent doses (from the NTP, 1990 bioassay with re-evaluation of liver
14 histopathology by PWG [1992]) were taken and arrived at similar estimates of doses associated
15 with extra risk levels of 10^{-4} , 10^{-5} , and 10^{-6} : 0.0002-0.0005, 0.00002-0.00005, and 0.000002-
16 0.000005 mg/kg/day, respectively (0.2-0.5, 0.02-0.05, 0.0002-0.005 $\mu\text{g/kg/day}$). The approach
17 that included time-to-tumor as an explanatory variable was selected as the linear approach of
18 choice for these data, because there was statistically significantly reduced survival of male rats in
19 the two highest dose groups in the NTP (1990) bioassay. With this approach, predicted doses
20 associated with extra risks of 10^{-4} , 10^{-5} , and 10^{-6} are: 0.0002, 0.00002, and 0.000002 mg/kg/day,
21 respectively (0.2, 0.02, and 0.002 $\mu\text{g/kg/day}$).

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1 **APPENDIX B: BENCHMARK DOSE MODELING RESULTS**

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4 All dichotomous models in the Benchmark Dose Software (version 1.3.2) were fit to the
5 data for the incidence of toxic hepatitis in F344/N male and female rats fed Mirex in the diet for
6 2 years (Table 1). As assessed by the chi-square goodness-of-fit statistic, the log-logistic,
7 gamma, 5-degree polynomial multistage, quantal linear, and Weibull models for the male rats,
8 and the log-logistic model for the female rats adequately fit the data for the incidence of toxic
9 hepatitis. The log-logistic models provided the best fit as assessed by Aikake's Information
10 Criteria (AIC) for both the male and female data (Table 2).
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16 **Table 1. Incidence of toxic hepatitis in F344/N male and female rats fed Mirex**
17 **in the diet for 2 years**
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Rat dose mg/kg-day						
	0	0.007	0.08	0.7	1.9	3.9
20 Male	1/52	2/52	5/52	11/52	28/52	29/52
21 Female	1/52	1/52	3/52	25/52	34/52	35/52

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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

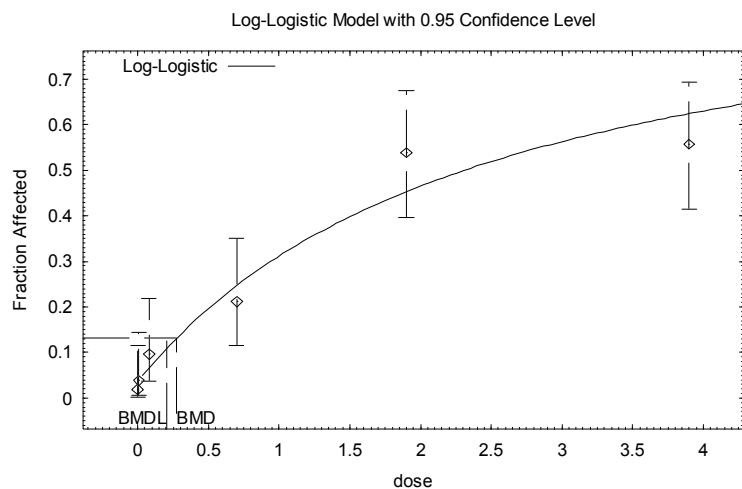
MODEL	ED ₁₀	LED ₁₀	x2 p-value	AIC
Male rats				
Log-logistic ^b	0.28	0.20	0.40	264.63
Gamma ^a	0.40	0.32	0.13	267.57
Multi-stage ^c	0.40	0.32	0.13	267.57
Quantal linear	0.40	0.32	0.13	267.57
Weibull ^a	0.40	0.32	0.13	267.57
Log-probit ^b	0.68	0.55	0.02	271.90
Logistic	0.99	0.84	0.00	283.99
Probit	0.93	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

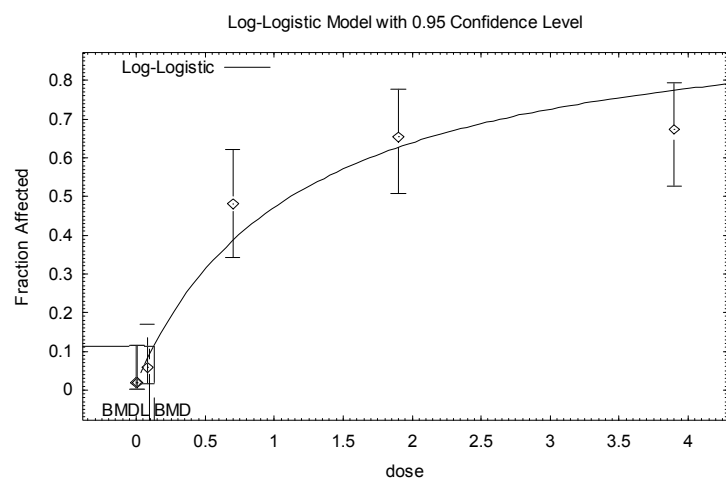
c = Restrict betas >=0; Degree of polynomial = 5

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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 = ED₁₀; BMDL = LED₁₀; Dose unit = mg/kg-day



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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 = ED₁₀; BMDL = LED₁₀; Dose unit = mg/kg-day