2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO TOXAPHENE IN THE UNITED STATES

Toxaphene is a manufactured pesticide comprising a complex mixture of hundreds of chlorinated terpenes. After DDT was banned from use in the United States in 1972, toxaphene became the most popular substitute. Control of pests on cotton crops was the principal use of toxaphene in the United States, although the pesticide was used to control a variety of insects on a range of crops and to eradicate undesirable fish species in some aquatic environments. In November of 1982, EPA canceled the registration of toxaphene for most uses as a pesticide or pesticide ingredient. All registered uses of toxaphene mixtures in the United States and its territories were canceled in 1990.

Toxaphene was widely released to the environment mainly as a result of its past use as an insecticide. Toxaphene has been transported over long distances in the atmosphere. The presence of toxaphene in surface waters of the Great Lakes has been attributed to aerial transport of the mixture from application sites in the southern United States. Atmospheric toxaphene is transported back to soil and water surfaces by wet and dry deposition processes. Toxaphene strongly adsorbs to particles and is relatively immobile in soils. In water, toxaphene is strongly adsorbed to suspended particulates and sediments and is bioconcentrated by aquatic organisms to fairly high levels, with bioconcentration factors (BCFs) on the order of 4,200–60,000. Toxaphene also appears to be biomagnified in aquatic food chains.

The composition of technical toxaphene released to the environment has changed over time since toxaphene congeners degrade at different rates, resulting in what is commonly termed weathered toxaphene. Degradation proceeds mainly through dechlorination and dehydrochlorination resulting in a shift in composition toward lower chlorinated homologs. Presently, exposure to persistent toxaphene congeners and degradation products is the primary health concern for the general population. Toxaphene congeners that have been found to persist in fish, marine mammals, and human serum and breast milk include those identified as Parlars p-26, p-40/41, p-44, p-50, and p-62. Pooled results of studies that assessed levels of these congeners in human serum and/or breast milk indicate that p-26, p-50, and p-62 comprise most of the total toxaphene body burden.

The major source of exposure for the general population appears to be ingestion of low concentrations of persistent toxaphene congeners in food, particularly fish, and toxaphene-contaminated drinking water. Subpopulations with increased potential for significant exposure to persistent toxaphene congeners

include northern Native American groups that eat toxaphene-contaminated aquatic mammals, recreational or subsistence hunters in the southern United States that consume significant amounts of game animals (especially species like raccoons), and people who consume certain types of sport-caught fish (such as trout, salmon, herring, smelt, and walleye) from the Great Lakes.

2.2   SUMMARY OF HEALTH EFFECTS

This toxicological profile for toxaphene summarizes health effects information for toxaphene based on exposure to technical toxaphene that was formerly widely used as a pesticide in the United States. Since being banned for use as a pesticide in the United States in 1990, the greatest present concern for the general population would be exposure to persistent toxaphene congeners and degradation products of technical toxaphene formerly released to the environment.

Limited information is available regarding noncancer health effects in humans or laboratory animals following inhalation exposure to toxaphene. Pulmonary hypersensitivity and hematological alterations were indicated in two Egyptian agricultural pesticide workers involved in spraying a pesticide formulation (68% toxaphene, 35% kerosene, 3% xylol, and 2% emulsifier). One controlled study found no signs of toxicity in a group of 25 volunteers exposed to an aerosol containing a maximum of 500 mg toxaphene/m^3 30 minutes/day for 30 days. The nervous system and the liver have been identified as targets of toxaphene toxicity in limited animal studies that employed the inhalation exposure route.

Ingestion of toxaphene has resulted in death in some cases of acute poisoning. Mortalities have also been observed in animals following single- and repeated-dose oral exposure.

Clinical signs of central nervous system stimulation including convulsions have been reported in humans following accidental or intentional ingestion of toxaphene. Similar effects have been observed in animals following oral exposure to toxaphene. Dogs appear to be particularly sensitive to the neurological effects of toxaphene; convulsions were elicited at oral doses as low as 10 mg/kg/day.

Animal studies provide evidence that toxaphene can also affect the liver, kidney, endocrine system, immunological system, and body weight, as well as the nervous system. Morphological and degenerative changes were observed in the livers of dogs, rats, and mice following repeated oral exposure to toxaphene; doses as low as 1.8 mg/kg/day caused nuclear changes in hepatocytes of rats. Renal tubular injury was reported in the kidneys of rats receiving toxaphene at 8.6 mg/kg/day for 13 weeks.

Histopathological evidence of effects on the thyroid gland of male rats was observed following 13 weeks of oral administration of toxaphene at 1.8 mg/kg/day. Significant reductions in IgM responses (indicative of depressed humoral immunity) were reported in female cynomolgus monkeys administered toxaphene by oral capsule at a dose level of 0.4 mg/kg/day during a 75-week treatment period. Pregnant animals may be particularly sensitive to toxaphene-induced effects on body weight; for example, average body weight gain of rat dams administered toxaphene by gavage at 15 mg/kg/day during pregnancy was 22% lower than that of pregnant control rat dams.

Toxaphene-induced reproductive effects have not been demonstrated in animal studies. Effects such as decreased early postnatal body weight and increased incidences of supernumary ribs were observed in offspring of rat dams that were fed toxic doses of toxaphene. Decreased renal protein was noted in the kidneys of 21-day-old rat fetuses whose mothers had been administered toxaphene by gavage at 12.5 mg/kg/day during gestation. Suppression of macrophage phagocytic function was reported in 8-week-old offspring of mouse dams administered toxaphene at doses ≥2 mg/kg/day during premating and throughout mating, gestation, and lactation. In one developmental toxicity study, slight delays in successful responses to a righting reflex test were observed in nursing pups from rat dams administered toxaphene by gavage at 6 mg/kg/day during mating and throughout gestation. Retarded swimming ability and righting reflex were reported in young pups of rat dams administered toxaphene at 0.05 mg/kg/day during gestation and lactation; however, the effect was transient, observed only during postpartum days 10–12 and of uncertain toxicological significance. However, these studies do not provide convincing evidence of toxaphene-induced reproductive or developmental effects.

Limited animal data indicate that dermal exposure to high levels of toxaphene can cause clinical signs of neurotoxicity, liver and kidney effects, and death at very high doses.

Some case-control studies of farm workers and prospective cohort studies of pesticide applicators have reported statistically significant associations between exposure to toxaphene and risk of cancers such as leukemia, certain types of non-Hodgkin's lymphoma, rectal cancer, and melanoma. However, these results were based on relatively small numbers of cancer cases. It should be noted that all uses of toxaphene in the United States were canceled in 1990. Increased incidences of thyroid tumors were observed in rats administered toxaphene at approximately 80 mg/kg/day for 80 weeks; in similarly-treated mice, increased incidences of hepatocellular tumors were noted at toxaphene doses of 17 and 34 mg/kg/day. One unpublished study reported increased incidences of hepatocellular tumors in male mice administered toxaphene orally at approximately 8.6 mg/kg/day for 18 months. Available *in vitro*

assays provide equivocal evidence for toxaphene-induced genotoxicity. Information regarding the potential genotoxicity of toxaphene *in vivo* is extremely limited; available results have not suggested a toxaphene-induced genotoxic response. The International Agency for Research on Cancer (IARC) has classified toxaphene as Group 2B (possibly carcinogenic to humans). The National Toxicology Program (NTP) considers toxaphene as "reasonably anticipated to be a human carcinogen". EPA has given toxaphene a classification of B2 (probable human carcinogen). The cancer classifications are based on the findings of hepatocellular tumors in toxaphene-treated mice, thyroid tumors in toxaphene-treated rats, and evidence of mutagenicity in *in vitro* bacterial assays.

2.3   MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for toxaphene. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990a), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

No inhalation MRLs were derived for toxaphene due to the lack of reliable human and animal data. One controlled human study found no evidence of adverse effects among 25 human subjects exposed to an aerosol containing a maximum of 500 mg toxaphene/m^3 for 30 minutes/day for 10 days (Keplinger 1963). However, exposures were brief, air concentrations were not measured, and assessments were limited to

clinical examinations and incomplete blood and urinalysis testing. Acute pulmonary insufficiency was noted in two agricultural workers involved in spraying a formulation consisting of (60% toxaphene, 35% kerosene, 3% xylol, and 2% emulsifier) for 2 months. No other information was located regarding health effects in humans exposed to toxaphene in the air.

Available animal data are limited to summaries of unpublished data cited in a secondary unpublished bulletin and a report of clinical signs of neurotoxicity in several animal species following repeated inhalation exposure to toxaphene dust (Industrial Biotest 1965). Some studies conducted by Industrial Biotest have been shown to be less than reliable.

Oral MRLs

- An MRL of 0.05 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to toxaphene.

Information on effects of acute-duration oral exposure to toxaphene in humans is limited to reports of cardiac dilatation and swelling of the kidneys in a 2-year-old boy who ingested an unspecified lethal amount of toxaphene (McGee et al. 1952), temporarily-compromised hepatic and renal function in a 26-year-old male who attempted suicide by ingesting an insecticide containing toxaphene as the active ingredient (Wells and Milhorn 1983), and signs of neurotoxicity (convulsive seizures, temporary memory loss, nausea) in several females who had ingested collard greens coated with toxaphene (McGee et al. 1952).

Available acute-duration oral toxicity animal studies include single-dose studies in rats (Garcia and Mourelle 1984; Peakall 1976), guinea pigs (Chandra and Durairaj 1992, 1995), and dogs (Chu et al. 1986; Lackey 1949); and multiple-dose studies in rats (Chernoff and Carver 1976; Chernoff et al. 1990; Kavlock et al. 1982; Mehendale 1978; Rao et al. 1986; Trottman and Desaiah 1980; Waritz et al. 1996), mice (Chernoff and Carver 1976; Hedli et al. 1998), and dogs (Chu et al. 1986; Lackey 1949). Targets of acute oral toxaphene toxicity include the nervous system, liver, body weight, immunological system, endocrine system, and development.

Several animal studies include reports of treatment-related liver weight changes following acute-duration oral exposure to toxaphene. Chernoff and Carver (1976) reported 23, 25, and 32% increased mean relative liver weight in mouse dams administered toxaphene by gavage at 15, 25, and 35 mg/kg/day during gestation days 7–16; the 35 mg/kg/day dose level represented a no-observed-adverse-effect level

(NOAEL) for liver weight in similarly-treated rat dams. Trottman and Desaiah (1980) reported 20% increased mean liver weight in rats receiving toxaphene from the diet at 18 mg/kg/day for 14 days; the NOAEL for effects on liver weight was 13.5 mg/kg/day. Hedli et al. (1998) reported significantly increased mean relative liver weights in mice gavaged at 50 and 100 mg/kg/day for 7 days; the NOAEL for effects on liver weight was 25 mg/kg/day. Peakall (1976) reported a 9% increase in relative liver weight and significantly increased microsomal enzyme activity in rats at 5 days following administration of a single 120 mg/kg oral dose of toxaphene. Mehendale (1978) reported decreased biliary excretion of imipramine metabolites from perfused livers of rats that had received toxaphene from the diet at an estimated dose of 10 mg/kg/day for 8 days.

Chu et al. (1986) observed convulsions in dogs following oral administration of toxaphene at 10 mg/kg/day for 2 days; convulsions were not elicited after the dose was reduced to 5 mg/kg/day for the remainder of a 13-week treatment period. Lackey (1949) reported convulsions in dogs administered a single 10 mg/kg dose of toxaphene. Mild tremors and nervousness were noted in rats receiving toxaphene by gavage at 25 mg/kg/day for 3 days (Rao et al. 1986).

Toxaphene-induced effects on maternal body weight were observed in rat and mouse dams administered toxaphene via gavage during organogenesis (Chernoff and Carver 1976; Chernoff et al. 1990). The mouse study identified a NOAEL of 15 mg/kg/day and a serious lowest-observed-adverse-effect level (serious LOAEL) of 25 mg/kg/day for 22% decreased maternal body weight gain (Chernoff and Carver 1976). The rat studies identified serious LOAELs of 32 mg/kg/day (the only dose tested by Chernoff et al. 1990) and 15 mg/kg/day (the lowest dose tested by Chernoff and Carver 1976) for the effect.

Trottman and Desaiah (1980) reported a 36% decrease in mean thymus weight in rats receiving toxaphene from the diet at an estimated dose of 13.5 mg/kg/day for 14 days; this effect was not observed at the lower dose (9 mg/kg/day). The study report made no mention of histopathological evaluations.

A greater than 2-fold increase in serum TSH and histopathologic thyroid lesions were reported in rats administered toxaphene by gavage at 75 mg/kg/day (the only dose tested) for 14 days (Waritz et al. 1996).

Significantly increased incidence of supernumerary ribs (17% greater than controls) was reported in fetuses of rat dams administered toxaphene by gavage at 32 mg/kg/day (the only dose tested) during gestation days 6–15 (Chernoff et al. 1990). Significantly decreased fetal renal protein and slight, but statistically significant retardation in kidney development were reported in fetuses of rat dams

administered toxaphene by gavage at 12.5 or 25 mg/kg/day during gestation days 7–16; lower dose levels were not tested (Kavlock et al. 1982).

The most sensitive effects of acute oral toxaphene toxicity were observed at doses in the range of 10–15 mg/kg/day and include increased liver weights, clinical signs of neurotoxicity, depressed maternal body weight gain, and indicators of treatment-related effects on developmental end points. There is some degree of uncertainty regarding the toxicological significance of the reported effects on liver weight, fetal renal protein, thymus weight, and biliary excretion of imipramine metabolites. The reported 22% decreased maternal body weight gain in the toxaphene-treated rat dams of the Chernoff and Carver (1976) study is clearly a serious adverse effect. However, this effect was observed at the lowest dose tested, and the study did not identify a NOAEL. ATSDR does not derive MRLs based on a serious LOAEL in the absence of an identified NOAEL. The dog study of Chu et al. (1986) identified a NOAEL (5 mg/kg/day) and a LOAEL (10 mg/kg/day) for clinical signs of toxaphene-induced neurotoxicity, but did not include histopathological investigations. Support for a NOAEL of 5 mg/kg/day for neurological effects is provided by the results of another dog study in which a single 5 mg/kg dose of toxaphene elicited no clinical signs of neurotoxicity, whereas a single 10 mg/kg dose resulted in convulsions (Lackey 1949). Although both studies identified a serious LOAEL of 10 mg/kg/day for neurological effects, the NOAEL of 5 mg/kg/day (identified in both studies) is considered adequate basis for deriving an acute-duration oral MRL for toxaphene. The dog study of Chu et al. (1986) is selected as the principal study, and the NOAEL of 5 mg/kg/day is selected as the critical effect and point of departure (POD) for MRL derivation. An acute-duration oral MRL derived in this manner is expected to be protective of toxaphene-induced effects on the nervous system, liver, endocrine system, and developmental end points.

In the principal study (Chu et al. 1986), groups of male and female beagle dogs (6/sex/group) were given gelatin capsules containing toxaphene at 0, 0.2, 2.0, or 5.0 mg/kg daily for 13 weeks. During the first 2 treatment days, the high-dose group received toxaphene at 10 mg/kg/day. This dose was reduced to 5 mg/kg/day on treatment day 3 because the 10 mg/kg/day dose level elicited convulsions, salivation, and vomiting in 1/6 males and 2/6 females. These clinical signs were not observed in any of the toxaphene-treated dogs throughout the remainder of the scheduled 13-week treatment period. This study identified a serious LOAEL of 10 mg/kg/day for neurological effects elicited during the first 2 days of oral treatment and a NOAEL of 5 mg/kg/day. Using the NOAEL of 5 mg/kg/day as the POD, application of a total uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) yields an acute-duration oral MRL of 0.05 mg/kg/day for toxaphene.

- An MRL of 0.002 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to toxaphene.

No human studies were located regarding the effects of intermediate-duration oral exposure to toxaphene.

Intermediate-duration oral toxicity studies are available for rats (Chu et al. 1986, 1988; Crowder et al. 1980; Garcia and Mourelle 1984; Kennedy et al. 1973; Koller et al. 1983; NCI 1979; Olson et al. 1980; Ortega et al. 1957; Peakall 1976; Waritz et al. 1996), mice (Allen et al. 1983; NCI 1979), dogs (Chu et al. 1986; Lackey 1949), and cynomolgus monkeys (Bryce et al. 2001; Tryphonas et al. 2000, 2001). The animal studies identified the nervous system, liver, kidney, thyroid gland, and immunological system as targets of toxaphene toxicity from intermediate-duration oral exposure. Lackey (1949) reported occasional convulsions in groups of dogs (2/group) administered toxaphene by capsule at 4 mg/kg/day for 44 or 106 days, but did not include more specific details. Study limitations preclude the usefulness of this study for quantitative risk assessment. One developmental toxicity study reported inferior swimming ability at postnatal days 10–12 in pups of rat dams receiving toxaphene from the diet at 0.05 mg/kg/day throughout gestation and for 30 days postpartum; however, swimming behavior appeared normal by postnatal day 16 (Olson et al. 1980). No additional studies were located to support the finding of toxaphene-related effects on postnatal development at such low dose levels.

Identified LOAELs for liver, kidney, and thyroid effects range from 0.5 to 45 mg/kg/day. A 13-week dietary study of male and female rats (Chu et al. 1986) identified the lowest LOAELs for these effects. Significantly increased incidences of histopathologic lesions of the liver (anisokaryosis) and kidney (renal tubular injury) were observed in the females at a 0.5 mg/kg/day dose level in the absence of an identified NOAEL. Similar effects were observed in the males at the dose level of 1.8 mg/kg/day; the lowest dose tested in the male rats (0.35 mg/kg/day) represented a NOAEL for liver and kidney effects. The same study identified NOAELs of 0.35 and 12.6 mg/kg/day and LOAELs of 1.8 and 63 mg/kg/day for morphologic lesions in the thyroid (angular collapse of follicles, increased epithelial height with multifocal papillary proliferation, and reduced colloid density) of the males and females, respectively. Chu et al. (1986) also observed hepatomegaly in dogs administered toxaphene by capsule at 5 mg/kg/day for 13 weeks, but no evidence of treatment-related liver effects at a daily 2 mg/kg dose level.

Depressed humoral immune responses have been observed in rats, mice, and cynomolgus monkeys administered toxaphene orally for periods ranging from 8 to 52 weeks. In an enzyme-linked immunosorbent assay (ELISA) performed on female mice that received toxaphene from the diet at doses ≥19 mg/kg/day for up to 8 weeks, Allen et al (1983) reported suppressed antibody production, indicating

depressed humoral immunity; the study identified a NOAEL of 2 mg/kg/day for the effect. Koller et al. (1983) reported a 46% decrease in the IgG primary antibody response in male rats receiving toxaphene from the diet at 2.6 mg/kg/day for up to 9 weeks and challenged twice (after 8 and 15 days on test) with keyhole limpet hemocyanin (KLH).

Tryphonas et al. (2001) reported a NOAEL of 0.1 mg/kg/day and a LOAEL of 0.4 mg/kg/day for toxaphene-induced decreased anti-SRBC (IgM) titers as an indicator of depressed humoral immunity. In the study, groups of 10 female cynomolgus monkeys/dose group (approximately 7 years of age on average) received toxaphene via oral capsules at 0, 0.1, 0.4, or 0.8 mg toxaphene/kg/day for up to 75 weeks. Groups of five males dosed at 0 or 0.8 mg/kg/day (approximately 12.5 and 6 years of age on average, respectively) were included in the study. Testing for immune effects was initiated on treatment week 33 and included flow cytometry, lymphocyte transformation, natural killer cell activity and determination of serum cortisol during treatment weeks 33–46 and immunizations with sheep red blood cells (SRBC) treatment at week 44 for a primary response and week 48 for a secondary response (observations made through treatment week 52). Treatment with toxaphene at 0.4 mg/kg/day resulted in significant (p<0.05) reductions in mean primary anti-SRBC IgM responses at weeks 1 and 4 following primary immunization (27 and 35% lower than that of controls) and secondary anti-SRBC IgM responses at week 1 following secondary immunization (10% lower than that of controls). The dose level of 0.8 mg/kg/day resulted in significantly reduced mean primary anti-SRBC IgM responses at weeks 1–4 following primary immunization, significantly reduced mean secondary anti SRBC IgM response at weeks 1 and 4 following secondary immunization, and significantly reduced primary anti-SRBC IgG responses at weeks 2 and 3 following primary immunization (51 and 43% lower than that of controls). In males, 0.8 mg/kg/day toxaphene induced a significant reduction in mean primary anti-SRBC IgM response at weeks 1–3 following primary immunization. Flow cytometry tests showed that the only effect on leukocyte and lymphocyte subsets was a reduction in absolute B lymphocytes (CD20) in 0.8 mg/kg/day females (62% lower than controls). There were no detectable treatment-related effects on natural killer cell activity, lymphoproliferative response to mitogens, or serum cortisol levels. This study identified the lowest LOAEL (0.4 mg/kg/day for depressed humoral immunity) among reliable LOAELs for intermediate-duration oral exposure to toxaphene, and is selected as the critical effect for deriving an intermediate-duration oral MRL for toxaphene.

To derive a POD for MRL derivation, BMD modeling was conducted using data for depressed humoral immunity from the female cynomolgus monkeys (Tryphonas et al. 2001). All continuous variable models in the EPA Benchmark Dose Software (Version 2.1.1) were fit to the mean anti-SRBC (IgM) titre data at

week 1 post-immunization at treatment week 44; the modeled data are presented in Table A-3 of
Appendix A. A default benchmark response (BMR) of 1 standard deviation (1SD) from the control mean
was selected in the absence of a toxicological rationale for selecting an alternative BMR. As discussed in
detail in Appendix A, the polynomial and power models converged on the linear model and provided
identical fit to the data. These models predicted BMD_{1SD} and $BMDL_{1SD}$ values of 0.34 and
0.22 mg/kg/day, respectively. The $BMDL_{1SD}$ of 0.22 mg/kg/day was divided by an uncertainty factor of
100 (10 for interspecies extrapolation and 10 for human variability). The resulting intermediate-duration
oral MRL is 0.002 mg/kg/day.

A chronic-duration oral MRL was not derived for toxaphene for the following reasons:

1. No human studies were located regarding the effects of chronic-duration oral exposure to
    toxaphene.

2. A study designed to assess the effect of toxaphene on the immune system of cynomolgus
    monkeys identified a LOAEL of 0.4 mg/kg/day for decreased anti-SRBC IgM response during
    intermediate-duration oral exposure (<52 weeks) that is lower than the LOAEL of 0.8 mg/kg/day
    for decreased anti-TT response to chronic-duration oral exposure (testing initiated after 53 weeks
    of treatment).

3. Toxaphene doses used in chronic duration oral toxicity studies in rats and mice (NCI 1979) were
    2 orders of magnitude higher than doses eliciting immunological effects in cynomolgus monkeys
    treated for intermediate and chronic durations.