S,S,S-TRIBUTYL PHOSPHOROTRITHIOATE
(Tribufos)

RISK CHARACTERIZATION DOCUMENT

(Revision No. 1)

Medical Toxicology and Worker Health and Safety Branches

Department of Pesticide Regulation

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I. SUMMARY

S,S,S-Tributyl phosphorotrithioate (tribufos) is an organophosphate chemical which was first registered in 1960 for cotton defoliation (U.S. EPA, 1981). Tribufos induces early leaf abscission through changes in the levels of plant hormones. Defoliation occurs 4 to 7 days after treatment. Dietary exposure to tribufos may occur from consumption of cottonseed products such as cottonseed oil or cottonseed meal or from consumption of meat or milk from livestock that are fed cottonseed products in their feed. A Risk Characterization Document evaluating potential occupational and dietary exposure to tribufos was completed by the Department of Pesticide Regulation (DPR) in the California Environmental Protection Agency in 1998. Since then U.S. EPA has completed a Human Health Risk Assessment (1999) and an Interim Reregistration Eligibility Document (2000) for tribufos. Subsequent to the completion of DPR's RCD for tribufos, the registrant has submitted a new dermal absorption study in monkeys and several neurotoxicity studies in rats (acute, subchronic and developmental) which could potentially alter the risk estimates for tribufos. This revised Risk Characterization Document represents a reevaluation of the potential health risks from occupational and dietary exposure to tribufos based on the new data.

Tribufos appears to be readily absorbed by the oral route and rapidly metabolized in the species examined. The oral absorption rate was assumed to be 70% based on the average urinary excretion in rats on all dosing regimens. The dermal absorption for tribufos was assumed to be 7.1% in humans based on a study conducted in monkeys and 47.5% in animals based on a study conducted in rats. A default assumption of 50% respiratory retention and 100% absorption was used with occupational exposure based on the assumption that tribufos is primarily in the vapor phase. Several metabolic pathways have been proposed for tribufos based on a few metabolites; however, the metabolism of tribufos by the various routes of exposure is still highly speculative. One explanation for the inability to identify metabolites was that most of the parent compound had been extensively metabolized into natural constituents, such as fatty acids and proteins. n-Butyl mercaptan (nBM) was identified in the excreta of hens administered tribufos orally. It was proposed that tribufos was hydrolyzed to nBM in the gut causing the hematological effects which were only observed with oral administration of tribufos. Apparently nBM inhibits glucose-6-phosphate dehydrogenase leading ultimately to red blood cell (RBC) lysis through the formation of methemoglobin. nBM is thought to be a product of the normal metabolism of tribufos in tissues. Tribufos also readily degrades to nBM in the environment and may be responsible for complaints by residents in communities near cotton fields due to its strong skunk-like odor (odor threshold ~ 0.01 to 1 ppb). However, limited data on nBM preclude a thorough toxicological evaluation.

The acute effects of tribufos in experimental animals are due primarily to its inhibition of various esterases including cholinesterase (ChE) and neuropathy target esterase. In general, DPR considers brain ChE inhibition to be indicative of overt toxicity since it is one of the primary target sites and more subtle neurological signs, such as memory and learning losses, may not be easily detected in animals unless they are specifically tested for these effects. The toxicological significance of plasma and RBC ChE inhibition is less certain because the physiological functions of ChEs in blood have not been clearly established, but several possible physiological functions have been proposed including drug metabolism, neural development and hematopoiesis. The clinical signs observed include both cholinergic signs and delayed neuropathy, although delayed neuropathy was only observed in hens. Hematological changes were also seen with acute exposure to tribufos. The no-observed-effect level (NOEL) for the hematological changes appears to be higher than the NOEL for the neurological effects. The lowest acute NOEL observed in a well-conducted study was 2 mg/kg based on reduced motor

activity, neurobehavioral effects and plasma and RBC ChE inhibition in a rat neurotoxicity study after a single oral dose. This NOEL was selected as the critical NOEL to evaluate occupational and dietary exposure. After correcting for oral absorption, the adjusted acute NOEL was 1.4 mg/kg/day. The NOEL for dermal irritation was estimated to be 8.3 mg formulation/cm^2.

The neurological effects were also the predominant adverse effects seen with subchronic exposure, although hematological changes (reduced RBC count, hemoglobin and hematocrit), some ocular effects (reduced electroretinographic (ERG) responses and pale retinal fundus) and fatty droplets in the adrenal gland were observed in a rat inhalation study. In a rat reproductive toxicity study, several reproductive effects were observed including a reduction in fertility, birth, and viability indices, an increase gestation length, reduced pup weights, cannibalism of pups, and discolored pup livers. However, the neurological effects were the most sensitive endpoints with subchronic exposure. The lowest subchronic NOEL in a well-conducted study was 0.14 mg/kg/day based on plasma, RBC and brain ChE inhibition in a neurotoxicity study where rats were fed tribufos in the diet for 90-days. This NOEL was selected as the critical NOEL for evaluating seasonal occupational exposure to tribufos. After correcting for oral absorption, the adjusted subchronic NOEL was 0.1 mg/kg/day.

Hematological changes, brain ChE inhibition, reduced weight gain, and transient hypothermia were observed in laboratory animals with chronic exposure to tribufos. Hematological changes were seen in mice, rats, and dogs. Significant brain ChE inhibition was detected in rats and mice. The reduced weight gain and hypothermia were only observed in rats. In mice, there were dose-related increases in numerous non-neoplastic lesions in the gastrointestinal tract (small intestine vacuolar degeneration, dilated/distended small intestine and cecum, rectal necrosis/ulceration), liver (hypertrophy), adrenal glands (degeneration/ pigmentation), and spleen (hematopoiesis). Dose-related increases in several pre-neoplastic lesions were also seen in the small intestine (mucosal hyperplasia and focal atypia) and lungs (focal hyperplasia and epithelialization). Histological changes in the small intestine (hyperplasia and vacuolar degeneration), liver (cytoplasmic vacuolation), and adrenal glands (vacuolar degeneration) were also observed in rats. In addition, numerous ocular effects were observed in one rat study including corneal opacity, lens opacity, cataracts, corneal neovascularization, iritis, uveitis, bilateral flat ERG responses, bilateral retinal atrophy, and optical nerve atrophy. The corneal opacity, cataracts, and optic nerve atrophy appear to be secondary to the degenerative changes in the retina. The lowest chronic NOEL observed in a well-conducted study was 0.1 mg/kg/day based on plasma ChE inhibition in dogs. This NOEL was selected as the critical NOEL for evaluating chronic dietary exposure to tribufos. After correcting for oral absorption (70%), the adjusted chronic NOEL was 0.07 mg/kg/day.

There was a dose-related increase in adenocarcinomas of the small intestine of both sexes, hemangiosarcomas in the liver of males, and alveolar/bronchiolar adenomas in females in a 90-week mouse feeding study. There were no dose-related increases in tumors in two rat chronic feeding studies and all the genetic toxicity studies for tribufos were negative. Because the increase in tumors occurred in both sexes of mice at multiple sites (one of which was rare), a quantitative assessment of the oncogenic potency was conducted based on the incidence of hemangiosarcomas in male mice. After adjusting for oral absorption, the estimated oncogenic potency of tribufos ranged from 4.7×10^{-2} to 8.4×10^{-2} $(mg/kg/day)^{-1}$.

Daily, seasonal, and lifetime exposure dosages were estimated for 11 different scenarios for pesticide workers potentially exposed to tribufos. An annual exposure dosage was not calculated for occupational exposure since exposure was clearly limited to a few months during the year. Five of these job scenarios are for handlers (aerial mixer/loaders, pilots, flaggers, ground mixer/loaders and ground applicators) and six are for field workers (irrigators and

weeders with 4-day or 7-day reentry intervals (REIs), picker operators, module builder operators, rakers, and trampers). The estimated mean absorbed daily dosages (ADDs) for workers ranged from 0.7 µg/kg/day for ground applicators to 25.5 µg/kg/day for irrigators and weeders with a 4-day REI. The highest dermal exposure to tribufos was to the hands and ranged from 0.23 mg formulation/cm^2 for ground applicators to 5.48 µg formulation/cm^2 for pilots. Assuming the pesticide workers were exposed for an average of 21 days during a 45-day-day use season, the seasonal average daily dosages (SADDs) ranged from 0.3 µg/kg/day for ground applicators to 11.9 µg/kg/day for irrigators and weeders with a 4-day REI. Assuming a worker is exposed for 40 years of a 70-year lifespan, the lifetime average daily dosages (LADDs) ranged from 0.02 µg/kg/day for ground applicators to 0.84 µg/kg/day for irrigators and weeders with a 4-day REI. Combined occupational, dietary and ambient air exposure was initially evaluated for workers. However, the dietary and ambient air exposure was minor for most pesticide workers when compared to their occupational exposure (0.7 to 8.4% for acute exposure, 0.4 to 5.1% for seasonal exposure, and 0.9 to 11.7% for chronic exposure), so no further analysis was performed.

Dietary exposure to tribufos may occur from the consumption of cottonseed products, such as cottonseed oil or cottonseed meal, or from consumption of meat or milk from livestock that were fed cottonseed products or gin trash in their feed. The potential dietary exposure was estimated for various population subgroups using anticipated residues derived from residues on whole cottonseed. The ADDs ranged from 52 to 224 ng/kg/day for the different population subgroups. The Annual Average Daily Dosages (AADDs) ranged from 3 to 37 ng/kg/day. Children, 1 to 6 years old, had the highest potential acute and chronic dietary exposure to tribufos. Combined dietary and ambient air exposure was evaluated for the general population. The ADDs for combined exposure ranged from 151 to 528 ng/kg/day. The AADDs for combined exposure ranged from 14 to 57 ng/kg/day. Children, 1 to 6 years old, also had the highest combined exposure to tribufos in the diet and ambient air.

The risk for non-oncogenic health effects in humans is expressed as a margin of exposure (MOE). The MOE is the ratio of the NOEL from experimental animal studies to the human exposure dosage. Generally, a margin of exposure greater than 100 is considered protective of human health when it is calculated from a NOEL derived from an animal study. The MOEs for acute neurological effects with occupational exposure ranged from approximately 55 for irrigators and weeders with a 4-day REI to 2,000 for ground applicators. The MOEs for dermal irritation ranged from 1,500 to 36,000. The seasonal MOEs were less than 100 for most pesticide workers (10 to 75), except ground applicators and module builder operators who had MOEs of 400 and 133, respectively. The estimated oncogenic risk from occupational exposure to tribufos was approximately 10^{-5} for most pesticide workers, except for ground applicators whose oncogenic risk was approximately 10^{-6}. An oncogenic risk level less than 10^{-6} is generally considered negligible.

The MOEs for acute dietary exposure to tribufos in the various population subgroups ranged from 6,300 to 27,000 based on anticipated residues in cottonseed products. The MOEs for the chronic dietary exposure ranged from 1,900 to 20,000. The MOEs for combined acute dietary and ambient air exposure to tribufos ranged from 2,700 to 9,300. The MOEs for combined chronic dietary exposure ranged from 1,200 to 5,000. The estimated oncogenic risk from dietary exposure to tribufos was between 10^{-6} and 10^{-7} for the U.S. population. However, the dietary exposure is based entirely on anticipated residues that were estimated from whole cottonseed using processing and distribution factors. The chronic dietary exposure may have been overestimated due to several assumptions including not adjusting the residue levels in cottonseed oil for the deodorization process, assuming cattle consumed cottonseed by-products in their feed at the maximum allowable level on a long-term basis, and not correcting for the

percent of crop treated. The estimated oncogenic risk from combined dietary and ambient air exposure was also between 10^{-6} and 10^{-7}.

A tolerance assessment for tribufos was conducted assuming commodities were consumed at the tolerance levels. After adjusting for oral absorption (70%), the estimated acute dietary intakes for various population subgroups ranged from 148 to 995 ng/kg/day. The resultant MOEs ranged from 1,400 to 9,500. Chronic consumption of commodities containing tribufos residues at the tolerance level was considered highly improbable based on the small percentage of samples (<1%) that had residues at or above tolerance in the DPR and California Department of Food and Agriculture pesticide monitoring programs. Therefore, a tolerance assessment for chronic dietary exposure to tribufos was not conducted.

II. INTRODUCTION

A. CHEMICAL IDENTIFICATION

S,S,S-Tributyl phosphorotrithioate (tribufos) is an organophosphate chemical used as a cotton defoliant. Tribufos induces early leaf abscission through changes in the levels of plant hormones (Ware, 1978). Defoliation occurs 4 to 7 days after treatment.

The toxicity of tribufos to animals is primarily due to its inhibition of various esterases, including acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), neuropathic target esterase (NTE), and carboxylesterase. AChE is also called specific or true cholinesterase and is found near cholinergic synapses, in some organs (e.g., lung, spleen, gray matter) and in red blood cells (RBCs) (Lefkowitz *et al.*, 1990). Normally, AChE metabolizes acetylcholine to acetate and choline, which results in the termination of stimulation to dendritic nerve endings and motor endplates. Acetylcholine is the neurochemical transmitter at endings of postganglionic parasympathetic nerve fibers, somatic motor nerves to skeletal muscle, preganglionic fibers of both parasympathetic and sympathetic nerves, and certain synapses in the central nervous system (CNS) (Murphy, 1986).

The inhibition of AChE results in the accumulation of endogenous acetylcholine in nerve tissue and effector organs. In acutely toxic episodes, muscarinic, nicotinic and CNS receptors are stimulated with characteristic signs and symptoms occurring throughout the peripheral and central nervous systems (Ellenhorn and Barceloux, 1997; Murphy, 1986). Muscarinic effects can include increased intestinal motility, bronchial constriction and increased bronchial secretions, bladder contraction, miosis, secretory gland stimulation and bradycardia. Nicotinic effects include muscle weakness, twitching, cramps and general fasciculations. Accumulation of acetylcholine in the CNS can cause headache, restlessness, insomnia, anxiety and other non-specific symptoms. Severe poisoning results in slurred speech, tremors, ataxia, convulsions, depression of respiratory and circulatory centers and, eventually, coma.

Butyrylcholinesterase (BuChE), sometimes referred to as plasma cholinesterase (ChE), pseudo-cholinesterase, or serum esterase, is also inhibited by tribufos. Any reference in this document to "cholinesterase", without specifically indicating that the enzyme is serum or plasma ChE, should be interpreted as AChE. BuChE only occurs to a limited extent in neuronal elements of the central and peripheral nervous systems in adults, but it appears to be important in the developing nervous system of birds and mammals where it is the predominant form of cholinesterase (Brimijoin and Koenigsberger, 1999). As neuroblasts switch from cell proliferation to neural differentiation, there is concomitant switch from BuChE to AChE. Li *et al.* (2000) speculated that BuChE functions in the adult nervous system as a replacement for AChE based primarily on the survival of $AChE^{-/-}$ knockout mice for several weeks after birth. Unlike AChE, BuChE occurs primarily in non-neuronal or non-synaptic sites in adults like the liver, lung, and plasma and its function has not been clearly established (Lefkowitz et al., 1990; Brimijoin, 1992; U.S. EPA, 1993; Pantuck, 1993). BuChE may protect the nervous system by acting as a scavenger or a detoxification enzyme in these non-neuronal sites. Administration of exogenous BuChE has been demonstrated to provide significant protection against several organophosphate compounds in rats, mice, guinea pigs and non-human primates (Raveh et al., 1993 & 1997; Allon et al., 1998). However, rats that were depleted of plasma AChE by injecting them intravenously with antibodies specific to this enzyme were not more susceptible to paraoxon toxicity than untreated controls based on their performance in a functional observational battery and AChE activity in the brain and diaphragm (Padilla et al., 1992). Jbilo *et al.* (1994) noted that BuChE has characteristics similar to other detoxification enzymes. It

concentrates in major organs of entry such as the liver, and lung and it has a broad substrate specificity relative to AChE due to its larger active. Naturally occurring ChE inhibitors include esters (cocaine), carbamates (physostigmine), peptides (fasciculin) and alkaloids (solanine).

An atypical genetic variant of plasma cholinesterase has been associated with an increased susceptibility to various drugs, such as succinylcholine and cocaine (Lockridge, 1990; Pantuck, 1993; Lockridge and Masson, 2000). The atypical BuChE has a single amino acid substitution in which aspartic acid 70 is replaced by glycine 70, resulting in a decreased affinity for positively charged ChE inhibitors compared to neutral compounds. This evidence suggests that individuals with atypical BuChE would only more be susceptible to OPs and carbamates if they were positively charged. Other genetic variants of BuChE have been identified including some that have normal catalytic activity, but a reduced number of molecules. Some silent genetic variants have essentially no BuChE activity. Individuals with these genetic variants are probably more susceptible to most ChE inhibitors. Sparks *et al.* (1999) found that BuChE inhibited by OPs or carbamates potentiated the toxicity of succinylcholine in mice. The potentiation was greatest with the most potent BuChE inhibitors (not necessarily the most potent AChE inhibitors). These investigators also noted that increased sensitivity to succinylcholine was reported in two cases where patients were poisoned by OPs.

NTE inhibition in the hen brain of greater than 70% is associated with the organophosphate-induced delayed neuropathy (OPIDN) produced by some organophosphate compounds with acute exposure (Carrington, 1989; Abou-Donia and Lapadula, 1990). Slightly lower levels of inhibition (~50%) are needed with chronic exposure. The physiological or biochemical role of NTE is unknown at this time. Aging of the phosphorylated enzyme (loss of an alkyl group) apparently is also important in the induction of this neuropathy.

Carboxylesterase is involved in the detoxification of various chemicals, including pesticides. Inhibition of carboxylesterase by tribufos has resulted in the potentiation of organophosphate pesticides such as malathion that contain a carboxylic ester group (Murphy *et al.*, 1976). However, tribufos also markedly potentiated the toxicity of azinphos-methyl which does not contain any carboxylic ester groups (Gaughan *et al.*, 1980). Inhibition of other detoxification enzymes may be involved. Inhibition of liver microsomal esterases is thought to be responsible for the potentiation of permethrin toxicity by tribufos (Gaughan *et al.*, 1980). The absorption of phthalate diesters is reduced by tribufos apparently due to its inhibition of esterase activity in the intestinal mucosa (White *et al.*, 1980). Tribufos is also a potent inhibitor of liver arylamidase *in vitro* (Satoh and DuBois, 1973).

B. REGULATORY HISTORY

Tribufos was first registered in 1960 for cotton defoliation and this has remained its only use (U.S. EPA, 1981). In 1981, the U.S. EPA issued a decision not to initiate a Rebuttable Presumption Against Registration (RPAR) review of tribufos despite evidence of irreversible neurotoxic effects in laboratory animals exposed to tribufos. This decision was based on the lack of evidence of neurotoxic symptoms among applicators exposed to tribufos and adequate margins of exposure when specified protective clothing is worn.

In 1983, the Department of Pesticide Regulation[1] (DPR) in the California Environmental Protection Agency (Cal/EPA) limited the concentration of nBM in formulations of tribufos sold or

[1] Prior to 1991, DPR was part of the California Department of Food and Agriculture.

used in California to less than 0.1% due to public concern about the odor associated with the use of tribufos (California Administrative Code, Title 3, Section 6361). Despite the use of low-odor formulations, DPR continued to receive odor-related complaints from residents in cotton-growing regions. Public health concerns were also raised because of evidence that tribufos caused delayed neuropathy (see Neurotoxicity section under the Toxicology Profile).

In August 1991, DPR placed tribufos in reevaluation based on inadequate acute toxicity data to assess the appropriateness of the signal words and precautionary statements on the label. Primary eye and dermal irritation studies for the technical grade material and a complete set of acute toxicity studies for the formulations were submitted by the registrants to DPR. The registrants were informed that the signal words and precautionary language on the current label were not adequate to mitigate possible eye and skin irritation hazards from the use of these products. The registrants have submitted proposed label amendments which have been approved by the U.S. EPA and DPR.

Section 13131 of the Food and Agricultural Code of California requires DPR to conduct an assessment of dietary risks associated with the consumption of produce and processed foods treated with pesticides. This assessment integrates data on acute health effects and the mandatory health effects studies specified in subdivision (c) of Section 13123, appropriate dietary consumption estimates, and relevant residue data to quantify consumer risk.

DPR completed a Risk Characterization Document (RCD) for tribufos in 1998 which addressed occupational and dietary exposure (Lewis, 1998a). No mitigation was required for dietary exposure to tribufos; however, mitigation was needed for some handler exposure scenarios and all harvester exposure scenarios. As a result of the 1998 RCD, mitigation measures were proposed in 1999 to reduce occupational exposure. The proposed mitigation consisted of increasing the reentry interval (REI) for cotton field workers to 10 days. Later that year, the registrant submitted a primate dermal absorption study which significantly lowered the dermal absorption assumed for humans. As a result of this new dermal absorption study, the exposure assessment was revised in 2000. Based on the revised exposure assessment, an REI of 7-days was determined to be adequate to protect field workers. The revised dermal absorption also resulted in lower exposure estimates for handlers, such that the existing personal protective equipment (PPE) was considered adequate to protect handlers. The registrant submitted a label amendment which increased the REI to 7 days. The 7-day REI on the labels negated the need for any mitigation.

DPR was given responsibility to identify and control pesticides that are toxic air contaminants (TACs) under Assembly Bill 1807 (AB1807) that was passed in 1983. In 1986, tribufos was added to the candidate list of TACs under AB1807. About the same time the RCD for tribufos was completed in 1998, a Toxic Air Contaminant (TAC) document was completed which addressed exposure to tribufos in ambient air (Lewis, 1998b). The ambient air concentrations for tribufos were not of sufficient magnitude to require mitigation; however, they were sufficient to recommend listing tribufos as a TAC. In July of 1999, DPR proposed amending section 6860 in Title 3 of the California Code of Regulations (3 CCR) to designate tribufos as a TAC pursuant to Food and Agricultural Code (FAC) section 14023. In January of 2000, the Office of Administrative Law (OAL) approved the regulation designating tribufos as a TAC. This regulation became effective in March of 2000.

In 1999, U.S. EPA completed a human health risk assessment for tribufos in which they evaluated the occupational and dietary exposure (Travaglini, 1999). They also found the dietary exposure to tribufos to be of little concern. However, they found the occupational dermal exposures for all handler exposure scenarios to be of concern. The occupational exposure for

post-application exposure scenarios were not of concern if the REIs were increased to 20-30 days. In 2000, U.S. EPA issued an Interim Reregistration Eligibility Document (IRED) for tribufos which revised their previous risk assessment for tribufos based on the new primate dermal absorption study (U.S. EPA, 2000a). They also reduced the uncertainty factor they used to estimate the acute dermal NOEL used in evaluating occupational exposure. Despite these changes, U.S. EPA remained concerned about dermal exposure for several handler scenarios for aerial application: flaggers, mixers, loaders and applicators because they were below their target MOE of 300. However, they were no longer concerned about dermal exposure from mixing, loading and application by groundboom. U.S. EPA still had risk concerns about several post-application exposure scenarios (rakers, trampers and pickers) with the current application rate and 7-day REI. However, they had no risk concerns for these exposure scenarios at their proposed lower application rate of 1.125 lb a.i./acre with a 7-day REI.

C. TECHNICAL AND PRODUCT FORMULATIONS

There are two products currently registered in California which contain tribufos as the active ingredient, DEF 6 and Folex 6 EC. DEF 6 is manufactured by Bayer Corp. (formerly Miles Inc. or Mobay Corp.) while Folex 6 EC is currently manufactured by Amvac Chemical Co. Folex used to contain S,S,S-tributyl phosphorotrithioite which is rapidly converted to tribufos by oxidation within a few hours after exposure to air (Obrist and Thornton, 1978). However, it was reformulated after the DPR limited the amount of nBM that could be in these formulations and now Folex contains only tribufos. The concentration of tribufos in these formulations is approximately 70%. The Material Safety Data Sheet (MSDS) for DEF 6 indicates that the other inert ingredients are trimethylbenzenes (20-30%), Ingredient 1923 (1-10%), xylenes (1-5%), and ethylbenzene (1-2%) (National Agricultural Chemicals Association, 1990). The inert ingredients for Folex 6 are not identified in its MSDS.

D. USAGE

The recommended application rate for tribufos is approximately 1 to 2.5 pints (0.75 to 1.9 lb. active ingredient)/acre/year. It can be applied as a dilute spray in 5-10 gallons of water per acre by air or in 20-25 gallons of water per acre with ground equipment. It cannot be used through any type of irrigation system. Tribufos is applied predominantly by air in California. Under favorable conditions tribufos gives effective defoliation within 4 to 7 days after application. When continued low temperatures prevail at night (< 60^{o}F), complete defoliation may require 9 to 14 days. In 2002, 190,149 lbs. of tribufos were used in California in 1,780 applications over 129,570 acres (DPR, 2003). Tribufos represented approximately 3 percent of the total pounds of pesticides applied to cotton fields in 2002.

The labels for these formulations require that applicators and other handlers wear the following protective clothing: coveralls over a long-sleeved shirt and long pants, chemical-resistant gloves, chemical-resistant footwear over socks, protective eyewear, chemical-resistant headgear, chemical-resistant apron when mixing/loading and cleaning equipment, and a MSHA/NIOSH-approved respirator in enclosed areas or MSHA/NIOSH-approved dust/mist filtering respirator for outdoors. According to the federal worker protection standards for agricultural pesticides, the protective clothing requirements for mixer/loaders may be reduced to work clothing (long-sleeved shirt and long pants), chemical-resistant apron, and chemical-resistant gloves when using closed mixing/loading systems. Applicators may also reduce the protective clothing to work clothing when closed systems are used. California regulations require that a closed system be used for all mixing and transfers of cotton defoliants and that

there is a ½-mile buffer zone between residential areas and sprayed fields (California Code of Regulations, Title 3, Section 6470). In addition, California regulations require that the level of n-butyl mercaptan (nBM) in formulated products containing tribufos cannot exceed 0.1 percent (California Code of Regulations, Title 3, Section 6361). The restricted reentry interval in areas treated with tribufos is 7 days.

E. ILLNESS REPORTS

In 1977, the California Department of Food and Agriculture (CDFA) published a report summarizing several hundred complaints it had received that were associated with tribufos (Maddy and Peoples, 1977). The complaints usually involved wheezing, coughing, nausea, and other discomforts that were attributed to the degradation product, nBM, a volatile degradation product of tribufos with a strong skunk-like odor. This odor apparently can be detected by humans at air concentrations as low as 0.01 ppb (Santodonato *et al.*, 1985).

Approximately 13 drums containing tribufos and S,S,S-tributyl phosphorotrithioite (merphos) were damaged on a ship in transit from Mexico to Australia (McLeod, 1975). Caustic soda was used in the process of cleaning up the damaged drums in Auckland, New Zealand, which resulted in increased liberation of nBM. Over 600 people were seen at a local hospital with various complaints. Symptoms observed in 49 cases were attributed to organophosphate poisoning (excessive salivation, sweating, muscle weakness, fatigue, nausea, vomiting, diarrhea, and miosis); however, no cholinesterase inhibition was found. Symptoms attributed to nBM (headache, dizziness, dry mouth and throat constriction) were reported in another 192 cases. It was estimated that the air levels of nBM exceeded 0.5 ppm (ACGIH TWA-TLV) and in some places exceeded 10 ppm. It was not possible to categorize the symptoms observed in the remaining cases. It was reported that panic may have been a factor in some of these cases since there had been widespread coverage of the spill in the news media.

More recently, the California Department of Health Services also did an epidemiology study in which they examined the relationship of the health symptoms and community exposure to cotton defoliants (Scarborough *et al.*, 1989). Four-hundred and six residents in six agricultural communities in the San Joaquin Valley were surveyed by phone during the time of cotton defoliation. They found a significantly greater risk for eye and throat irritation, rhinitis, fatigue, shortness of breath, nausea and diarrhea in the high exposure group (people who lived or worked within one mile of a cotton field that had been treated within the previous two weeks). In the high-exposure group, there was also a significantly greater risk for these self-reported symptoms in the subgroup noticing a strong odor, suggesting that tribufos or nBM was the causative agent.

There were a total of 31 illness and/or injury cases that were definitely (7), probably (8) or possibly (16) associated with exposure to tribufos or merphos alone or in combination with other pesticides in California from 1982 through 2002 (Mehler, 2004). Of the 31 cases, 24 were systemic illnesses, and the remaining 7 involved respiratory, eye or skin illnesses or injuries. All but 5 cases were considered occupationally related. Most of the cases (20) involved exposure to tribufos or merphos in combination with other pesticides. There have only been 8 reports since 1992 and of these, 7 cases were related to one incidence in 1999 in which an agronomist mistakenly approved entry of 8 workers into a cotton field sprayed with defoliants 5 hours earlier. A company supervisor saw them in the field 3 hours later and told them to leave.

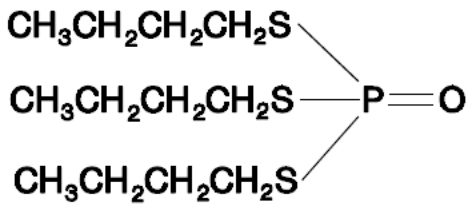
No cases of delayed neurotoxicity in humans have been reported for tribufos, although there was one case where a 28-year-old agricultural worker spilled merphos (S,S,S-tributyl

phosphorotrithioite) on his arm (Fisher, 1977). He did not develop any acute symptoms, but four days later his hands and arms became weak. He finally sought medical attention six days after exposure when he could barely move his arms or legs. After admission to the hospital, his plasma cholinesterase level was normal despite his symptoms. Eight days later, complete facial paralysis developed. Electromyography demonstrated decreased voltage of action potentials, delayed conduction velocity, increased insertional activity, and denervation potentials. Recovery was complete after fourteen weeks of intensive physical therapy.

F. PHYSICAL/CHEMICAL PROPERTIES

Tribufos (Talbott, 1990)

1. Common Names: Tribufos, DEF, Butiphos, Merphos Oxide
2. Chemical Name: S,S,S-Tributyl phosphorotrithioate
3. Trade Names: DEF, Folex
4. CAS Registry No.: 78-48-8
5. Empirical Formula: $C_{12}H_{27}OPS_3$
6. Molecular Structure:



7. Molecular Weight: 314.5
8. Physical State: Colorless to yellow liquid
9. Odor: Skunk-like
10. Melting Point: < -25°C
11. Boiling Point: 150°C at 0.3 mm Hg
12. Density: 1.057 g/cm at 20°C
13. Solubility: Water - 2.3 ppm at 20°C (Leimkuehler, 1980)
    Solvents - Completely miscible with n-hexane, dichloromethane, toluene, and 2-propanol (Betker, 1985)
14. Vapor Pressure: 6.5×10^{-6} mm at 25°C (Talbott and Mosier, 1987)
15. Octanol-Water Partition Coefficient: 3.31×10^{5} at 25°C (D'Harlingue, 1987)

16. Henry's Law Constant: 2.9×10^{-7} atm x m^3/mole at 20°C (Talbott, 1987)

n-Butyl Mercaptan (ACGIH, 1986)

1. CAS Registry No.: 109-79-5
2. Empirical Formula: $C_4H_{10}S$
3. Molecular Structure: **$CH_3CH_2CH_2CH_2SH$**
4. Molecular Weight: 90.19
5. Density: 0.83679 at 25°C
6. Melting Point: -115.9°C
7. Boiling Point: 97.2 – 101.7°C
8. Vapor Pressure: 83 mg Hg at 25°C
9. Solubility: Slightly soluble in water, but very soluble in alcohols, ether, and liquid hydrogen sulfide

G. ENVIRONMENTAL FATE

Field Dissipation

Tribufos was applied once to soil at 3.375 lb. a.i./acre in two different sites in California, one near Fresno and one near Watsonville (Grace and Cain, 1990). Soil samples were analyzed for tribufos and its metabolite, dibutyldisulfide. None of the samples contained $\geq$ 0.01 ppm dibutyldisulfide. Only two samples had tribufos more than 6 inches below the surface and tribufos in these two samples was still less than 12 inches below the surface. The half-lives were 15.3 and 47.7 days for the Watsonville and Fresno sites, respectively.

Hydrolysis

Tribufos was relatively stable in aqueous solutions at pH 5 and 7 up to 32 days (94.5% and 94.6% recovered, respectively), but degraded slightly at pH 9 (80.8% recovered after 32 days) (Schocken and Philippson, 1987). The half-life at pH 9 was estimated to be 124 days. The polar breakdown product was identified as desbutylthio tribufos.

Photolysis

Tribufos was stable in a sandy loam soil exposed to natural sunlight for 30 days (Jackson *et al.*, 1988); however, it degraded in aqueous solutions (pH 7) exposed to natural sunlight for 30 days (Kesterson and Lawrence, 1990). The estimated half-life was 44 days. No photodegradation products were identified.

Soil Adsorption and Mobility

The soil adsorption coefficient (K_d) and constant (K_{oc}) were determined for tribufos with four different soil types (sand, sandy loam, silty loam, and clay loam) (Daly, 1987). The estimated K_d values ranged from 60.6 for sandy loam soil to 106 for clay loam. The estimated K_{oc} values ranged from 4,870 for silt loam to 12,684 for sand. In a column leaching study, tribufos was applied to the top of columns (1.6 cm by 45 cm) containing clay loam, sandy loam, loam or muck soil (Church and Shaw, 1969). Tribufos remained in the top 4 cm of soil regardless of soil type. Tribufos was detected only in the leachate of the sandy loam soil at less than 1% of the applied dose. In another study, ^{14}C-tribufos was incubated aerobically in sandy loam soil at room temperature for 32 days (aged) and then applied to the top of a column (5.4 cm by 45 cm) containing sandy loam soil (Schocken and Parker, 1987). The vast majority (94.7%) of the applied radioactivity was found in the upper 6 cm of the soil columns with most (74.7%) of this radioactivity identified as the parent compound. Less than 1% of the applied radioactivity was found in the leachate.

Soil Metabolism

The estimated half-life for tribufos was 198 days when incubated with sandy loam soil in the dark under aerobic conditions (Olson *et al.*, 1990). The half-life of tribufos under anaerobic conditions was 64.8 days (Olson *et al.*, 1989). However, in both studies the material balance was less than 50%, so interpretation of the results is difficult.

Groundwater Monitoring

Pursuant to the Pesticide Contamination Prevention Act (AB 2021), DPR has not identified tribufos as a potential groundwater contaminant based on its high soil adsorption (K_{oc} > 1900 cm^3/g). Some groundwater monitoring for tribufos was conducted by DPR, the Department of Health Services and the U.S. Geological Services between 1986 and 1994 (DPR, 2004a). A total of 465 wells were sampled in 16 counties (Colusa, Fresno, Kern, Kings, Los Angeles, Madera, Merced, Orange, Riverside, San Bernardino, San Diego, San Mateo, Santa Cruz, Stanislaus, Tulare, Ventura). All the samples had non-detectable residues.

Surface Water Monitoring

Some surface water monitoring was also conducted by DPR (DPR, 2004b). Only 2 samples among 810 analyzed between 1991 and 2003 had detected residues. The two detectable samples were from Stanislaus County. Approximately one third of the samples came from Stanislaus County. The remainder came from Butte, Colusa, Contra Costa, Merced, Monterey, Sacramento, San Joaquin, Santa Cruz, Shasta, Solano, Sutter, Tehama, Yolo and Yuba Counties. The two samples with detectable residues had residues right at the limit of quantitation (0.01 ppb).

Summary

The water solubility, soil adsorption, hydrolysis and aerobic soil metabolism data suggest that tribufos is not likely to be a ground water contaminant. The available well monitoring data for tribufos support this conclusion. The surface water monitoring indicate that it is also not likely to be a surface water contaminant. Tribufos may become airborne from drift after aerial application or ground spraying. Tribufos is not a very volatile compound based on its vapor pressure; however, the degradation product, nBM, is volatile with a skunk-like odor and is probably responsible for a number of complaints in areas near where tribufos has been applied.

III. TOXICOLOGY PROFILE

A. PHARMACOKINETICS

Summary: Tribufos appears to be readily absorbed by the oral route and rapidly metabolized in the species examined. The oral absorption was assumed to be 70% based on the average urinary excretion in rats on all dosing regimens. The dermal absorption for tribufos was assumed to be 47.5% in animals based on a study in rats and 7.1% in humans based on a study in monkeys. A default assumption of 50% respiratory retention and 100% absorption was used with tribufos based on the assumption that tribufos is primarily in the vapor phase with occupational exposure.

Absorption

[^{14}C] Tribufos was administered to 5 rats/sex/dose in a single dose by oral gavage at 5 or 100 mg/kg or in 14 consecutive doses at 5 mg/kg/day (Kao *et al.*, 1991). Approximately 95-98% of the total dose was excreted in the urine and feces within 72 hours after dosing. Most of the radioactivity was excreted within 24 hours after a single dose at 5 mg/kg (M:91%;F:87%) or 100 mg/kg (M:75%;F:57%). A similar percentage (M:89%;F:85%) was excreted within 24 hours after 14 consecutive doses at 5 mg/kg/day. The majority of the radioactivity was excreted in the urine after a single dose at 5 mg/kg (M:55%;F:66%) or 100 mg/kg (M:60%;F:70%). A slightly higher percentage (M:73%;F:80%) was excreted in the urine after 14 consecutive doses at 5 mg/kg/day.

Male rats had [^{14}C] tribufos (98.9% - mixed in distilled water with DEF 6 blank formulation) applied to their shaved backs at 1.93, 12.4 and 100 µg/cm^2 for 10 hours (Schroeder, 1992). The application site was protected by a non-occlusive cover of a Teflon-laminated filter and a carbon-impregnated material. Four rats/dose were sacrificed at 1, 4, 10 and 168 hours (7 days). The amount excreted in the urine over 7 days ranged from 25.8 to 36.0% of the applied dose decreasing from the low to high dose level. On the other hand, the amount excreted in the feces was fairly similar (3.2 to 3.6% of applied dose) at the different dose levels. After correcting for recoveries, the mean dermal absorption rates were 47.5, 47.9 and 33.9% at 1.93, 12.4, and 100 µg/cm^2, respectively. The dermal absorption rate at the lowest concentration was selected for adjusting NOELs for dermal studies to absorbed dosages.

The dermal absorption of [^{14}C] tribufos (99.3% - mixed in distilled water with DEF 6 blank formulation) was also evaluated in rhesus monkeys (Wills, 2000). Radiolabeled tribufos was applied to the shaved backs (4 cm x 6 cm) of 5 male monkeys (ages 1.4 to 3.2 years) at 3.5 µg/cm^2 (83.3 µg/animal) and covered by a Duoderm® patch and aluminum dome. The monkeys were placed in primate restraint chairs during the exposure period (8 hours). The urine and feces were collected for 5 days or until the radioactivity in the urine was less than twice background level. Since the average recovery in this study was 105.3%, intravenous injection was not required. Approximately 6.24% of the applied dose was recovered in the urine with the majority excreted between 12 and 72 hours. Only 0.72% of the applied dose was excreted in the feces. The sum of the mean radioactivity recovered in the urine, feces and biscuits was 7.44%. After adjusting for the recovery, the dermal absorption was estimated to be 7.1%. This dermal absorption rate was used to adjust the occupational exposure for workers to an absorbed dosage.

The pharmacokinetics of tribufos after intravenous administration was not studied due to the low water solubility of tribufos. However, based on the nearly complete elimination of

tribufos by the urinary route when applied dermally to rats (Schroeder, 1992), it was assumed the amount excreted by the biliary route is insignificant when tribufos is administered orally. Based on this finding, DPR assumed that with oral administration most of the radioactivity in the feces was unabsorbed material. Therefore, the oral absorption rate for tribufos was estimated to be 70% based on the approximate average urinary excretion for all dosing regimens.

There were no data available on the absorption of tribufos by the inhalation route.

Distribution

In laying hens administered tribufos at 50 mg/kg by the oral and dermal routes, the half-lives were 2.7 and 3.8 days, respectively, based on the plasma concentration curve (Abou-Donia *et al.*, 1984). Hall (1991) measured residues in the liver, fat, muscle, and eggs of 6 laying hens given [^{14}C] tribufos and 4 laying hens given [^{35}S] tribufos at 4 mg/kg/day in gelatin capsules for 3 consecutive days. Four hours after the last dose, the highest residues were found in liver followed by internal eggs, muscle, and fat.

Tissue residues were also determined in two lactating goats 21 hours after receiving [^{14}C] tribufos at 0.82 or 0.85 mg/kg/day in gelatin capsules for 3 consecutive days (Sahali, 1991). Of the four tissues examined (muscle, fat, kidney, liver), the liver had the highest residues and the muscle had the lowest. The residues in milk were between those of fat and muscle.

The most extensive residue analysis of tissues was conducted by Kao *et al.* (1991) in rats administered [^{14}C] tribufos by oral gavage at 5 or 100 mg/kg. Less than 3% of the total dose was found in the tissues and carcasses 72 hours after dosing. The highest residue levels were found in the liver, followed by fat, lung, kidney, blood, gastrointestinal tract, spleen, bone, heart, gonads, muscle, and brain.

Biotransformation

One of the initial steps in the metabolism of tribufos appears to be its oxidation to an active metabolite, such as a sulfoxide. Tribufos was converted to a more potent ChE inhibitor with the addition of microsomal fractions or purified cytochrome P-450 isozymes from mouse liver *in vitro* (Wing *et al.*, 1984; Levi and Hodgson, 1985). Hall (1991) and Sahali (1991) were unable to identify any of the metabolites in the tissues analyzed from laying hens and goats, respectively. Based on the complexity of the metabolite profiles and the extreme polarity of many of the metabolites in these tissues, these investigators suggested that most of the parent compound had been incorporated into natural constituents. Radioactive residues were detected in fatty acids and proteins in goat tissues (Sahali, 1991).

Kao *et al.* (1991) detected over 18 radioactive metabolites in the urine of rats, but only one was identified, butyl-gamma-glutamylcysteinylglycine disulfide. In feces, the parent compound and an unidentified non-polar metabolite accounted for 15 to 31% and 1% of the total dose, respectively. They proposed a metabolic pathway for tribufos, which involves the initial hydrolysis of tribufos to S,S-dibutyl phosphorodithioate and nBM (Figure 1). nBM is converted to the fatty acid, butyric acid, which may be further metabolized through the usual metabolic pathways for fatty acids. S,S-Dibutyl phosphorodithioate is further metabolized to nBM and phosphate. Hur and coworkers (1992) proposed a similar metabolic pathway based

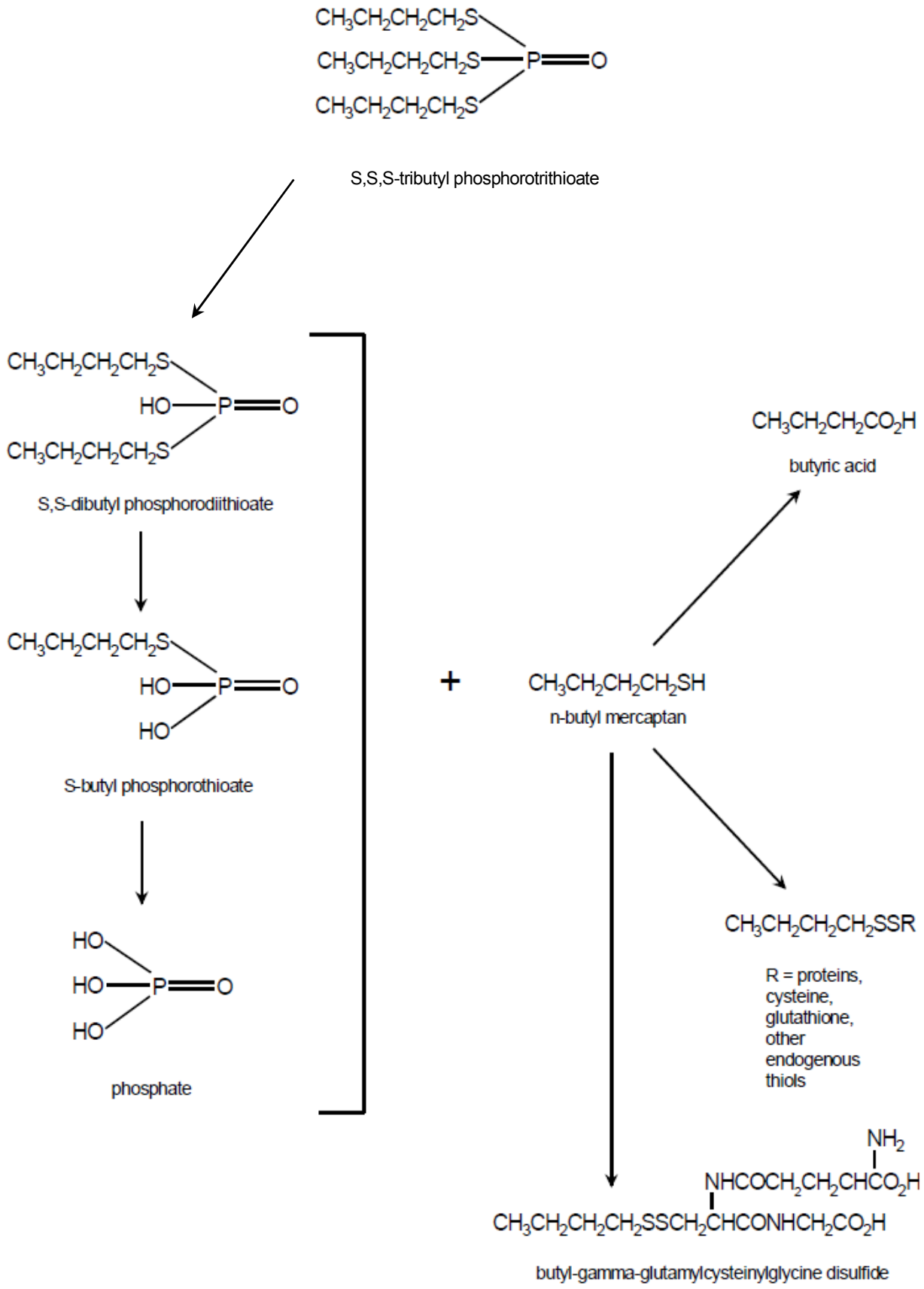


Figure 1. Proposed Metabolic Pathway for Tribufos (Kao *et al.*, 1991)

on the isolation of two metabolites (S,S-dibutyl phosphorodithioate and S,S-dibutyl phosphorothioic acid) after incubation of tribufos with mouse liver microsomes and in rat urine after intraperitoneal injection of tribufos at 100 mg/kg. They proposed that these metabolites were formed via tribufos sulfoxide and S,S-dibutyl,S-1-hydroxybutyl phosphorotrithioate, respectively, which are reactive intermediates formed by microsomal mixed function oxidases (MFOs), such as cytochrome P-450.

Abou-Donia and coworkers isolated nBM in the plasma and excreta of hens administered a single oral dose of tribufos at 400 mg/kg or 30 daily oral doses at 20-80 mg/kg/day (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). These investigators concluded that a portion of orally administered tribufos is converted to nBM in the gastrointestinal tract through hydrolysis. These studies are discussed in more detail in the Neurotoxicity Section of the Toxicology Profile.

Excretion

As mentioned previously under absorption, the major route of excretion in rats was the urinary route with an average excretion rate at 72 hours between 55 to 80% (*Kao et al.*, 1991). In the first 24 hours, the urinary excretion rate was affected by both dosage (decreased with increasing dosage) and sex (lower in males). The average amount excreted in the urine at 24 hours was lowest (M:44%;F:40%) in rats administered a single dose at 100 mg/kg; however, by 72 hours the total amount excreted in the urine was similar (M:60%;F:70%) to rats administered a single dose at 5 mg/kg (M:55%;F:66%). The urinary excretion was highest after administration of tribufos at 5 mg/kg/day for 14 consecutive days at both 24 hrs (M:67%;F:72%) and 72 hrs (M:73%;F:80%), suggesting more efficient absorption and/or metabolism with continued exposure. The average urinary excretion was higher in females on all of the dosing regimens at 72 hours (M:55-73%; F:66-80%), suggesting the absorption and/or metabolism of tribufos is more efficient in females than males. A significant amount of tribufos was also eliminated in the feces of rats within 72 hours following a single oral dose of tribufos at 5 mg/kg (M:42%;F:30%) or 100 mg/kg (M:38%;F:27%). The fecal excretion was slightly lower after 14 consecutive doses at 5 mg/kg/day (M:24%;F:15%). Only 1% was excreted as CO_2 in expired air for either sex.

B. ACUTE TOXICITY

Summary: The standard battery of acute toxicity tests was available for both the technical grade tribufos and the formulations. Nine of the 17 available acute toxicity tests were acceptable based on FIFRA guidelines. The clinical signs observed in animals after acute exposure to tribufos were typical cholinergic signs (e.g., ataxia, tremors, facial and urogenital stains). With inhalation exposure, dyspnea, red turbinates, and firm zones in the lungs were also reported. Erythema was also observed with dermal exposure. When comparing LD_{50}/LC_{50} values for the different routes in animals, technical grade tribufos appears to be slightly more toxic by the inhalation route than the oral route and least toxic by the dermal route. The higher dermal LD_{50} values also suggest that absorption of tribufos by the dermal route is slower or incomplete. For technical grade tribufos, the lowest-observed-effect level (LOEL) in an acceptable inhalation LC_{50} study with rats was 1,590 mg/m^3 (254 mg/kg) based on death, cholinergic signs, red turbinates and "firm zones." In an acceptable rat oral LD_{50} study with rats, the LOEL was 192 mg/kg based on cholinergic signs. The LOEL in an acceptable dermal LD_{50} study with rabbits was 500 mg/kg based on cholinergic signs and erythema. No-observed-effect levels (NOELs) were not established in any of the acceptable acute studies for technical grade tribufos. Acute effects observed in subchronic, developmental, and neurotoxicity studies are not

included here, but are discussed later under those sections. All acute effects are summarized under Acute Toxicity in the Hazard Identification section.

Several acute toxicity tests were also conducted on the degradation product, nBM, and the metabolite, 3-hydroxybutylmethyl sulfone; however, none of these tests met FIFRA guidelines. The effects observed in animals exposed to nBM were typical of CNS depression. The pathological findings included kidney and liver damage with all routes of exposure and lung damage with inhalation exposure. Ocular irritation was also observed. A comparison of LC_{50}/LD_{50} values suggests that nBM is less acutely toxic than tribufos. LOELs and NOELs could not be established in any acute toxicity studies for nBM.

Technical Grade Tribufos

The acute toxicity of technical grade tribufos is summarized in Table 1. In one acute inhalation study with technical grade tribufos, the LOEL was 2,920 and 1,590 mg/m^3 (234 and 127 mg/kg)[2] for male and female rats, respectively with a 4-hour, nose-only exposure (Warren, 1990). Analysis of particle size indicated that the mass mean aerodynamic diameter was approximately 1.5 µm and that greater than 87% of the particles were less than 2 µm. Based on the particle size analysis, DPR assumed that 100% of tribufos in the inhaled air reached the alveoli and was absorbed. Unthriftiness, hypoactivity, urine stains, nasal discharge, red eye discharge, lacrimation, ataxia, tremors, death, excitability, vocalization, dyspnea, red turbinates, and firm zones in the lungs were observed at the LOEL. A NOEL was not established in this study, although it was acceptable based on the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines. In another 4-hour (nose only) acute inhalation study in rats, a NOEL was established at 77 mg/m^3 (6.2 mg/kg)[1] based on abnormal behavior, including decreased preening and lethargy (Thyssen, 1978a). However, this study had several deficiencies including no summary of clinical signs by dose group, no gross necropsy, and no analysis of particle size.

In an acceptable acute oral toxicity study, death occurred at 235 and 429 mg/kg and higher in female and male rats, respectively (Sheets, 1991a). The NOEL was less than 192 mg/kg for females and 294 mg/kg for males based on urine stain (M:3/5,F:4/5), red lacrimal stain (M:4/5,F:2/5), clear lacrimation (M:3/5,F:3/5), diarrhea (M:0/5,F:5/5), perianal stains (M:1/5,F:4/5), red nasal stain (M:4/5,F:0.5), decreased activity (M:2/5,F:0/5), salivation (M:1/5,F:0/5), dyspnea (M:1/5,F:0/5), wheezing (M:1/5,F:0/5), and clear nasal stain (M:1/5,F:0/5).

After dermal administration, deaths were observed at 1,000 mg/kg and higher in rabbits (Sheets and Phillips, 1991). The NOEL was less than 500 mg/kg based on tremors (1/10), muscle fasciculations (10/10), erythema at the site of application (9/10), hypoactivity (2/10), clear nasal discharge (2/10), white nasal discharge (1/10), ataxia (1/10), increased reactivity (1/10), clear lacrimation (1/10), and clear lacrimal stain (1/10). This study was also acceptable based on FIFRA guidelines.

Technical grade tribufos was only mildly irritating to the skin and eyes of rabbits (Crawford and Anderson, 1972a; Sheets and Fuss, 1991; Sheets and Phillips, 1992a). Tribufosdid not induce a sensitization response in guinea pigs using the Buehler patch test (Sheets, 1990).

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[2] Estimated assuming a respiratory rate of 0.16 m^3/kg/4 hrs for a rat (Zielhuis and van der Kreek, 1979).

Table 1. The Acute Toxicity of Technical Grade Tribufos (95-99.7%)

Species	Sex	Results	References[a]
		Acute Inhalation LC_{50}	
Rat	M	4,000 mg/m^3 (4-hr, nose only)	1
	F	1,600 mg/m^3 (4-hr, nose only)	
	M	4,650 mg/m^3 (4-hr, nose only)	2*
	F	2,460 mg/m^3 (4-hr, nose only)	
		Acute Oral LD_{50}	
Rat	M	435 mg/kg	3*
	F	234 mg/kg	
		Acute Dermal LD_{50}	
Rabbit	M/F	1,093 mg/kg	4*
		Primary Dermal Irritation	
Rabbit	M/F	Mild Irritant	5,6*
		Primary Eye Irritation	
Rabbit	M/F	Mild Irritant	5,7*
		Dermal Sensitization	
Guinea Pig	M/F	Non-Sensitizer	8

[a] References: 1. Thyssen, 1978a; 2. Warren, 1990; 3. Sheets, 1991a; 4. Sheets and Phillips, 1991; 5. Crawford and Anderson, 1972a; 6. Sheets and Fuss, 1991; 7. Sheets and Phillips, 1992a; 8. Sheets, 1990.

* Acceptable study based on the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) guidelines

At environmental temperatures below 30°C, hypothermia has been observed in rats, mice, and guinea pigs, but not rabbits after a single dose of tribufos between 20 and 200 mg/kg by the oral, intraperitoneal or intravenous route (Ray, 1980; Ray and Cunningham, 1985). At doses greater than 100 mg/kg, the hypothermia persisted for several days. The hypothermia was associated with piloerection, sluggishness, and irritability, but a high degree of motor control even when body temperature reached 30°C. As body temperatures dropped below 30°C, deaths occurred usually after prolonged hypothermia. The hypothermia appears to be due to a block of shivering and non-shivering thermogenesis with little effect on basal metabolism, heat conservation or motor control. The investigators suggested a selective action on a central thermogenic control process may be involved. Other research indicates that the hypothermia associated with organophosphates is due to central AChE inhibition because it is antagonized by centrally active antiChE drugs, such as atropine, but not by peripherally active antiChE drugs, such as 2-PAM (Kenley *et al.*, 1982). A NOEL could not be established for this effect from these studies, although the effect was minimal with intraperitoneal injection of tribufos at 20 mg/kg.

Tribufos Emulsifiable Concentrates

The acute toxicity of tribufos emulsifiable concentrates is summarized in Table 2. The signs observed with tribufos emulsifiable concentrates were similar to those observed with technical tribufos. With inhalation exposure in rats, the LOEL was 540 mg formulation/m^3 (4-hr, nose-only) (Warren and Tran, 1992). Hypoactivity, lacrimation, red nasal discharge, and unthriftiness were observed at this dose, but no mortalities or gross lesions. Red lungs and nasal turbinates were observed at necropsy at higher doses. A NOEL was not established for this study. The lowest LOEL by the oral route was 290 mg formulation/kg in female rats (Sheets and Phillips, 1992b). The effects reported at the LOEL were tremors, hypoactivity, increased reactivity, vocalizations, hunched back, labored breathing, muscle fasciculations, lacrimation, nasal, ocular, oral and perianal stains. Compound-related gross lesions in animals that died included discolored stomach zones, red fluid in the bladder, gas/fluid in the intestines, and fluid in the abdomen. There appears to be a species difference in sensitivity based on the dermal LD_{50} values for rabbits and rats (300 vs. >2,000 mg formulation/kg, respectively); however, the NOELs appear to be similar. With rabbits, a NOEL was established at 106 mg formulation/kg based on unspecified cholinergic signs (Crawford and Anderson, 1972c). In rats, a NOEL was not established; however, the LOEL was 500 mg formulation/kg in both sexes of rats based on ataxia, increased reactivity, irritation at application site, and nasal and perianal stains (Astroff

Table 2. The Acute Toxicity of Tribufos Emulsifiable Concentrates (70%)

Species	Sex	Results	References[a]
		Acute Inhalation LC_{50}	
Rat	M	> 1,350 mg/m^3 (1-hr)	1
	F	> 1,450 mg/m^3	
	M	3,550 mg/m^3 (4-hr, nose only)	2*
	F	2,340 mg/m^3	
Mice	M	2,120 mg/m^3 (30-min)	3
		Acute Oral LD_{50}	
Rat	M	570-712 mg/kg	4,5*
	F	349 mg/kg	
		Acute Dermal LD_{50}	
Rabbit	M/F	300 mg/kg	6
Rat	M/F	> 2,000 mg/kg	7*
		Primary Dermal Irritation	
Rabbit	M/F	Corrosive	8,9*
		Primary Eye Irritation	
Rabbit	M/F	Severe Irritant	8

[a] References: 1. Kimmerle, 1972; 2. Warren and Tran, 1992; 3. DuBois and Meskauskas, 1968; 4. Crawford and Anderson, 1972b; 5. Sheets and Phillips, 1992b; 6. Crawford and Anderson, 1972c; 7. Astroff and Phillips, 1992a; 8. Crawford, 1971; 9. Sheets and Phillips, 1992c.

* Acceptable study based on the FIFRA guidelines.

and Phillips, 1992a). No mortalities or gross lesions were seen at the LOEL. A tribufos emulsifiable concentrate produced severe skin and eye irritation in rabbits (Crawford, 1971; Sheets and Phillips, 1992c) which appears to be due primarily to the inert ingredients since the technical grade tribufos was only mildly irritating (Crawford and Anderson, 1972a). No studies were available on the dermal sensitization potential of the tribufos emulsifiable concentrates.

N-Butyl Mercaptan

The acute toxicity of nBM, a major degradation product of tribufos, was examined by one laboratory (Table 3) (Fairchild and Stokinger, 1958). Based on the LD_{50} and LC_{50} values, nBM appears to be less toxic than tribufos. The inhalation LC_{50} estimates for nBM ranged from 2,500-4,020 ppm (9,202-14,798 mg/m^3) which were significantly higher than those for tribufos which ranged from 1,600-4,650 mg/m^3. The oral LD_{50} estimated for nBM (1,500 mg/kg) was also significantly higher than those for tribufos (234-435 mg/kg). The clinical signs observed after exposure to nBM were indicative of CNS depression. The signs observed in approximate order of appearance included restlessness, increased respiration, incoordination, muscular weakness, skeletal muscle paralysis, cyanosis, lethargy, sedation, respiratory depression, coma, and death. The signs were similar regardless of the route of exposure, except that with oral exposure, where diarrhea was also observed and with inhalation exposure, where watery eyes and sneezing were also observed. nBM also produced slight irritation in an ocular irritation test with rabbits. The pathological findings with all routes of exposure included indications of kidney damage (cloudy swelling of the tubules and hyaline casts in the lumina) and liver damage (lymphocytic infiltration and necrotic foci with small hemorrhages). With inhalation exposure, hyperemia of the trachea and lungs, capillary engorgement, edema and occasional hemorrhage in the lung were also observed. The systemic and local toxicity after exposure to nBM by the dermal route was not examined. The lungs appear to be an important route of excretion for nBM because a strong odor was detected in the expired air of animals regardless of the route of exposure. It was not possible to establish a NOEL by any route since the incidences of clinical signs and pathological lesions were not summarized by dose level.

Table 3. The Acute Toxicity of Technical Grade n-Butyl Mercaptan

Species	Sex	Results	Reference[a]
		Acute Inhalation LC_{50}	
Rat	M	4,020 ppm (4-hr)	1
Mice	M	2,500 ppm	
		Acute Oral LD_{50}	
Rat	M	1,500 mg/kg	1
		Acute Intraperitoneal LD_{50}	
Rat	M	399 mg/kg	1
		Primary Eye Irritation	
Rabbit	M	Slight Irritant	1

[a] Reference: 1. Fairchild and Stokinger, 1958.

3-Hydroxybutylmethyl Sulfone Metabolite

In an acute oral toxicity study, 5 female Sprague-Dawley rats were given 3-hydroxybutylmethyl sulfone in water by gavage at 0 or 2,000 mg/kg (Astroff and Phillips, 1992b). There were no mortalities, reduction in body weights or treatment-related gross lesions. Ataxia, lacrimation, hypoactivity, hyperactivity, increased reactivity, and hunched back were observed in the treated animals. The LD_{50} was greater than 2,000 mg/kg. A NOEL could not be established for this study. The toxicological significance of this metabolite is uncertain at this time, but it appears to be less toxic than tribufos. This study had several deficiencies including only females tested and no analysis of dosing material.

C. SUBCHRONIC TOXICITY

Summary: Seven subchronic studies of variable exposure duration were available for tribufos, 3 inhalation studies, 3 oral studies and one dermal study. Only a 13-week inhalation study in rats and a 3-week dermal study in rabbits met the FIFRA guidelines. The clinical signs observed with subchronic exposure to tribufos were primarily cholinergic signs. Unlike the acute toxicity studies, ChE inhibition data were available for most of the subchronic studies. In general, DPR considers brain ChE inhibition to be indicative of overt toxicity since it is one of the primary target sites and more subtle neurological signs, such as memory and learning losses, may not be easily detected in animals unless they are specifically tested for these effects. The toxicological significance of plasma and RBC ChE inhibition is less certain because the physiological functions of ChEs in blood have not been clearly established, but several possible physiological functions have been proposed including drug metabolism, neural development and hematopoiesis (Lockridge and Masson, 2000; Brimijoin and Koenigsberger, 1999, and Grisaru *et al.*, 1999). In the one acceptable subchronic inhalation study in rats, the NOEL for overt toxicity was 12.2 mg/m^3 (2.9 mg/kg/day) based on cholinergic signs, brain ChE inhibition (60% of control activity), impaired retinal function, pale retinal fundus, and fatty droplets in the adrenal cortex. The NOEL for plasma and RBC ChE inhibition in this study was 2.4 mg/m^3 (0.6 mg/kg). In an acceptable 3-week dermal toxicity study in rabbits, the NOEL for overt toxicity was 2 mg/kg/day based on cholinergic signs, brain ChE inhibition (85% of control activity), acanthosis and hyperkeratosis at the dosing site. The NOEL for plasma and RBC ChE inhibition was less than 2 mg/kg/day. Other subchronic effects are described under the Reproductive and Developmental Toxicity sections and are summarized under Subchronic Toxicity in the Hazard Identification section.

Inhalation-Rat

Ten Wistar-II rats/sex/group were exposed (nose only) to tribufos (95%) at analytical air concentrations of 0, 2, 7 or 32 mg/m^3 (0, 0.5, 1.7 or 7.7 mg/kg/day)[3] for 6 hour/day, 5 days/week for 3 weeks (Thyssen, 1978b). Animals exposed to 32 mg/m^3 exhibited slight behavioral abnormalities, including lethargy and decreased preening. The high-dose animals also had increased absolute and relative adrenal gland and spleen (females only) weights and slight inflammatory lung alterations at necropsy. The increased organ weights were not considered toxicologically significant since there were no apparent treatment-related histological changes in the adrenal gland and spleen. There was a significant reduction in the mean plasma ChE activity at 7 mg/m^3 (M:64%; F:52% of controls) and 32 mg/m^3 (M:36%; F:15% of controls) at

[3] Dose was estimated from air concentration in mg/m^3 using Equation 1 in Appendix A. The respiratory rate for a rat was assumed to be 0.24 m^3/kg/6 hrs (Zielhuis and van der Kreek, 1979).

study termination. The mean RBC ChE activity was reduced at 32 mg/m^3 (M:76%; F:73% of controls). The mean brain ChE activity was also reduced at 32 mg/m^3 (F:73% of controls). No effects on body weights, hematology, clinical chemistry, urinalyses or gross pathology were reported. The NOEL for overt toxicity was 7 mg/m^3 (1.7 mg/kg/day) based on the brain and RBC ChE inhibition, clinical signs, and histological changes in the lung. The NOEL for plasma ChE inhibition was 2 mg/m^3 (0.5 mg/kg/day). This study had major deficiencies including inadequate exposure duration (< 90 days), inadequate hematology, clinical chemistry, and histopathology, and no analyses of airflow, particle size or temperature in the chambers during exposure.

Inhalation-Rat

Ten Bor: WISW (SPF-Cpb) rats/sex/dose were exposed (nose only) to tribufos (98%) at analytical air concentrations of 0, 0.27, 2.6, 13.3 or 62.5 mg/m^3 (0, 0.06, 0.6, 3.2 or 15 mg/kg/day)^2 for 6 hrs/day, 5 days/wk in a two-week range-finding study (Pauluhn, 1991). Particle size analysis indicated that greater than 99% of the particles were less than 3 µm. Therefore, DPR assumed that 100% of the tribufos in inhaled air reached the alveoli and was absorbed. At 62.5 mg/m^3, rats exhibited hypoactivity, aggressive behavior, vocalization, piloerection, exophthalmos, bradypnea, dyspnea, and slight hypothermia. At 2.6 mg/m^3 and higher, some females displayed a more pronounced tail-pinch response on day 7. The toxicological significance of this effect is unknown. The mean plasma ChE activity was reduced at 62.5 mg/m^3 (M:42%; F:13% of controls) at the study termination. The mean RBC ChE activity was reduced at 13.3 mg/m^3 (M:62% of controls) and 62.5 mg/m^3 (M:27%; F:25% of controls). Significantly reduced mean brain ChE activity (61% of control activity) was seen in females at 62.5 mg/m^3. In males, a significant reduction in relative liver weights was observed at 13.3 and 62.5 mg/m^3. In females, a significant reduction in absolute spleen weights was observed at 62.5 mg/m^3. The reductions in organ weights were not considered toxicologically significant since there were no treatment-related histological changes in these organs. The acute NOEL was 13.3 mg/m^3 (3.2 mg/kg) based on reduced activity, bradypnea, and vocalization by day 3. The subchronic NOEL for overt toxicity was 13.3 mg/m^3 (3.2 mg/kg) based on the clinical signs and plasma and brain ChE inhibition. The NOEL for RBC ChE inhibition was 2.6 mg/m^3 (0.6 mg/kg/day). This study was designed only to be a range-finding study; therefore, it did not meet FIFRA guidelines for a subchronic study because the exposure period was short and there was no hematology, clinical chemistry or histopathology examination.

Inhalation-Rat

In a 13-week subchronic inhalation toxicity study, 10 Bor:WISW (SPF-Cpb) rats/sex/dose were exposed (nose only) to analytical air concentrations of tribufos at 0, 0.9, 2.4, 12.2 or 59.5 mg/m^3 (0, 0.2, 0.6, 2.9 or 14.3 mg/kg/day)^2 for 6 hours/day, 5 days/week (Pauluhn, 1992). The particle size analysis indicated that greater than 99% of the particles were less than 3µm. Therefore, DPR assumed that 100% of the tribufos in inhaled air reached the alveoli and was absorbed. Various clinical signs including reduced motility, bradypnea, dyspnea, increased aggressiveness, miosis, exophthalmos, vocalization, piloerection, convulsions, blepharospasm (spasm in the eyelid muscle resulting in more or less complete closure of the eyelid), and hypothermia (females only) were observed in animals at 59.5 mg/m^3. Some of these signs (reduced motility, bradypnea, piloerection, ungroomed coat, vocalization, irregular breathing and increased startle response) were observed within the first three days of exposure and , therefore, were considered acute effects. Most of these signs do not appear to be cholinergic in origin, but may reflect a localized response in the lungs. However, they appear to be treatment-related since none of these were observed in the lower treatment groups or the controls. There was no treatment-related effect on reflexes, body weight or clinical chemistry. The mean RBC

count, hematocrit, and hemoglobin values were reduced significantly in males at 59.5 mg/m^3 (8%, 7%, and 8%, respectively). The mean RBC count, hematocrit and hemoglobin values were also reduced in females (3%, 7%, and 6%, respectively), but these differences were not statistically significant. At study termination, the mean plasma ChE activity was reduced at 12.2 mg/m^3 (F:60% of controls) and 59.5 mg/m^3 (M:51%; F:33% of controls). The mean RBC ChE activity was also reduced at 12.2 mg/m^3 (M:35%; F:36% of controls) and 59.5 mg/m^3 (M:19%; F:13% of controls). The mean brain ChE activity was significantly reduced at 59.5 mg/m^3 only (M&F:60% of controls). Pale or mottled retinal fundus were noted in females at 59.5 mg/m^3 with the ophthalmological examination. Animals at 59.5 mg/m^3 had evidence of impaired retinal function based on reduced a and b waves in the electroretino-graphic (ERG) examination; however, histological examination of the eye revealed no evidence of retinal degeneration. Fine fatty droplets in the adrenal cortex and elevated absolute and relative adrenal gland weights were also seen in rats at 59.5 mg/m^3. The NOEL for overt toxicity was 12.2 mg/m^3 (2.9 mg/kg/day) based on the clinical signs, brain ChE inhibition, impaired retinal function, pale retinal fundus, fatty droplets in the adrenal gland, and increased adrenal weights. The NOEL for plasma and RBC ChE inhibition was 2.4 mg/m^3 (0.6 mg/kg/day). This study was found acceptable by DPR based on FIFRA guidelines.

Diet-Mouse

In a pilot study, 15 CD-1 mice/sex/group were fed tribufos (97.7%) in the diet at 0, 10, 30, 90 or 270 ppm (M: 0, 3.4, 9.4, 40 or 140 mg/kg/day; F: 0, 5.6, 14.3, 54 or 132 mg/kg/day) for 8 weeks (Hayes, 1985). No clinical signs or deaths were observed. The mean food consumption was higher in the males at 90 ppm (33%) and 270 ppm (51%) and in females at 90 ppm (29%). There was no effect on body weight gain. The mean plasma ChE activity was reduced at 10 ppm (M:36%; F:29% of controls), 30 ppm (M:13%; F:8% of controls), 90 ppm (M:7%; F:4% of controls), and 270 ppm (M:5%; F:4% of controls). The mean RBC ChE activity was reduced at 30 ppm (M:63%; F:56% of controls), 90 ppm (M&F:36%), and 270 ppm (M:27%; F:31% of controls). The mean brain ChE activity was only reduced at 270 ppm (M:74%; F:71% of control activity). The NOEL for overt toxicity was 90 ppm (M: 40 mg/kg/day; F: 54 mg/kg/day) based on the brain ChE inhibition. The NOEL for RBC ChE inhibition was 10 ppm. The NOEL for plasma ChE inhibition was less than 10 ppm (M: 3.4 mg/kg/day; F: 5.6 mg/kg/day). This study was designed as a pilot study for an oncogenicity study and, therefore, the exposure period was short. In addition, there was no histopathological examination, no clinical chemistry or hematology, and no analysis of the diet.

Diet-Rat

Groups of male and female Sprague-Dawley rats were fed diets containing tribufos at 0, 5, 10, 20, 50 or 100 ppm (0, 0.25, 0.5, 1.0, 2.5 or 5.0 mg/kg/day)[4] for 3 months (Root and Doull, 1966). A NOEL of 5 ppm (0.25 mg/kg/day) was reported, but the toxic effects were not indicated. This study had major deficiencies including no summary of the incidence of clinical signs, body weights, food consumption, pathology findings and no clinical chemistry or hematology.

[4] Estimated assuming that for a rat 1 ppm in the diet is equivalent to 0.05 mg/kg/day (FDA, 1959).

Diet-Dog

Tribufos was also administered to groups of male and female beagle dogs in the feed at 0, 5, 10, 20, 50 or 100 ppm (0, 0.125, 0.25, 0.5, 1.25 or 2.5 mg/kg/day)[5] for 3 months (Root and Doull, 1966). Again, a NOEL of 5 ppm (0.125 mg/kg/day) was reported, but the toxic effects were not indicated. This study also had major deficiencies including no summary of the incidence of clinical signs, body weights, food consumption, pathology findings and no clinical chemistry or hematology.

Dermal-Rabbit

A subchronic dermal toxicity study was conducted in which tribufos (99%) was applied topically to the shaved backs of 5 New Zealand white rabbits/sex/dose at 0, 2, 11 or 29 mg/kg/day (actual) for 6 hrs/day, 5 days/wk for 3 weeks (Sheets *et al.*, 1991). An additional 5 rabbits/sex were added to the control and high-dose group for a recovery study. Animals in the recovery groups were held for another 2 weeks after the last exposure. Clinical signs (muscle fasciculations, dried, cracked or flaking skin, erythema, tremors, decreased motor activity, anal stain, red conjunctiva, clear lacrimation, clear nasal discharge, edema, urine stain, and increased reactivity) were seen in both sexes at either 11 or 29 mg/kg/day (Table 4). Red conjunctiva, lacrimation, and anal stains were also seen in a few animals at 2 mg/kg/day. The investigators attributed the red conjunctiva and lacrimation to the plastic collars the rabbits wore during exposure to prevent licking of the application site. To support this conclusion, they noted that these signs resolved within one day in all of the recovery groups after the collars were removed. The investigators considered the incidence of anal stains to be treatment-related; however, the toxicological significance of this sign at 2 and 11 mg/kg/day is uncertain because the incidence was similar to the control group. A reduction in mean body weights (M:15%; F13%) and food consumption (M:30%; F:29%) was seen in both sexes at 29 mg/kg/day by study termination. No compound-related effects were observed with the ophthalmological and gross pathological examinations. Clinical pathological findings included an increased number of segmented white blood cells, a decreased number of lymphocytes, and an increase in blood urea nitrogen (BUN) levels in animals at 29 mg/kg/day. The toxicological significance of the changes in hematological and clinical chemistry values is uncertain without accompanying histological changes. The mean plasma ChE activity was reduced at 2 mg/kg/day (M:82%; F:89% of controls), 11 mg/kg/day (M:43%; F:46% of controls), and 29 mg/kg/day (M:27%; F:26% of controls). A reduction in the mean RBC ChE activity was also observed at 2 mg/kg/day (M:89%; F:80% of controls), 11 mg/kg/day (M&F:30% of controls), and 29 mg/kg/day (M:28%; F:20% of controls). The mean brain ChE activity was significantly reduced at 11 mg/kg/day (M:86%; F:85% of controls) and 29 mg/kg/day (M:68%; F:62% of controls). Microscopic findings were limited to acanthosis and hyperkeratosis, which were observed in the skin at the dosing site of both sexes at 11 and 29 mg/kg/day. The hyperkeratosis was of minimal severity at 2 mg/kg/day and apparently reversible based on the decreased incidence in the high-dose recovery group. Therefore, they were not considered toxicologically significant. Based on the muscle fasciculations, brain ChE inhibition (85-86% of controls), and microscopic lesions in the skin seen at 11 mg/kg/day, the subchronic NOEL for overt toxicity was 2 mg/kg/day. The subchronic NOEL for plasma and RBC ChE inhibition was less than 2 mg/kg/day. An acute NOEL was estimated to be 11 mg/kg/day for this study based on the onset of muscle fasciculations in 9 of 10 animals at 29 mg/kg/day on day 2. DPR found this study acceptable based on the FIFRA guidelines.

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[5] Estimated assuming that for a dog 1 ppm in the diet is equivalent to 0.025 mg/kg/day (FDA, 1959).

Table 4. Incidence of Mortalities, Clinical Signs and Microscopic Lesions in Rabbits with Dermal Exposure to Tribufos for 3 Weeks

	Dose Level (mg/kg/day)							
	0		2		11		29	
	M	F	M	F	M	F	M	F
Death	0[a]	0	0	0	0	0	1(18)	4(12)
Clinical Signs								
Red conjunctiva	2(5)	9(3)	2(7)	2(3)	2(3)	3(2)	3(3)	5(2)
Muscle Fasciculations	0	0	0	0	2(8)	4(5)	10(2)	10(1)
Dried, cracked or flaking skin	0	0	0	0	3(17)	2(14)	10(10)	10(11)
Tremors	0	0	0	0	0	0	9(6)	10(5)
Hypoactivity	0	0	0	0	0	0	7(4)	10(5)
Anal stain	0	1(16)	2(8)	0	2(8)	1(12)	9(13)	1(10)
Erythema	0	0	0	0	2(13)	1(18)	3(6)	7(7)
Clear lacrimation	2(3)	2(3)	1(1)	0	0	1(6)	4(2)	3(3)
Clear nasal discharge	0	0	0	0	0	0	1(10)	4(6)
Edema	0	0	0	0	0	0	2(5)	0
Urine stain	0	0	0	0	0	0	1(13)	1(11)
Increased reactivity	0	0	0	0	1(4)	0	0	1(5)
Salivation	0	0	0	0	0	0	0	1(13)
Microscopic Lesions								
Acanthosis	0	0	0	0	4	1	5	2
Hyperkeratosis	0	0	1	1	5	4	8	7

[a] Five animals/sex/dose, except for the control and high-dose group which had 10 animals/sex/dose, half of which were used for a recovery study. Day of onset for each clinical sign indicated in parentheses next to incidence for each group.

D. CHRONIC TOXICITY/ONCOGENICITY

Summary: Four chronic toxicity studies were available, including a 90-week mouse study, two 2-year rat studies, and a 1-year dog study. All four studies administered tribufos to the animals in the diet. All of the studies met FIFRA guidelines, except one of the rat studies. Mice, rats, and dogs exposed to tribufos orally for one year or longer all had evidence of marked anemia based on reduced hematological values. Significant brain ChE inhibition was observed in all three species. Gastrointestinal effects were seen in both mice and rats including vacuolar degeneration and hyperplasia of the small intestine. Degeneration or pigmentation of the adrenal glands were also seen in both mice and rats. Liver effects were also noted in both

species (hypertrophy in mice and cytoplasmic vacuolation in rats). Other treatment-related histopathological changes were only seen in one species. Extramedullary hematopoiesis was observed in the spleen of mice. Ocular lesions were also seen in rats, including cataracts, lens opacity, corneal opacity, corneal neovascularization, iritis/uveitis, bilateral unrecordable ERG responses, bilateral retinal atrophy, and optical nerve atrophy. There was no evidence of oncogenicity in rats; however, in mice there was a significant increase in adenocarcinomas of the small intestine in both sexes, liver hemangiosarcomas in males, and alveolar/bronchiolar adenomas in females. Not only was there an increase in tumors at more than one site, but there was a significant increase in one tumor type (adenocarcinomas of the small intestine) in both sexes by trend analysis ($p < 0.01$). The lowest NOEL for overt toxicity was 4 ppm (0.2 mg/kg/day) in rats based on hyperplasia and vacuolar degeneration of the small intestine, anemia and RBC ChE inhibition. The lowest NOEL for plasma ChE inhibition was established in dogs at 4 ppm (0.1 mg/kg/day).

Diet-Mouse

In a 90-week study, 50 CD-1 mice/sex/group were fed tribufos (98.6% purity) in the diet at 0, 10, 50 or 250 ppm (M: 0, 1.5, 8.4 or 48.1 mg/kg/day; F:0, 2.0, 11.3 or 63.1 mg/kg/day) (Hayes, 1989). The survival rate was significantly reduced in both sexes at 250 ppm (M:50%; F:38%). The early deaths occurred primarily in the last five months, although there was a significant increase in deaths during months 12 to 18. Enlarged abdomens were seen in both sexes at 250 ppm during weeks 14 to 26. Paleness, loose stools and perineal staining were common in the 250 ppm animals in the second year and coincided with the period of increased mortality. The loose stools and perineal staining were not attributed to cholinesterase inhibition due to the late onset of these effects. Body weights increased for both sexes at 250 ppm after week 13. At necropsy, a significant increase in fluid-filled or dilated intestines and cecum was observed macroscopically in the 250 ppm animals. The males at 50 and 250 ppm had a significant increase in the incidence of an enlarged spleen. A significant increase in the absolute weights of the liver, spleen (males only) and heart (males only) was found in the 250 ppm animals. At study termination, the mean plasma ChE activity was significantly reduced at 10 ppm (M:33%; F:35% of controls), 50 ppm (M:9%; F:7% of controls), and 250 ppm (M:6%; F:3% of controls). The mean RBC ChE activity was also reduced at 10 ppm (M&F:82% of controls), 50 ppm (M:58%; F:63% of controls), and 250 ppm (M:45%; F:50% of controls). There was a statistically significant reduction in the mean brain ChE activity at 10 ppm (M:91% of controls), 50 ppm (M:87% of controls), and 250 ppm (M:62%; F:73% of controls). Both sexes at 250 ppm had evidence of anemia based on significant reductions in their mean RBC counts (M:29%; F:13%), hemoglobin (M:18%; F:13%) and hematocrits (M:20%;F:11%) values and increases in their mean corpuscular volumes (M:16%) and mean corpuscular hemoglobin (M:20%) values at study termination. Females at 50 ppm also had significant reductions in their mean RBC counts (10%), hemoglobin (8%), and hematocrits (8%) at study termination.

A significant increase in numerous non-neoplastic lesions in the intestines, liver, adrenal gland, and spleen were observed microscopically in animals at 250 ppm (Tables 5 and 6). The incidence of mucosal hyperplasia in the small intestine, dilated/edematous cecum or small intestine, necrosis/ulceration of the rectum, and adrenal degeneration/ pigmentation were significant at 250 ppm with dose-related trends. The incidences of vacuolar degeneration in the small intestine and extramedullary hematopoiesis in the spleen (males only) were significant at 50 and 250 ppm with dose-related trends. The incidence of focal atypia (group of abnormal appearing cells) in the small intestine exhibited a dose-related trend, although the increase was not significant when compared with the concurrent controls. The study pathologist considered the focal atypia pre-neoplastic, although he made no comment about the mucosal hyperplasia

Table 5. Incidence of Non-neoplastic Microscopic Lesions in Male Mice Fed Tribufos for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Vacuolar degeneration	0/50^{+++}	1/50	8/50**	28/50***
	(0%)	(2%)	(16%)	(56%)
Mucosal hyperplasia	0/50^{+++}	0/50	1/50	22/50***
	(0%)	(0%)	(2%)	(44%)
Focal atypia	0/50^{+++}	0/50	0/50	4/50
	(0%)	(0%)	(0%)	(8%)
Dilated/distended	0/50^{+++}	0/50	2/50	7/50**
	(0%)	(0%)	(4%)	(14%)
Cecum				
Dilated/edematous	4/50^{++}	8/50	6/50	13/50*
	(8%)	(16%)	(12%)	(26%)
Rectum				
Necrosis/ulceration	0/45^{+++}	1/49	1/47	10/46***
	(0%)	(2%)	(2%)	(22%)
Liver				
Hypertrophy	1/50^{++}	0/50	1/50	4/50
	(2%)	(0%)	(2%)	(8%)
Adrenal				
Degeneration/pigment.	17/50^{+++}	15/50	21/50	39/50***
	(34%)	(30%)	(42%)	(78%)
Spleen				
Hematopoiesis	6/50^{+++}	6/50	14/50*	19/50**
	(12%)	(12%)	(28%)	(38%)

[a] The denominator is the number of animals examined; the number in parentheses represents the incidence in percentage.

$^{++}$, $^{+++}$ A significant trend based on a dose-weighted chi-square test at $p < 0.01$, and 0.001, respectively (Peto *et al.*, 1980).

*, **, *** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$, 0.01, and 0.001, respectively.

Table 6. Incidence of Non-neoplastic Microscopic Lesions in Female Mice Fed Tribufos for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Vacuolar degeneration	0/50^{+++}	0/50	11/50***	28/50***
	(0%)	(0%)	(22%)	(56%)
Mucosal hyperplasia	1/50^{+++}	0/50	0/50	19/50***
	(2%)	(0%)	(0%)	(38%)
Focal atypia	0/50^{+}	0/50	0/50	1/50
	(0%)	(0%)	(0%)	(2%)
Dilated/distended	2/50^{+++}	0/50	1/50	18/50***
	(4%)	(0%)	(2%)	(36%)
Cecum				
Dilated/edematous	7/50^{+++}	3/50	4/50	20/50**
	(14%)	(6%)	(8%)	(40%)
Rectum				
Necrosis/ulceration	2/50^{+++}	0/46	1/50	14/49**
	(4%)	(0%)	(2%)	(29%)
Liver				
Hypertrophy	0/50^{+++}	2/50	0/50	6/50*
	(0%)	(4%)	(0%)	(12%)
Adrenal				
Degeneration/pigment.	18/50^{+++}	26/50	22/50	38/49***
	(36%)	(52%)	(44%)	(78%)
Spleen				
Hematopoiesis	16/50	14/50	18/50	20/50
	(32%)	(28%)	(36%)	(40%)

[a] The denominator is the number of animals examined; the number in parentheses represents the incidence in percentage.

$^{+}$, $^{+++}$ A significant trend based on a dose-weighted chi-square test at $p < 0.05$ and 0.001, respectively (Peto *et al.*, 1980).

*, **, *** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$, 0.01, and 0.001, respectively.

Table 7. Incidence of Neoplastic Microscopic Lesions in Male Mice Fed Tribufos for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Adenocarcinoma[b]	0/47^{+++}	0/48	0/47	9/46***
	(0%)	(0%)	(0%)	(20%)
Liver				
Hemangiosarcoma[c]	1/47^{++}	1/48	4/47	7/46*
	(2%)	(2%)	(9%)	(15%)
Lungs				
Alveolar/bronchiolar	11/47	9/48	5/47	9/46
adenoma[d]	(23%)	(19%)	(11%)	(20%)
Alveolar/bronchiolar	3/47	5/48	4/47	3/46
carcinoma[e]	(6%)	(10%)	(9%)	(7%)
Alveolar/bronchiolar	11/47	13/48	9/47	11/46
tumors - combined	(23%)	(27%)	(19%)	(24%)

[a] The denominator is the number of animals at risk (excluding those that died before the first tumor was observed or 52 weeks, whichever came first); the number in parentheses represents the incidence in percentage.
[b] First small intestine adenocarcinoma observed on week 75 at 250 ppm.
[c] First liver hemangiosarcoma observed on week 59 at 50 ppm.
[d] First alveolar/bronchiolar adenoma observed on week 57 at 10 ppm.
[e] First alveolar/bronchiolar carcinoma observed on week 57 at 10 ppm.
$^{++}$, $^{+++}$ A significant trend based on a dose-weighted chi-square test at $p < 0.01$ and 0.001, respectively.
*, *** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$ and 0.001, respectively.

which could also be considered pre-neoplastic. The study pathologist attributed the vacuolar degeneration to the inability of the epithelial cells to "absorb or secrete products", resulting in fluid accumulation. The extramedullary hematopoiesis in the spleen may be related to the anemia and enlarged spleen, but these findings were not usually present in the same animal at the same time. The increase in adrenal degeneration/pigmentation was considered by the study pathologist to be an enhancement of a common age-related lesion that may be due to stress. The incidence of liver hypertrophy exhibited a dose-related trend in both sexes, but was only significant in females at 250 ppm by pairwise statistical comparison to the concurrent controls. There was no correlation of the liver hypertrophy observed histologically with the increased liver weights.

A significant increase in several neoplastic lesions was reported in mice fed tribufos in the diet at the high dose (Tables 7 and 8) (Hayes, 1989). There was a significant increase in liver hemangiosarcomas in males at 250 ppm that exhibited a dose-related trend. Among the

Table 8. Incidence of Neoplastic Microscopic Lesions in Female Mice Fed Tribufos for 90 Weeks[a]

	Dose Level (ppm)			
	0	10	50	250
Small Intestine				
Adenocarcinoma[b]	0/49[++]	1/45	0/44	4/47[c]
	(0%)	(2%)	(0%)	(9%)
Liver				
Hemangiosarcoma[d]	2/49	2/47	2/47	1/48
	(4%)	(4%)	(4%)	(2%)
Lungs				
Alveolar/bronchiolar	5/49[+++]	5/45	2/44	15/47**
adenoma[e]	(10%)	(11%)	(5%)	(32%)
Alveolar/bronchiolar	1/49	2/45	0/44	2/47
carcinoma[f]	(2%)	(4%)	(0%)	(4%)
Alveolar/bronchiolar	6/49[+++]	7/45	2/44	16/47**
tumors - combined	(12%)	(16%)	(5%)	(34%)

[a] The denominator is the number of animals at risk (excluding those that died before the first tumor was observed or 52 weeks, whichever came first); the number in parentheses represents the incidence in percentage.
[b] First small intestine adenocarcinoma observed on week 69 at 250 ppm.
[c] Not significantly different (p = 0.054) from the control group based on Fisher's exact test.
[d] First liver hemangiosarcoma observed on week 46 at 50 ppm.
[e] First alveolar/bronchiolar adenoma observed on week 74 at 250 ppm.
[f] First alveolar/bronchiolar carcinoma observed on week 75 at 250 ppm.
++, +++ A significant trend based on a dose-weighted chi-square test at p < 0.01 and 0.001, respectively.
** Significantly different from the control group based on the Fisher's exact test at p < 0.01.

animals with liver hemangiosarcomas most also had hemorrhage and/or necrosis in the liver (M: 0/1, 1/1, 4/4, 6/7; F: 2/2, ½, 2/2, 1/1). The incidence of liver hemangiosarcomas in the males at 250 ppm was outside the historical control range for males reported by this laboratory (0-6%). However, the historical control data consisted of only 50 mice/sex/study from three studies.

There was an increase in adenocarcinomas of the small intestine in both sexes which were significant by trend analysis primarily due to the response at the high dose. The increase in these tumors was very highly significant by pairwise comparison with concurrent controls in males ($p < 0.001$), but not in females at 250 ppm ($p = 0.054$). Some of these tumors were associated with inflammatory responses. The reported historical control range for this laboratory was 0% for both sexes. In addition, mice with adenocarcinomas often had focal atypia, mucosal hyperplasia, and/or vacuolar degeneration of the small intestines, too. The investigators suggested that these lesions in the small intestine are interrelated based on their multiplicity and dose relationship.

The incidence of alveolar/bronchiolar adenomas was also significantly higher in females at 250 ppm and was significant by trend analysis essentially due to the response at the high dose. The incidence at the high dose was outside the laboratory's historical control range for these tumors in females (0-14%). There was also a significant positive trend in other potentially pre-neoplastic lesions in the lungs of females including epithelialization (0/50, 1/50, 4/50, 5/50) and focal hyperplasia (3/50, 4/50, 3/50, 8/50). The increase in epithelialization was significant at 250 ppm. The multiplicity of the lung lesions (focal hyperplasia and alveolar/bronchiolar adenomas and carcinomas) was elevated in the females at 250 ppm (0/50, 0/50, 1/50, 9/50).

Small intestine adenocarcinomas and liver hemangiosarcomas were present in several males at 250 ppm that died during the study (M - 1/16, 0/14, 3/21, 12/30; F - 1/19, 1/17, 2/22, 2/31) and may account for some of the early deaths. There was no association with the early deaths and the tumor incidence in females. The liver hemangiosarcomas, small intestine adenocarcinomas and alveolar/bronchiolar adenomas were first seen in females on week 46 (50 ppm), 69 (250 ppm), and 74 (250ppm), respectively.

The overall NOEL for the study was less than 10 ppm (M:1.5 mg/kg/day; F: 2.0 mg/kg/day) based on plasma, RBC and brain ChE inhibition. DPR found this study acceptable based on the FIFRA guidelines.

Diet-Rat

Groups of 24 Sprague-Dawley rats/sex/group were fed tribufos (97.7%) in the diet at 0, 5, 25, 100 or 250 ppm (0, 0.25, 1.25, 5.0 or 12.5 mg/kg/day)[6] for 2 years (Root *et al.*, 1967). There was no effect on survival even at the highest dose level. The mean body weight gain in males at 100 ppm was reduced (~12% relative to controls) by the end of the study. The females fed tribufos at 250 ppm also had significantly reduced (~20% relative to controls) mean body weight gain. The mean plasma ChE activity was significantly reduced at 25 ppm (M:69%; F:65% of controls), 100 ppm (M:31%; F:26% of controls), and 250 ppm (M:22%; F:18% of controls). A reduction in the mean RBC ChE activity was seen at 25 ppm (M:53%; F:42% of controls), 100 ppm (M:27%; F:18% of controls), and 250 ppm (M:15%; F:12% of controls). The mean brain ChE activity was significantly reduced at 100 ppm (F:69% of controls) and 250 ppm (M:57%; F:32% of controls). An increased incidence of liver cytoplasmic vacuolation was found in females at the 100 and 250 ppm dose levels. The NOEL for overt toxicity was 25 ppm (1.25 mg/kg/day) based on the liver cytoplasmic vacuolation, reduced weight gain and brain ChE inhibition. The NOEL for plasma and RBC ChE inhibition was 5 ppm (0.25 mg/kg/day). This study had major deficiencies including incomplete histopathological examination, no hematology or clinical chemistry data, no analysis of dosing material, no individual data, and intercurrent disease.

Diet-Rat

A combined chronic toxicity/oncogenicity/neurotoxicity study was conducted in which Fischer 344 rats were fed tribufos (98.5%) in the diet at 0, 4, 40 or 320 ppm (M: 0, 0.2, 1.8 or 16.8 mg/kg/day; F: 0, 0.2, 2.3 or 21.1 mg/kg/day) for 2 years (Christenson, 1992). Fifty rats/sex/dose were assigned to the oncogenicity study, 20 rats/sex were assigned to the control and 320 ppm groups as interim sacrifice animals for the chronic toxicity study, and 20 rats/sex/dose were assigned to the neurotoxicity study. The incidences of a number of clinical signs were higher in

[6] Estimated assuming that for a rat 1 ppm in the diet is equivalent to 0.05 mg/kg/day (FDA, 1959).

the 320 ppm rats, including pale eyes, ocular opacity, rough coats, rash, raised zones of the skin, urine stains, clear discharge (origin not reported), soft feces, and diarrhea. The mean body weight gains were reduced in both sexes at 320 ppm (~15%) at study termination. Body temperature reductions occurred more frequently in the 40 and 320 ppm rats, although not in a consistent or dose-related manner.

Significant decreases in several hematological values (RBC counts, hemoglobin, and hematocrits) were found in blood drawn from the 40 and 320 ppm rats at 6 and 12 months, but by 18 and 24 months some of these values had returned to normal levels. In fact, these values had actually increased in the 320 ppm rats when compared to controls, possibly from some compensatory mechanism(s). These hematological changes were considered an adverse effect based on the evidence in hens that tribufos is hydrolyzed in the gut to nBM which can cause methemoglobinemia and eventual cell lysis (Abou-Donia, 1979: Abou-Donia *et al.*, 1979a&b). The NOEL for the hematological changes was 4 ppm (M & F: 0.2 mg/kg/day). Several clinical chemistry values, including a decrease in plasma glucose, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, total protein, albumin, and globulin and an increase in BUN, triglycerides, and creatine kinase (CK), were also significantly different in the 40 and 320 ppm groups compared to controls at 6 months. A few of these values had also returned to control levels at both dose levels by study termination, including AST, ALT, CK, and triglycerides. Other values had only returned to control levels in the 40 ppm group (total protein, albumin, globulin, and BUN). The toxicological significance of these changes in clinical chemistry values is uncertain, especially in the absence of any histological changes in the liver, kidney or heart. There was a reduction in the mean plasma ChE activity at 4 ppm (M:84%; F:94% of controls), 40 ppm (M:44%; F:40% of controls), and 320 ppm (M:20%; F:17% of controls) at study termination. The mean RBC ChE activity was reduced at 40 ppm (M:73%; F:72% of controls) and 320 ppm (M:52%; F:53% of controls) while the mean brain ChE activity was only reduced in the 320 ppm rats (M:40%; F:32% of controls). The NOEL for brain ChE inhibition was 40 ppm. The NOEL for RBC ChE inhibition was 4 ppm. A NOEL was not established for plasma ChE inhibition.

Ophthalmologic examination revealed an increased incidence of cataracts, lens opacity, corneal opacity, corneal neovascularization, iritis and/or uveitis in both sexes at 320 ppm at study termination (Tables 9 and 10). These effects were not seen in the 1-year interim sacrifice animals. An increased incidence of bilateral unrecordable (flat) ERG responses was seen in 2-year-old rats of both sexes at 320 ppm. Microscopic examination of the eye also revealed bilateral retinal atrophy (1- and 2-year) and optical nerve atrophy (2-year) in both sexes at 320 ppm. Because the cataracts, lens opacity, corneal opacity, corneal neovascularization, iritis/uveitis, and optical nerve atrophy were not seen in the one-year rats, the study pathologist concluded that these effects were secondary to the retinal atrophy. The NOEL for ocular lesions was 40 ppm.

No dose-related increases were found in the microscopic lesions of the brain, spinal cord and sciatic nerve of rats assigned to the neurotoxicity study. Some studies suggest that rodents (especially Fischer 344 rats) are less sensitive to OPIDN (Abou-Donia, 1981; Somkuti *et al.*, 1988; De Bleeker *et al.*, 1992). The susceptibility of rodents to OPIDN appears to be variable based on studies by other investigators (Padilla and Veronesi, 1988; Veronesi *et al.*, 1991; Moretto *et al.*, 1992; Inui *et al.*, 1993). Differences in age, regeneration of peripheral nerves, aging and resynthesis of NTE, and metabolism have been suggested as possible explanations for the variable response among rodents (Moretto *et al.*, 1992; Veronesi *et al.*, 1991). Since chemicals that produce OPIDN can affect both sensory and motor nerves (Abou-

Table 9. Incidence of Ophthalmologic and Microscopic Lesions in Male Rats Fed Tribufos for 2 Years

	Dose Level (ppm)			
	0	4	40	320
Ophthalmology Examination				
Posterior, subcapsular	5/36^{+++}	4/30	5/36	27/32***
or complete cataract	(14%)	(13%)	(14%)	(84%)
Lens Opacity	6/36	4/30	3/36	8/32
	(17%)	(13%)	(8%)	(25%)
Diffuse or focal corneal opacity	21/36^{+++}	20/30	26/36	31/32***
	(58%)	(67%)	(72%)	(97%)
Corneal neovascularization	2/36^{+++}	6/30	1/36	15/32***
	(5%)	(20%)	(3%)	(47%)
Iritis and/or uveitis	3/36^{+++}	5/30	7/36	31/32***
	(8%)	(17%)	(19%)	(97%)
Electroretinographic Examination				
Bilateral unrecordable responses	0/15^{+++}	2/9	0/15	11/13***
	(0%)	(22%)	(0%)	(85%)
Microscopic Examination				
Bilateral retinal atrophy	1/50^{+++}	0/50	0/50	50/50***
	(2%)	(0%)	(0%)	(100%)
Optic nerve atrophy	10/50^{+++}	6/50	6/50	32/50***
	(20%)	(12%)	(12%)	(64%)
Small intestine				
Vacuolar degeneration	0/50^{+++}	1/50	24/50***	37/50***
	(0%)	(2%)	(48%)	(74%)
Hyperplasia	0/50^{+++}	3/50	23/50***	34/50***
	(0%)	(6%)	(46%)	(68%)
Adrenal vacuolar degeneration	6/50^{+++}	6/49	9/50	35/49***
	(12%)	(12%)	(18%)	(71%)

$^{+,+++}$ Significant trend based on a dose-weighted chi-square test at $p < 0.05$ and 0.001, respectively (Peto *et al.*, 1980).

*,*** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$ and 0.001, respectively.

Table 10. Incidence of Ophthalmologic and Microscopic Lesions in Female Rats Fed Tribufos for 2 Years

	Dose Level (ppm)			
	0	4	40	320
Ophthalmology Examination				
Posterior, subcapsular	4/41^{+++}	6/38	6/34	15/32***
or complete cataract	(10%)	(16%)	(18%)	(47%)
Lens Opacity	9/41^{+++}	8/38	5/34	20/32***
	(22%)	(21%)	(15%)	(62%)
Diffuse or focal corneal opacity	20/41^{+++}	27/38*	20/34	31/32***
	(49%)	(71%)	(59%)	(97%)
Corneal neovascularization	11/41^{+++}	7/38	4/34	19/32**
	(27%)	(18%)	(12%)	(59%)
Iritis and/or uveitis	3/41^{+++}	5/38	5/34	29/32***
	(7%)	(13%)	(15%)	(91%)
Electroretinographic Examination				
Bilateral unrecordable responses	1/16^{+++}	2/16	0/13	7/8***
	(6%)	(12%)	(0%)	(88%)
Microscopic Examination				
Bilateral retinal atrophy	0/50^{+++}	2/50	0/50	40/50***
	(0%)	(4%)	(0%)	(80%)
Optic nerve atrophy	15/50^{+++}	12/50	12/50	34/50***
	(30%)	(24%)	(24%)	(68%)
Small intestine				
Vacuolar degeneration	0/50^{+++}	0/50	19/50***	35/50***
	(0%)	(0%)	(38%)	(70%)
Hyperplasia	1/50^{+++}	0/50	11/50**	30/50***
	(2%)	(0%)	(22%)	(60%)
Adrenal vacuolar degeneration	10/50^{+++}	6/50	16/50	41/50***
	(20%)	(12%)	(32%)	(82%)

$^{+,+++}$ Significant trend based on a dose-weighted chi-square test at $p < 0.05$ and 0.001, respectively (Peto *et al.*, 1980).

*,**,*** Significantly different from the control group based on the Fisher's exact test at $p < 0.05$, 0.01, and 0.001, respectively.

Donia, 1981), it is possible that the degeneration of the retina and optic nerve observed in this study is another, perhaps more sensitive, sign of OPIDN in rats.

There was an increase in other non-ocular lesions in 2-year-old rats of both sexes at 320 ppm, including vacuolar degeneration of the adrenal glands and small intestine and hyperplasia of the small intestine. The incidence of the vacuolar degeneration and hyperplasia of the small intestine was also increased in 2-year-old rats at 40 ppm. An increase incidence of vacuolar degeneration of the small intestine was also seen in rats of both sexes at the 1-year interim sacrifice. The incidence in 1-year-old males was 0/20, 0/10, 7/10, and 18/20 at 0, 4, 40 and 320 ppm, respectively. The incidence in 1-year-old females was 0/20, 0/10, 8/10, and 16/20 at 0, 4, 40, and 320 ppm, respectively. The lesions in the small intestine correlated with the gross findings of thickened and white discoloration. The adrenal lesions correlated with the gross finding of enlargement and increased adrenal weights. The NOELs for the lesions in the small intestine and adrenal glands were 4 ppm (M & F: 0.2 mg/kg/day) and 40 ppm (M: 1.8 mg/kg/day; F: 2.3 mg/kg/day), respectively. A decrease in the incidence of chronic nephropathy was seen in the 2-year-old rats. The incidence among males was 50/50, 50/50, 46/50, and 34/50 at 0, 4, 40 and 320 ppm, respectively. The incidence among females was 39/50, 45/50, 30/50, and 25/50 at 0, 4, 40, and 320 ppm, respectively. There were no dose-related increases in the incidence of benign or malignant tumors. The NOEL for overt toxicity was 4 ppm (M & F: 0.2 mg/kg/day) based on hyperplasia and vacuolar degeneration of the small intestine, and hematological changes. The NOEL for RBC ChE inhibition was also 4 ppm. The NOEL for plasma ChE inhibition was less than 4 ppm. This study was acceptable to DPR.

Diet-Dog

In a chronic dog study, 4 beagle dogs/sex/group were administered tribufos (98.5%) in the feed at 0, 4, 16 or 64 ppm (M: 0, 0.1, 0.4 or 1.7 mg/kg/day; F: 0, 0,1, 0.4 or 2.0 mg/kg/day) for 1 year (Christenson, 1991). There were no treatment-related differences in body weights, food consumption, clinical signs, clinical chemistry, brain ChE, urinalysis, palpable masses, gross pathologic, histopathologic and ophthalmologic lesions. At study termination, the mean plasma ChE activity was significantly depressed at 16 ppm (M:67% of controls) and 64 ppm (M:38%; F:52% of controls). The mean RBC ChE activity was also reduced (M:87%; F:84% of controls) at 64 ppm. Slight reductions in the mean RBC count (9-14%), hemoglobin value (6-13%), and hematocrit (8-12%) were observed in females at 64 ppm on days 91, 182, 273 and 364. Although the reductions in the means were greatest on day 364, the differences were only statistically significant on day 273. The overall NOEL for overt toxicity was 16 ppm (0.4 mg/kg/day) based on the hematological changes in females and RBC ChE inhibition in both sexes. The NOEL for plasma ChE inhibition was 4 ppm (0.1 mg/kg/day). This study was acceptable to DPR based on the FIFRA guidelines.

E. GENOTOXICITY

Summary: Five genotoxicity tests were available for tribufos including an Ames assay, an *in vitro* chromosomal aberrations assay with Chinese hamster ovary cells, two *in vitro* sister chromatid exchange assay with Chinese hamster V79 cells, and an unscheduled DNA synthesis assay with rat primary hepatocytes. Three of these tests met FIFRA guidelines (which refer to the Toxic Substances Control Act (TSCA) guidelines for genotoxicity studies). There was no evidence of genotoxicity in any of the five available studies.

Gene Mutation

Tribufos (98.5%) did not produce an increase in the mutation frequency in a mutagenicity assay using *Salmonella typhimurium* strains, TA98, TA100, TA1535, TA1537 and TA1538 at concentrations ranging from 667 to 10,000 µg/plate with and without metabolic activation (Curren and Gentry, 1989). The assay was acceptable to DPR based on the FIFRA guidelines.

Chromosome Effects

No increase in chromosomal aberrations was seen in Chinese hamster ovary cells exposed to tribufos (98.5%) at concentrations of 0.007 to 0.1 µl/ml with metabolic activation and at 0.004 to 0.05 µl/ml without activation (Putman and Morris, 1989). This study was acceptable to DPR.

Other Genotoxic Effects

Chen *et al.* (1982a&b) found no increase in sister chromatid exchanges in Chinese hamster V79 cells exposed to tribufos (95.7%) at concentrations from 2.5 to 20 µg/ml with and without metabolic activation. Nicholas and Van Den Berghe (1982) also reported no increase in sister chromatid exchanges in Chinese hamster V79 cells exposed to tribufos at concentrations up to 60 µM (18.9 µg/ml) without metabolic activation.

In an unscheduled DNA synthesis assay, no increase in the average grains per nucleus was observed in rat primary hepatocytes exposed to tribufos (98.5%) at concentrations between 0.0001 and 0.03 µl/ml (Curren, 1989). DPR found this study acceptable.

F. REPRODUCTIVE TOXICITY

Summary: Only one reproductive toxicity study was available for tribufos. The test compound was administered to rats by the oral route. The study met FIFRA guidelines. Several reproductive effects were seen in this study. The reproductive effects included reductions in the fertility, birth, and viability indices, increased gestation length, reduced pup weight, clinical signs in pups, plasma, RBC and brain ChE inhibition in pups and gross pathological lesions in pups. Based on a cross-fostering study, the reproductive effects in the pups, such as reduced birth and viability indices, reduced neonatal pup weights, clinical signs and gross pathological lesions were probably related to maternal toxicity rather than a direct effect of tribufos on the pups. The reproductive NOEL was 32 ppm (2.4 mg/kg/day). The parental NOEL for overt toxicity was 4 ppm (0.3 mg/kg/day) based on brain ChE inhibition. The NOEL for plasma and RBC ChE inhibition in adults was less than 4 ppm.

Diet-Rat

In a two-generation rat reproduction study, 30 Sprague Dawley rats/sex/group/ generation were fed tribufos (98.5%) in the diet at 0, 4, 32 or 260 ppm (M: 0, 0.3, 2.2 or 19.1; F: 0, 0.4, 3.0 or 24.1 mg/kg/day) for 10 weeks/generation prior to mating (Eigenberg, 1991a). Body weight gains were significantly lower during all phases of the study in F_0 dams at 260 ppm and during lactation in F_1 dams at 260 ppm. The mean body weights were significantly reduced for the F_{1a} pups at 260 ppm from birth (11%) through lactation (21-29%) at 260 ppm. The investigators found in a subsequent cross-fostering study that the low birth weights were not due to a compound-related effect on development, but rather a weight loss that occurred

between birth and the time the birth weights were taken which was up to 24 hours later (Eigenberg, 1991b). There was no difference in the mean body weights for the F_{2a} pups at birth, but by day 4 the mean body weight at 260 ppm was reduced by 9%. The mean pup weights were significantly reduced at 260 ppm on days 7, 14, and 21 (14-22%). Maternal food consumption was reduced in both generations at 260 ppm. Tremors were observed in one F_0 dam at 260 ppm and abnormal head tilt was seen in three F_1 dams at 260 ppm. Clinical signs observed in F_{1a} and F_{2a} pups at 260 ppm included cannibalization, bite marks, bruised body, diffuse purple discoloration on head, shoulders and abdomen, dehydration, unkempt appearance and moribundity. The investigators attributed the increased cannibalization, bite marks and bruised bodies to some unknown effect of tribufos on the dams based on the cross-fostering study (Eigenberg, 1991b).

Several reproductive parameters were affected at 260 ppm (Table 11). There was a noticeable reduction in the fertility index in the F_1 generation (76% vs. 97% for controls), although it was not statistically significant. This effect was considered toxicologically significant based on a supplemental study in which a similar reduction (83% vs. 90% for controls) was observed in the F_1 generation at 260 ppm (Eigenberg, 1991c). There was a significant increase in gestation length in the F_{2a} litters at 260 ppm which was reproduced in the cross-fostering study (Eigenberg, 1991b). There were also significant reductions in the birth index, live birth index, and viability index in both generations at 260 ppm. The reductions in the birth index and live birth index are probably indirectly related to maternal toxicity. Based on the cross-fostering study, the reductions in the neonatal pup weights and the viability index were also probably due to some unknown effect of tribufos on the dams (Eigenberg, 1991b).

Cholinesterase activity was measured in adults at week 8 of premating for each generation and in both adults and pups at the terminal sacrifice. At week 8 of premating, there were significant reductions in the mean plasma ChE activity at 32 ppm (F_0M:67%; F_0F:32%; F_1F:41% of controls) and 260 ppm (F_0M:30%; F_1M:43%; F_0F:9%; F_1F:7% of controls). At the terminal sacrifice, a significant reduction in the mean plasma ChE activity was observed at 4 ppm (F_0F:75% of controls), 32 ppm (F_0M:82%; F_0F&F_1F:28% of controls) and 260 ppm (F_0M:22%; F_1M:32%; F_0F:10%; F_1F:7% of controls). At week 8, there were significant reductions in the mean RBC ChE activity at 4 ppm (F_1M: 91% of controls), 32 ppm (F_0M:65%; F_1M:74%; F_0F:63%; F_1F:72% of controls), and 260 ppm (F_0M:50%; F_1M:57%; F_0F:51%; F_1F:55% of controls). At the terminal sacrifice, the mean RBC ChE activity was also significantly reduced at 4 ppm (F_0F:88%; F_1F:93% of controls), 32 ppm (F_0M:69%; F_1M:72%; F_0F:54%; F_1F:51% of controls) and 260 ppm (F_0M:47%; F_1M:61%; F_0F:48%; F_1F:47%). The mean brain ChE activity was reduced only at 32 ppm (F_0F&F_1F:71% of controls) and 260 ppm (F_0M:63%; F_1M:67%; F_0F&F_1F:19% of controls). There were sex-related differences in ChE activity which were most pronounced at the terminal sacrifice. One explanation for these differences was the higher compound consumption in females during lactation. During lactation, the average compound consumption for females in both generations was approximately twice as high as their consumption during premating and gestation (0.7, 5.5, and 39.2 mg/kg/day at 4, 32, and 260 ppm, respectively). Significant reductions in the mean plasma ChE activity were observed in 21-day-old pups at 260 ppm (F_{1a}M:64%; F_{2a}M:49%; F_{1a}F:62%; F_{2a}F:36% of controls). The mean RBC ChE activity was also significantly reduced (F_{2a}M:75%; F_{1a}F:77%; F_{2a}F:62% of controls) at 260 ppm. There was also a significant reduction in the mean brain ChE activity in 21-day-old F_{2a} pups at 260 ppm (M&F:85% of controls).

There were no apparent compound-related increases in gross pathological findings in the adults. Sporadic gross ocular lesions (discoloration, opacity, reduced size, abnormal texture and enlargement) were observed in all groups of F_0 and F_1 adults which were attributed to the

Table 11. The Reproductive Effects of Tribufos in a Two-Generation Rat Study

Reproductive Effect	Generation	Dose Level (ppm)			
		0	4	32	260
Fertility Index	F_0	90	97	90	90
(%)	F_1	97	93	90	76
Mean Gestation Length	F_{1a}	21.8	22.0	21.9	22.2
(days)	F_{2a}	21.9	22.0	22.0	22.4*
Birth Index	F_{1a}	91	89	90	77*
(%)	F_{2a}	92	91	92	87*
Live Birth Index	F_{1a}	100	97	100	80*
(%)	F_{2a}	99	95	100	87*
Viability Index (day 4)	F_{1a}	96	96	100	90*
(%)	F_{2a}	97	100	97	81*
(day 21)	F_{1a}	100	99	99	83*
	F_{2a}	100	99	100	90*
Mean Pup Weight (day 4)	F_{1a}	7.1	7.1	7.2	6.3*
(g)	F_{2a}	6.8	7.2*	7.0	6.7
(day 21)	F_{1a}	49.5	50.2	50.1	35.2*
	F_{2a}	49.1	49.5	50.2	38.3*

* Significantly different from the control group by the Kruskal-Wallis and Mann-Whitney U test ($p < 0.05$).

orbital bleeding technique by the investigator. Possible compound-related retinal degeneration was observed microscopically in two females (one at 4 ppm and the other at 260 ppm) with gross ocular lesions (corneal opacity and enlargement, respectively). The eyes were examined in only a few rats with gross ocular lesions, probably because the effect of tribufos on the retina was not known when this study was conducted. Consequently, a dose-response was not apparent. In the pups, possible compound related effects observed at 260 ppm included cannibalism, discolored livers, uninflated lungs (stillbirths) and empty stomachs (non-suckling).

The reproductive NOEL was 32 ppm (3.0 mg/kg/day) based on the reduction in the fertility, birth, live birth and viability indices, increased gestation length, reduced pup weight, clinical signs in pups, reduced plasma (36-64% of controls) , RBC (62-77% of controls) and brain (85% of controls) ChE activity in pups and gross pathological lesions in pups. The parental NOEL for overt toxicity was 4 ppm (0.4 mg/kg/day) based on the reduced brain ChE activity (71% of control activity) in F_0 and F_1 females at 32 ppm (3.0 mg/kg/day). The parental NOEL for reduced plasma (7-32% of controls) and RBC (88-93%of controls) ChE activity was less than 4 ppm. This study was considered acceptable by DPR.

G. DEVELOPMENTAL TOXICITY

Summary: Two teratology studies were conducted with tribufos, one in rats and the other in rabbits. Both studies administered tribufos by oral gavage. These studies met FIFRA guidelines. No treatment-related increases in embryotoxicity, fetal malformations or variations were observed in rats and rabbits exposed to tribufos by the oral route. Maternal effects included plasma, RBC and brain ChE inhibition and reduced body weight gain. The maternal NOEL for overt toxicity in rats was 7 mg/kg/day based on brain ChE inhibition and reduced maternal weight gain. The rat maternal NOEL for plasma and RBC ChE inhibition was 1 mg/kg/day. The maternal NOEL for overt toxicity in rabbits was 3 mg/kg/day based on no weight gain during exposure. The rabbit maternal NOEL for plasma and RBC ChE inhibition was less than 1 mg/kg/day. ChE activity was not measured in the fetuses, except for rat fetal brain. No fetal brain ChE inhibition was observed at the highest dose tested, 28 mg/kg/day.

In addition, an inhalation teratology study was conducted in which mice and rats were exposed to vapors of nBM. This study did not meet FIFRA guidelines. An increased post-implantation loss was observed in mice exposed to vapors of nBM. The total number of malformations was also higher, although not on a litter basis. The developmental NOEL for nBM was 10 ppm (17 mg/kg/day). The maternal effects included increased mortalities, reduced body weight gain, and clinical signs. The maternal NOEL for nBM in mice was also 10 ppm. There were no treatment-related increases in developmental or maternal effects in rats exposed to vapors of nBM.

Gavage-Rat

In a teratology study, tribufos (98%) was administered to 33 mated female Sprague-Dawley rats/group by oral gavage in 0.5% carboxymethylcellulose at 0, 1, 7 or 28 mg/kg/day on gestation days 6 to 15 (Kowalski *et al.*, 1986). Excessive salivation was observed in two dams at 28 mg/kg/day on treatment days 3 and 6 (gestation days 9 and 12). There was a significant reduction in the mean body weight gain of the 28 mg/kg/day group. The mean plasma and RBC ChE activity in the dams was significantly reduced at 7 mg/kg/day (42 and 29% of controls, respectively) and 28 mg/kg/day (25 and 13% of controls, respectively) on day 16. Although the mean maternal brain ChE activity remained significantly reduced (54% of control) on day 20 at 28 mg/kg/day, fetal brain ChE activity was unaffected. Plasma and RBC ChE activity was not measured in the fetuses. No treatment-related teratogenic or other developmental effects were seen. The maternal NOEL for overt toxicity was 7 mg/kg/day based on the brain ChE inhibition and reduced body weight gain. The NOEL for plasma and RBC ChE inhibition was 1 mg/kg/day. The developmental NOEL was greater than or equal to 28 mg/kg/day, the highest dose tested, based on the lack of fetal effects including brain ChE inhibition. This was an acceptable study to DPR.

Gavage-Rabbit

Groups of 17 mated female American Dutch rabbits were given tribufos (98%) by oral gavage in carboxymethylcellulose at 0, 1, 3, or 9 mg/kg/day on days 7 to 19 of gestation (Clemens *et al.*, 1987). Although control animals gained 150 g on average from gestation days 7 to 21, animals at 9 mg/kg/day gained no weight on average during this time. The animals at 9 mg/kg/day also tended to consume less food during the treatment period, although the difference was not statistically significant. The mean plasma ChE activity was significantly reduced at 1 mg/kg/day (60% of controls), 3 mg/kg/day (46% of controls), and 9 mg/kg/day (33% of controls) on day 20. There was also a significant reduction in RBC ChE activity at 1 mg/kg/day (30% of controls), 3 mg/kg/day (15% of controls), and 9 mg/kg/day (7% of controls)

on day 20. The slight reduction in the mean brain ChE activity (95% of controls) at 9 mg/kg/day was not statistically significant. There was no treatment-related increase in embryotoxicity, fetal malformations or variations. Fetal ChE activity was not measured. The maternal NOEL for overt toxicity was 3 mg/kg/day based on no body weight gain during exposure. The maternal NOEL for reduced plasma (60% of controls) and RBC (30% of controls) ChE activity was less than 1 mg/kg/day. The developmental NOEL was greater than or equal to 9 mg/kg/day, the highest dose tested. DPR found this study acceptable.

N-Butyl Mercaptan

Inhalation-Mouse

In an inhalation teratology study, 25 pregnant female mice/dose were exposed to vapors of nBM (97.5%) for 6 hrs/day at 0, 10, 68 or 152 ppm (actual; 0, 17, 113 or 252 mg/kg/day)[7] during gestation days 6-16 (Thomas *et al.*, 1987). Seventeen mice at 68 and 152 ppm died. One dam at 152 ppm had limb paralysis and spasmodic respiratory appearance. Emaciation, unkempt appearance, lethargy and red/brown perianal staining were seen in dams at 68 and 152 ppm. There was a reduction in the terminal maternal body weights (>10%) and an increase in postimplantation losses at 68 and 152 ppm. The total number of fetuses with malformations (which included cleft palate, open eye, exencephaly, hydrocephaly, vertebral anomalies and bent bones) was also significantly higher at 68 ppm, although there was no significant difference in the number of malformations/group on a litter basis. Four of the 5 fetuses with cleft palate at 68 ppm occurred in two litters in which there was evidence of both maternal and fetal toxicity based on maternal weight loss and lower fetal weights. The maternal NOEL for nBM was 10 ppm (17 mg/kg/day) based on the mortality, reduced weight gain and clinical signs. The developmental NOEL was also 10 ppm based on the increased postimplantation losses and malformations. This study was only available as a published report and, therefore, it is not known if it met FIFRA guidelines.

Inhalation-Rat

Thomas *et al.* (1987) also exposed 25 pregnant female rats/dose to vapors of nBM (97.5%) at 0, 10, 68 or 152 ppm (actual; 0, 9, 60 or 135 mg/kg/day)[8] for 6 hr/day during gestation days 6-19 (Thomas *et al.*, 1987). There were no mortalities or significant treatment-related clinical signs. There was no evidence of developmental toxicity. The maternal and developmental NOELs in rats were greater than 152 ppm (135 mg/kg/day), the highest dose tested.

H. NEUROTOXICITY

Summary: Numerous neurotoxicity studies have been conducted in which hens were exposed to tribufos by the intraperitoneal, subcutaneous, inhalation, oral, or dermal routes (Tables 12 and 13). Due to the large number of studies, most of these studies will only be

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[7] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a mouse was assumed to be 0.45 m^3/kg/6 hrs (Zielhuis and van der Kreek, 1979).

[8] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a rat was assumed to be 0.24 m^3/kg/6 hrs (Zielhuis and van der Kreek, 1979).

discussed briefly. Most of these studies were available as published reports and did not follow the standard protocol recommended in the FIFRA guidelines. One subchronic dermal neurotoxicity study did meet FIFRA guidelines. Delayed neuropathy was observed in acute and subchronic studies in which hens were exposed by the inhalation, oral, and dermal routes. In addition, cholinergic signs and other effects described as "late acute" effects were seen; however, the late acute effects were only observed with oral exposure in hens. The late acute effects were attributed to the hydrolysis of tribufos to nBM which was found in the excreta of hens administered tribufos orally. In subsequent studies, it was shown in hens that nBM causes RBC deformation and lysis through the inhibition of glucose-6-phosphate dehydrogenase. With acute oral exposure in hens, the OPIDN occurred at approximately the same dose levels or higher than the cholinergic and late acute effects. However, the distinction between the cholinergic, late acute, and delayed neurotoxic effects was blurred in some studies because some effects such as leg weakness and unsteadiness were common to all syndromes and could only be separated based on their time of onset. There is also some uncertainty about the dose levels at which cholinergic signs occurred because many of the studies provided insufficient information about the incidence of cholinergic effects to accurately determine a NOEL. The lowest NOEL in hens with acute inhalation exposure to tribufos was less than 43 mg/kg based on mild cholinergic signs (Thyssen and Schilde, 1976a). With acute oral exposure, the lowest NOEL was 50 mg/kg based on death, unspecified toxic signs, late acute effects, and OPIDN (Thyssen, 1976; Abou-Donia *et al.*, 1979a). The lowest acute dermal NOEL was 100 mg/kg based on mild cholinergic signs and OPIDN (Abou-Donia *et al.*, 1984; Abdo *et al.*, 1983a). In the subchronic studies in hens, the OPIDN generally occurred at lower doses than the cholinergic or late acute effects. The lowest NOEL with subchronic inhalation exposure to tribufos was 3.6 mg/kg/day based on mild cholinergic signs and OPIDN (Thyssen and Schilde, 1978a). The lowest subchronic oral NOEL was 0.1 mg/kg/day based on OPIDN (Abou-Donia *et al.*, 1979b). With subchronic dermal exposure to tribufos, the lowest NOEL was 2.6 mg/kg/day based on OPIDN (Sheets, 1991b).

Several guideline neurotoxicity studies were also conducted in rats. The neurobehavioral effects observed in an acute neurotoxicity study included reduced motor activity and rearing, lacrimation and oral stains, reduced auditory response, uncoordinated righting response, and reduced temperature. The acute NOEL was 2 mg/kg based on reduced motor activity, reduced auditory response and reduced plasma and RBC ChE activity. In a subchronic neurotoxicity study in rats, urine stains, reduced motor and locomotor activity and bilateral retinal atrophy were observed at the high dose. The subchronic NOEL was 2 ppm (M: 0.14 mg/kg/day; F: 0.17 mg/kg/day) based on reduced plasma, RBC and brain ChE activity. In a developmental neurotoxicity study in rats, reduced gestation index, tremors and reduced body weights were observed in the dams at the highest dose level. The maternal NOEL was 4 ppm (0.4 mg/kg/day) based on plasma, RBC and brain ChE inhibition. The pup NOEL was 40 ppm (3.5 mg/kg/day) based on clinical signs (weakness, wound/cut, no milk in stomach during lactation), delayed developmental landmarks (surface righting and preputial separation), reduced body weights and food consumption, reduced acoustic startle response, reduced brain size and weight and plasma and brain ChE inhibition. All three rat neurotoxicity studies were found acceptable to DPR based on FIFRA guidelines.

Hen Studies

Parenteral Studies

Casida and coworkers (1963) first reported evidence of OPIDN when chickens developed ataxia 10-14 days after 7-10 daily intraperitoneal injections of tribufos at 100

Table 12. Acute Neurotoxicity Studies for Tribufos with Hens

Dosage	Hens/Dose	Effect	NOEL (mg/kg)	LOEL (mg/kg)	Ref.[a]
		Inhalation[b]			
391, 878 or 1,585 mg/m^3, single 4-hr exposure	5	Leg weakness, drowsiness, inactivity, breathing disorders	----	43	1
		Ataxia, paralysis (onset day 15), degeneration of sciatic nerve	97	174	
62, 145 or 246 mg/m^3, 4-hrs/day for 5 days	10	Ataxia (onset day 16-18) (paralysis, nerve degeneration at 27 mg/kg)	6.8	16	1
		Leg weakness, drowsiness, inactivity, breathing disorders	16	27	
		Subcutaneous			
200 or 1,060 mg/kg	2-3	Ataxia, paralysis (onset day 8)	200	1060	2
220 or 1,100 mg/kg	2	Ataxia (onset not reported)	220	1010	3
		Oral			
0, 50 - 500 mg/kg, gavage	10	Death and unspecified toxic effects	50	100	4
0, 50 - 1,000 mg/kg, one capsule	3	Late acute effects (onset day 2-14), ataxia (onset day 4-17)	50	100	5
0, 100 - 1,000 mg/kg, one capsule	5	Ataxia (onset not reported), peripheral demyelination (1 hen)	----	100	6
		Dermal			
0.5, 1 or 2 ml/kg, dorsal skin	5	Impaired general health, ataxia, paralysis (onset week 2-3)	~500	~1000	7
0, 400 or 1,000 mg/kg, comb	3	Brain ChE inhibition (74% of control), ataxia (onset day 6-11) (nerve degeneration at 1,000 mg/kg)	----	400	5
100 - 1,000 mg/kg, neck	5	Ataxia, paralysis (onset day 9-10)	100	250	8
		Unspecified mild cholinergic signs	250	500	
0, 100 - 1,000 mg/kg, back of neck	5	Unspecified mild cholinergic signs, ataxia, paralysis (onset not reported)	100	250	6

[a] References: 1. Thyssen and Schilde, 1976a; 2. Johnson, 1970a; 3. Johnson, 1970b; 4. Thyssen, 1976; 5. Abou-Donia *et al.*, 1979a; 6. Abou-Donia *et al.*, 1984; 7. Thyssen and Schilde, 1976b; 8. Abdo *et al.*, 1983a.

[b] Air concentrations were converted to mg/kg by assuming a respiratory rate of 0.11 m^3/kg/4 hrs (Dejours *et al.*, 1970).

Table 13. Subchronic Neurotoxicity Studies for Tribufos in Hens

Dosage	Hens/Dose	Effect	NOEL (mg/kg/day)	LOEL (mg/kg/day)	Ref.[a]
		Inhalation[b]			
8, 21 or 84 mg/m^3, 6 hr/day, 5 day/wk, 3 wks	10	Decreased preening, lethargy, ataxia, paralysis (onset week 4), degeneration of sciatic nerve (1 hen)	3.6	14.3	1
		Intraperitoneal			
100 mg/kg/day, 7 or 10 days	NR	Ataxia (onset day 10-14)	----	100	2
50 or 100 mg/kg/day, 3 to 15 days	5-28	Muscle weakness, ataxia, paralysis, nerve degeneration	----	50	3
		Oral			
50, 100 or 150 mg/kg/day, 4-15 days	1-7	Unspecified degenerative lesions in spinal cord and sciatic nerve (1 hen)	100	150	3
0, 100, 250 or 500 ppm, 30 days, diet	6	Focal liquefication of brain	34[c]	87	4
0, 25 - 400 ppm, 30 days, diet	10	Reduced food consumption, perivascular CNS[d] & PNS inflammation	6.1	10.9	5
0, 0.1 - 80 mg/kg/day, capsule, 90 days	5	Ataxia (onset day 30) Late acute effects (onset day 2-5), paralysis (onset day 19-30), nerve degeneration	0.1 10	0.5 20	6
3 - 40 mg/kg/day, capsule, 91-97 days	3-4	Death, ataxia, paralysis (onset day 10-26)	5-6	38-40	7
		Dermal			
0, 0.01 - 1 ml/kg/day, 6 hr/day, 5 days/wk, 3 wks, axilla	8	Ataxia, paralysis (onset week 3) Unspecified cholinergic signs	~30 ~100	~100 ~300	8
0, 20 or 40 mg/kg/day, 90 days, comb	3	Ataxia (onset day 8-22)	----	20	6
6 - 16 mg/kg/day, 91-101 days, comb	3	Ataxia (onset day 76-100); skin: thickening of keratin and epidermis, collagen deposition, inflammation	----	6-8	7
0, 2.6, 11, 42 mg/kg/day, 5 day/wk, 13 wks, comb	12	Axonal degeneration	2.6	11	9*

[a] References: 1. Thyssen and Schilde, 1978a; 2. Casida *et al.*, 1963; 3. Baron and Johnson, 1964; 4. Harris, 1965; 5. Thyssen *et al.*, 1977; 6. Abou-Donia *et al.*, 1979b; 7. Hansen *et al.*, 1982; 8. Thyssen and Schilde, 1978b; 9. Sheets, 1991b.

[b] Air concentrations were converted to mg/kg by assuming a respiratory rate of 0.17 m^3/kg/6 hrs (Dejours *et al.*, 1970).

[c] Using the mean food consumption for each group from the study and assuming a body weight of 2 kg.

[d] CNS = central nervous system; PNS = peripheral nervous system

* Acceptable study based on FIFRA guidelines

mg/kg/day with and without atropine protection. A similar study conducted by Baron and Johnson (1964) reported muscle weakness, ataxia, paralysis, and degenerative lesions in the sciatic nerve and spinal cord in hens after 3-15 intraperitoneal injections of tribufos at 50 and 100 mg/kg/day. Johnson (1970 a&b) also reported evidence of OPIDN in hens after a single subcutaneous injection of tribufos at approximately 1,000 mg/kg with an onset around day 8. NTE activity, measured in the brain of two hens 17 and 24 hours after dosing, was reduced to 23% of the control activity.

Inhalation Studies

Three inhalation studies were conducted in hens with the exposure ranging from a single 4-hr exposure to daily 6-hr exposures, 5 days/week for 3 weeks (Thyssen and Schilde, 1976a; Thyssen and Schilde, 1978a). Evidence of OPIDN (ataxia, paralysis and nerve degeneration) was observed in all 3 studies. Compared to cholinergic signs, the development of OPIDN appeared to be especially sensitive to repeated inhalation exposure. With a single inhalation exposure, the acute LOEL for OPIDN was 4-fold higher (878 mg/m^3 or 174 mg/kg[9]) than for cholinergic signs (391 mg/m^3 or 43 mg/kg). However, with 5 consecutive inhalation exposures, the LOEL for OPIDN was nearly 2-fold lower (145 mg/m^3 or 16 mg/kg/day) than for cholinergic signs (246 mg/m^3 or 27 mg/kg/day). The subchronic NOEL for OPIDN was 21 mg/m^3 (3.6 mg/kg/day)[10]. None of these studies met FIFRA guidelines.

Oral Studies

Several of the initial oral studies for tribufos suggested that OPIDN was not easily produced by this route. Baron and Johnson (1964) did not observe any evidence of OPIDN in hens when tribufos was administered by oral gavage at 50-150 mg/kg for 4-15 days with the possible exception of one hen. In a 30-day feeding study, there was equivocal histological evidence of OPIDN (demyelination of the spinal cord at 100 and 250 ppm only; focal liquefication of the brain at 250 and 500 ppm) in 1 of 6 hens per dose when fed tribufos at 100, 250 or 500 ppm (Harris, 1965). Thyssen (1976) found no clinical or histological evidence of OPIDN when hens were administered tribufos by oral gavage at 300 mg/kg twice with a 21-day interval between each dose. Equivocal histological evidence of OPIDN (perivascular CNS and PNS inflammation) was also seen in another 30-day feeding study in which hens were fed tribufos at 0, 25, 50, 100, 200 or 400 ppm (Thyssen *et al.*, 1977).

Evidence of OPIDN was observed in four other oral neurotoxicity studies with hens (Abou-Donia *et al.*, 1979a&b; Abou-Donia *et al.*, 1984; Hansen *et al.*, 1982). An acute NOEL of 50 mg/kg/day was established for OPIDN in one study (Abou-Donia *et al.*, 1979a). A significantly lower NOEL of 0.1 mg/kg/day was observed in an oral subchronic study with hens based on mild ataxia (Abou-Donia *et al.*, 1979b). However, there was limited histological evidence of delayed neuropathy with oral exposure even at high doses (Table 14). Unequivocal histological lesions in the spinal cord or peripheral nerve were not observed in this study until the dose was increased to 20 mg/kg. At 80 mg/kg/day, 1 out of 5 hens had unequivocal lesions and 2 out of 5 hens had equivocal lesions in the spinal cord indicative of OPIDN. The equivocal lesions were ones Abou-Donia *et al*. (1979b) suggested could be early signs of delayed neuropathy, but because they were occasionally observed in controls he could not be certain.

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[9] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a chicken was assumed to be 0.11 m^3/kg/4 hrs (Dejours *et al.*, 1970).

[10] Dose was estimated from air concentration in ppm using Equation 2 in Appendix A. The respiratory rate for a chicken was assumed to be 0.17 m^3/kg/6 hrs (Dejours *et al.*, 1970).

However, there was no dose-response relationship in the incidence of the equivocal lesions with oral exposure. More likely these lesions were age-related because the birds were relatively old (19 months). Abou-Donia *et al*. (1979b) did not report the incidence of the histological lesions at 0.1 mg/kg/day or in the controls, making interpretation of the equivocal lesions difficult. Hens receiving 20-80 mg/kg/day orally, developed severe ataxia, some became paralyzed and nearly all died from late acute effects within the first few weeks of exposure. Abou-Donia *et al*. (1979b) suggested that more histological lesions would have been seen with oral exposure if the hens had lived longer. However, if there was enough nBM to kill these hens, there probably was significantly less tribufos available to produce delayed neuropathy. Moreover, the nBM is probably responsible for the ataxia and paralysis with oral exposure because of the limited evidence of delayed neuropathy even at lethal doses. Abou-Donia *et al*. (1979b) and Fairchild and Stokinger (1958) reported that nBM caused muscle weakness, incoordination, paralysis, CNS depression and cyanosis all of which could affect gait. NTE activity was not measured in the Abou-Donia *et al.* (1979b) study which would have helped in the interpretation of the clinical and histopathological findings. A LOEL of 20 mg/kg/day for OPIDN would be more consistent with the LOEL reported for a similar oral subchronic neurotoxicity study in which death, paralysis, and nerve degeneration were observed in hens at approximately 40 mg/kg/day (Hansen *et al.*, 1982). None of the oral neurotoxicity studies for tribufos met FIFRA guidelines because of inadequate exposure duration, inadequate number of hens per group, age of hens, no analysis of test article or dosing material, inadequate or no histopathology data or no positive controls.

One explanation for the reduced incidence of OPIDN in hens administered tribufos by the oral route may be the hydrolysis of tribufos to nBM in the gastrointestinal tract. Abou-Donia *et al.* (1979a) first reported "late acute" effects in hens administered a single capsule containing tribufos at 100 mg/kg or higher. The hens exhibited leg weakness, unsteadiness and a yellowish watery liquid around the mouth by the second day after dosing. Their condition progressively worsened with malaise, general muscle weakness, loss of balance, diarrhea, loss of appetite, disorientation, tremors and loss of breath which were not responsive to atropine therapy. Just prior to death, their combs became dark and droopy. These late acute effects were distinguished from those associated with OPIDN in that the onset of death was earlier (2-14 days after dosing) and no histological lesions were found in the sciatic nerve. nBM was identified by mass spectrometry in the excreta of hens administered tribufos orally. These investigators tested the possibility that these effects were due to nBM by administering hens a single capsule containing nBM (98%) at 100, 400 or 1,000 mg/kg. The hens at 400 and 1,000 mg/kg developed clinical signs similar to the late acute effects observed when tribufos was administered orally; however, the onset of signs was earlier (6-12 hrs after administration). No degenerative changes in the sciatic nerve were present in any of the hens treated with nBM. Furthermore, there was a slight increase or no change in brain and plasma ChE activity of hens exposed to nBM. The half-life of nBM was estimated to be 8 days based on the plasma levels.

A mechanism of action for nBM toxicity was proposed by Abdo *et al.* (1983b) who found an increase in Heinz bodies and extensive RBC deformation and lysis 24 to 48 hrs after hens were given nBM in capsules at 500 mg/kg. Methemoglobin levels were significantly higher in the treated birds while the hemoglobin concentration, packed cell volume, RBCs and glucose-6-phosphate dehydrogenase (G-6-PD) activity were significantly lower. The time course for disappearance of hematological changes and late acute effects was similar and the investigators suggested that the inhibition of G-6-PD by nBM led to the hematological changes. This enzyme is required to regenerate nicotinamide adenine dinucleotide phosphate (NADPH) which is needed for the reduction of glutathione. Decreased levels of reduced glutathione resulted in the denaturation of hemoglobin (i.e., formation of methemoglobin and Heinz bodies), coagulation of surface proteins on RBCs leading to deformation, and eventual cell lysis. The

investigators concluded the late acute effects observed after oral administration of tribufos were directly related to the hematological changes.

Dermal Studies

Evidence of OPIDN was observed in nearly all of the acute dermal neurotoxicity studies in hens (Thyssen and Schilde, 1976b; Abou-Donia *et al.*, 1979a; Abou-Donia *et al.*, 1984; Abdo *et al.*, 1983a; Thyssen and Schilde, 1978b; Abou-Donia *et al.*, 1979b; Hansen *et al.*, 1982; Sheets, 1991b). The lowest acute NOEL for OPIDN by the dermal route was 100 mg/kg (Abdo *et al.*, 1983a; Abou-Donia *et al.*, 1984). None of the acute dermal neurotoxicity studies met FIFRA guidelines.

There was evidence of OPIDN was found in a number of subchronic dermal neurotoxicity studies in hens (Thyssen and Schilde, 1978b; Abou-Donia *et al*, 1979b; Hansen *et al.*, 1982; Sheets, 1991b). It is interesting to compare the findings with oral and dermal exposure from the Abou-Donia *et al.* (1979b) study at 40 mg/kg/day (Table 14). With oral exposure, 4 out of 5 hens had no lesions and 1 out of 5 hens had an equivocal lesion in the spinal cord suggestive of OPIDN. By contrast, with dermal exposure unequivocal lesions of OPIDN were observed in the spinal cord of all 3 hens. While all the hens administered tribufos at 40 mg/kg/day by the oral route died within the first few weeks of exposure, none of the hens administered tribufos at this same dose level by the dermal route died despite developing clear evidence of delayed neuropathy. A NOEL could not be established for delayed neuropathy with dermal exposure because animals at the lowest dose developed ataxia which, unlike with oral exposure, was probably related to OPIDN since significantly less nBM would likely be formed with this route of exposure.

Only one subchronic dermal study conducted by Sheets (1991b) was acceptable to DPR based on the FIFRA guidelines. In this study, 12 white leghorn hens/group were administered tribufos (97.7%) topically to the comb at 0, 2.6, 11, and 42 mg/kg/day for 5 days/week for 13 weeks. Whole blood cholinesterase activity was significantly reduced at 2.6 mg/kg/day (53% of controls), 11 mg/kg/day (43% of controls), and 42 mg/kg/day (43% of controls). Decreased motor activity and ataxia were observed in all hens at 42 mg/kg/day with an onset between days 12 and 39. There was a high background rate for axonal degeneration probably due to the age of the birds which were older (17 months) than recommended by FIFRA guidelines (8-14 months), thus making interpretation of the histological findings difficult. The axonal degeneration was identical to that encountered in older hens that have had contact with vaccines or other exogenous viral exposure, such as Marek's disease. There was a statistically significant increase in the severity of the axonal degeneration at 42 mg/kg/day. Although not statistically significant, there was a slight increase in the severity and incidence of axonal degeneration at 11 mg/kg/day. There were only two instances of mild ataxia on days 71 and 80 in 1 of 12 hens at 11 mg/kg/day, suggesting that most of axonal degeneration was age-related. DPR made the health protective assumption that the axonal degeneration was treatment-related and set the NOEL at 2.6 mg/kg/day. The LOEL in this study is consistent with the LOELs established in two other subchronic dermal neurotoxicity studies (Abou-Donia *et al.*, 1979b; Hansen *et al.*, 1982); however, the NOEL was the lowest subchronic NOEL observed for OPIDN by the dermal route.

Table 14. Incidence of Histological Lesions in Spinal Cord or Peripheral Nerve of Hens Administered Tribufos Orally or Dermally for 90 Days[a]

Dose mg/kg/day	Oral[b]			Dermal[c]		
	Positive	Equivocal	Negative	Positive	Equivocal	Negative
80	1	2	2	—	—	—
40	0	1	4	3	0	0
20	1	0	4	0	1	2
10	0	1	4	—	—	—
5	0	1	4	—	—	—
2.5	0	0	5	—	—	—
1	0	2	3	—	—	—
0.5	0	2	3	—	—	—

[a] Abou-Donia *et al.*, 1979b
[b] Five hens exposed by the oral route per dose level
[c] Three hens exposed by the dermal route per dose level

A study was conducted to evaluate the neurotoxic effects of tribufos in hens from normal field use. Scaleless hens were exposed to varying levels of tribufos over a 7-hour period based on their proximity to a cotton field that was sprayed with tribufos by a rig (Wilson *et al.*, 1980). Dermal exposure was estimated by measuring residues on mylar sheets placed next to the hens. The estimated dermal exposure ranged from 0.0092 µg/cm^2 in unsprayed rows of cotton to 47.8 µg/cm^2 on the rig near the hens exposed for one day. The dermal exposure for hens exposed daily in treated rows for a week was estimated to be 108 µg/cm^2. Air concentrations of tribufos were also measured and ranged from 0.111 mg/m^3 in untreated rows to 13.8 mg/m^3 near the rig. None of the hens exhibited ataxia or other signs of OPIDN.

Rat Studies

Acute

Eighteen Wistar rats/sex/dose were administered a single dose of tribufos (98.5%) by oral gavage in corn oil at 0, 2, 20 or 100 mg/kg (Sheets and Gilmore, 2000). Six rats/sex/dose were used for cholinesterase measurements 6 hours after dosing (time to peak effect). Clinical signs were observed in rats at 100 mg/kg, including reduced activity, salivation, ataxia, tremors, body cool-to-touch, diarrhea, abnormal urine color, clear lacrimation, and stains. Only perianal and urine stains were observed at 20 mg/kg. The main study animals were tested in the FOB one week prior to dosing, 6 hours, 7 days and 14 days after dosing. Similar neurobehavioral effects were observed in the FOB at the time to peak effect at 100 mg/kg (Table 15). Additional effects observed at 100 mg/kg in the FOB include abnormal posture, clear oral stain, reduced number of rears, uncoordinated righting response, and reduced body temperature. Only reduced auditory response (1F) and reduced activity in open field (1M) were seen at 20 mg/kg. Motor and locomotor activity was also reduced at the time to peak effect in the figure-8 maze at

Table 15. Neurological Effects in the Rats Administered a Single Oral Dose of Tribufos[a]

	Dose Level (mg/kg)			
	0	2	20	100
MALES				
Home Cage Decreased activity	0	0	0	2
Handling Clear lacrimation	0	0	0	1
Clear oral stain	0	0	0	1
Open Field Posture - sitting/lying	0	0	0	1
- lying flattened	0	0	0	1
Arousal - inactive	0	0	0	1
- sluggish, minimal	0	0	1	1
- sluggish, some exploration	10	8	10	10
Rearing	0.7±1.4	1.2±1.9	1.1±1.2	0.0±0.0
Reflex/Physiologic Uncoordinated righting reflex	0	0	0	0
Reduced auditory response	0	0	0	1
Body temperature	37.3±0.3	37.2±0.3	37.1±0.4	35.4±1.4
Motor activity	313±112	300±181	176*±92	98*±79
Locomotor activity	179±81	188±108	109±63	51±40
FEMALES				
Home Cage Decreased activity	0	0	0	4*
Handling Clear lacrimation	0	0	0	1
Clear oral stain	0	0	0	0
Open Field Posture - sitting/lying	0	0	0	1
- lying flattened	0	0	0	3
Arousal - inactive	0	0	0	3
- sluggish, minimal	0	0	0	1
- sluggish, some exploration	3	7	4	6
Rearing	2.3±2.0	2.7±2.8	3.0±2.7	0.8±1.1
Reflex/Physiologic Uncoordinated righting reflex	0	0	0	1
Reduced auditory response	0	0	1	1
Body temperature	37.6±0.4	37.5±0.6	37.6±0.3	34.8*±2.9
Motor activity	321±142	305±96	296±63	131*±109
Locomotor activity	192±74	169±39	166±41	62*±53

a Sheets and Gilmore, 2000. Behavioral effects observed at time of peak effect, 6 hours after dosing. Twelve rats/sex/dose were submitted to functional observational battery and motor/locomotor activity tests.

* Significantly different from controls at $p < 0.05$ based on either categorical modeling and an analysis of contrasts (categorical data) or an analysis of variance and Dunnett's test (continuous data).

20 and 100 mg/kg. Plasma and RBC ChE activity were significantly reduced at the time to peak effect at 20 and 100 mg/kg in both sexes (Table 16). The brain ChE activity was only significantly reduced in females at 100 mg/kg. A comparison of the FOB effects and motor activity with the cholinesterase inhibition suggests that tribufos is acting more peripherally than centrally at least on an acute basis. This finding is important in that it stresses the importance of the need for peripheral ChE data or a surrogate for it (i.e., RBC ChE data). The NOEL was 2 mg/kg based on the reduced motor activity, FOB effects and reduced plasma and RBC ChE activity. DPR toxicologist found this study acceptable based on FIFRA guidelines.

Table 16. Cholinesterase Activity in Blood and Brain of Rats Administered a Single Oral Dose of Tribufos[a]

	Dose Level (mg/kg)			
	0	2	20	100
MALES				
Plasma ChE[b] (IU/ml)	0.44±0.06 (100%[b])	0.44±0.05 (100%)	0.16*±0.04 (36%)	0.07*±0.04 (16%)
RBC ChE (IU/ml)	1.04±0.24 (100%)	0.94±0.22 (90%)	0.61*±0.21 (59%)	0.20*±0.16 (19%)
Brain ChE (IU/g)	13.6±0.4 (100%)	13.9±0.3 (102%)	13.8±0.6 (101%)	13.1±0.8 (96%)
FEMALES				
Plasma ChE (IU/ml)	1.80±0.62 (100%)	1.13±0.38 (63%)	0.15$±0.05 (8%)	0.03$±0.03 (2%)
RBC ChE (IU/ml)	1.17±0.18 (100%)	1.12±0.12 (96%)	0.53*±0.20 (45%)	0.18*±0.13 (15%)
Brain ChE (IU/g)	13.5±0.5 (100%)	13.9±0.8 (103%)	13.1±0.5 (97%)	11.3*±1.1 (84%)

a Sheets and Gilmore, 2000. Cholinesterase activity measured in 6/rats/sex/dose at time to peak effect, 6 hours after dosing.
b ChE = cholinesterase
c Percent of control activity.
* Significantly different from controls based on the Dunnett's test ($p < 0.05$).
$ Significantly different from controls based on the Mann Whitney U-tests ($p < 0.05$)

Subchronic

In a subchronic neurotoxicity screening study, tribufos (98% purity) was fed to 18 Wistar rats sex/dose at 0, 2, 40 or 500 ppm (M: 0, 0.14, 2.89 or 36.8 mg/kg/day; F: 0, 0.17, 3.54 or 42.6 mg/kg/day) for 13 weeks (Sheets and Gilmore, 2001). Six rats/sex were used for ChE measurements. The remaining 12 rats/sex/dose were used for neurobehavioral observations. Of these remaining rats, 6 rats/sex/dose were submitted for histopathology. Significant body weight reductions occurred on days 14-91 at 500 ppm in both sexes (5-15%). Body weight were also significantly reduced (6%) in females on day 14 at 40 ppm. Food consumption was also significantly reduced in both sexes at 500 ppm (8-17%) at several time points during the study and in females on day 84 at 40 ppm (9%). Clinical signs were observed at 500 ppm on

several occasions including red nasal stain (1 male), perianal stain (1 male) and urine stains (2 females). Some effects in the FOB were considered treatment-related by the investigators, but were not statistically significant (Table 17). Statistically significant reductions in motor and locomotor activity were observed at 500 ppm in both sexes at 4, 8 and 13 weeks. Plasma and RBC ChE activity was significantly reduced in both sexes at 20 and 500 ppm at 4 and 13 weeks (Table 18). Brain ChE activity was significantly reduced in both sexes at 500 ppm at week 13. There was also a slight reduction (92% of controls) in females at 20 ppm at week 13 that was statistically significant. There were no treatment-related increases in ophthalmologic findings or gross pathological lesions. Bilateral retinal atrophy was observed in 4 males and all 6 females examined microscopically at 500 ppm. Unilateral retinal atrophy was observed in another male

Table 17. Neurological Effects in Rats Fed Tribufos in the Diet for 13 Weeks[a]

	Dose Level (ppm)			
	0	2	40	500
MALES				
Week 4 FOB[b] - Open field Arousal - sluggish, minimal	0	0	1	1
Motor activity	455±88	430±125	426±136	287*±75
Locomotor activity	292±82	267±55	258±94	131*±43
Week 8 Motor activity	358±95	336±65	405±85	269*±96
Locomotor activity	215±65	217±48	236±62	124*±42
Week 13 Motor activity	315±96	276±70	302±66	250±76
Locomotor activity	176±64	163±48	167±54	120*±33
FEMALES				
Week 4 FOB - Open Field Rearing	10.3±3.5	11.0±4.7	10.7±3.1	8.3±2.6
Motor activity	546±148	622±148	601±183	353*±70
Locomotor activity	356±103	410±124	363±124	169*±39
Week 8 FOB - Handling Urine stain	0	0	0	1
Motor activity	500±123	494±119	535±163	343*±68
Locomotor activity	311±97	323±84	336±116	159*±33
Week 13 FOB - Handling Urine stain	0	0	0	2
Motor activity	458±141	531±140	450±113	324*±41
Locomotor activity	281±102	313±86	273±78	147*±28

a Sheets and Gilmore, 2001. Twelve rats/sex/dose were submitted to functional observational battery and motor/locomotor activity tests.

* Significantly different from controls at $p < 0.05$ based on either categorical modeling and an analysis of contrasts (categorical data) or an analysis of variance and Dunnett's test (continuous data).

Table 18. Cholinesterase Activity in Blood and Brain of Rats Fed Tribufos in the Diet for 13 Weeks[a]

	Dose Level (ppm)			
	0	2	40	500
MALES				
Week 4				
Plasma ChE[b] (IU/ml)	0.40±0.08 (100%[b])	0.44±0.04 (110%)	0.26*±0.04 (65%)	0.05*±0.02 (12%)
RBC ChE (IU/ml)	0.89±0.07 (100%)	0.79±0.33 (89%)	0.33$±0.06 (37%)	0.06$±0.07 (7%)
Week 13				
Plasma ChE (IU/ml)	0.54±0.14 (100%)	0.49±0.06 (91%)	0.33*±0.05 (61%)	0.07*±0.04 (13%)
RBC ChE (IU/ml)	0.83±0.18 (100%)	1.07*±0.20 (128%)	0.34*±0.08 (41%)	0.14*±0.13 (17%)
Brain ChE (IU/g)	12.1±0.7 (100%)	11.9±0.4 (98%)	12.2±0.5 (101%)	4.5*±1.0 (37%)
FEMALES				
Week 4				
Plasma ChE (IU/ml)	2.00±0.58 (100%)	1.89±0.48 (94%)	0.44$±0.04 (22%)	0.04$±0.02 (2%)
RBC ChE (IU/ml)	1.00±0.15 (100%)	0.86±0.31 (86%)	0.28*±0.12 (28%)	0.07*±0.09 (7%)
Week13				
Plasma ChE (IU/ml)	3.49±1.11 (100%)	2.99±0.86 (86%)	0.57$±0.09 (16%)	0.06$±0.07 (2%)
RBC ChE (IU/ml)	1.19±0.24 (100%)	0.94±0.19 (96%)	0.47*±0.33 (45%)	0.13*±0.12 (15%)
Brain ChE (IU/g)	12.8±0.5 (100%)	12.6±0.5 (98%)	11.8*±0.3 (92%)	3.3*±0.4 (26%)

a Sheets and Gilmore, 2001. Cholinesterase activity measured in 6 rats/sex/dose at time to peak effect, 6 hours after dosing.
b ChE = cholinesterase
c Percent of control activity.
* Significantly different from controls based on the Dunnett's test ($p < 0.05$).
$ Significantly different from controls based on the Mann Whitney U-tests ($p < 0.05$)

at 500 ppm. The study NOEL was 2 ppm (M: 0.14 mg/kg/day; F: 0.17 mg/kg/day) based on plasma, RBC and brain ChE inhibition. This study was found acceptable by DPR toxicologists based on the FIFRA guidelines.

A developmental neurotoxicity study was conducted in which approximately 30 Wistar Crl:W(HAN)BR female rats/dose were fed tribufos (98.0-98.1% purity) in the diet at 0, 4, 40 and 200 ppm (gestation: 0, 0.4, 3.5 and 17.4 mg/kg/day; lactation: 0, 0.8, 8.1 and 42.4 mg/kg/day) from gestation day 0 through to lactation day 21 (Lake, 2001). Dams were sacrificed on lactation day 21, but pups were kept on the study on untreated feed from day 21 to study termination (day 75) approximately 7 weeks later. Approximately 10 females/dose were assigned to a satellite group for ChE activity measurements. A reduced gestation index (number of dams with live pups relative to the number pregnant) at 200 ppm (67.9%) relative to

controls (85.2%) was considered treatment-related by the investigators, although it was not statistically significant. There were no treatment-related deaths during gestation or lactation. Tremors were observed in 5 females at 200 ppm at parturition. There was no treatment-related effects observed in dams during an abbreviated functional observational battery (FOB) conducted on gestation days 6, 13 and 20 and lactation days 4, 11 and 21. Significant reductions in maternal body weights (8-12%) occurred during lactation at 200 ppm. On lactation day 21, the plasma, RBC and brain ChE activity were all significantly reduced at 40 ppm (34%, 24% and 78% of controls, respectively) and 200 ppm (12%, 13% and 26% of controls, respectively).The maternal NOEL for this study appears to be 4 ppm (0.4 mg/kg/day) based on the blood and brain ChE inhibition at 40 ppm.

Treatment-related clinical signs were observed in pups during lactation at 200 ppm, including weakness (4), wound or cut (5), and no milk in stomach (2). No treatment-related clinical signs were observed after weaning. Several developmental landmarks were delayed in pups relative to controls at 200 ppm, including surface righting and preputial separation. Reduced pup body weights (16-22%) were observed during lactation at 200 ppm. Body weights after weaning were also reduced at 200 ppm (M: 12-21%; F 8-20%). During the first 2 weeks after weaning food consumption was reduced at 200 ppm (M: 11-19%; F: 8%). There were other occasions where statistical differences in food consumption were seen in various treatment groups, but they were considered incidental and unrelated to treatment by the investigators since the differences were marginal and/or not dose-related. Subsets of 16 pups/sex/dose (only one male and/or female per litter) were subjected to various neurobehavioral tests. No significant difference in motor and locomotor activity was seen in Set A pups tested on postnatal day (PND) 13, 17, 21, and 60. A significant reduction in acoustic startle response (43%) was observed in Set B pups at 200 ppm tested on PND 22, but not on PND 38 and 60. There was no difference from controls in Set C pups in the passive avoidance test on PND 22 and 29, in the water maze on PND 60 and in an abbreviated FOB on PND 4, 11, 21, 35, 45 and 60. No treat-related lesions were found in 10 pups/sex/dose from Sets A-C subjected to ophthalmology (PND 50-50), and electroretinography (ERG) (PND75). Ten satellite pups/sex/dose were used to provide samples for ChE activity measurements and brain tissues. At 200 ppm, significant reductions were seen in brain ChE activity (F: 94% of controls) on PND 11 and in plasma ChE activity (M: 79%; F: 78% of controls) on PND 21. No significant difference was found in microscopic brain lesions (PND 21). The anterior-to-posterior lengths of the cerebrum and the cerebellum were reduced at 200 ppm on PND 11, but not PND 21 or 75. There was a significant reduction in absolute brain weights on PND 11 and 21 (28% and 21%, respectively); however, relative brain weights were significantly higher at both ages since reductions in body weights exceeded reductions in brain weights. Absolute and relative brain weights were not significantly different in pups at PND 75. The pup NOEL for overt toxicity was 40 ppm (3.5 mg/kg/day) based on clinical signs, delayed development, reduced body weights and food consumption, reduced acoustic startle response, plasma and brain ChE inhibition, and reduced brain weights and size. The pup NOEL for RBC ChE inhibition was equal to or greater than 200 ppm (17.4 mg/kg/day), the highest dose tested. DPR toxicologists found this study acceptable based on FIFRA guidelines.

Human Studies

Kilgore *et al.* (1984) conducted a study with pesticide workers before and after a 7-week exposure period to tribufos in which medical examinations and neuro-psychological tests were performed. No significant effects were found including cholinesterase inhibition. Another worker exposure study was conducted by Lotti *et al.* (1983) in which pesticide workers were monitored before and after the normal use season. No differences were detected between pre- and post-exposure electromyographs and nerve conduction tests. The whole blood and plasma

ChE levels were all within 25% of pre-exposure levels. However, the lymphocyte NTE activity was reduced to between 40 and 60% of pre-exposure levels between days 25 and 30 of exposure. Neither of these studies provided sufficient information to accurately estimate total tribufos exposure.

IV. RISK ASSESSMENT

A. HAZARD IDENTIFICATION

Acute Toxicity

The effects observed in experimental animals after acute exposure to tribufos are summarized in Table 19. In addition to the effects observed in the LD_{50}/LC_{50} studies and the acute neurotoxicity studies, some findings observed in the 90-day inhalation, 21-day dermal and developmental toxicity studies were also considered as acute effects. These include maternal signs of toxicity observed within the first few days of exposure and fetal effects that could be the result of one or two days of exposure, such as pre- and post-implantation losses, and skeletal and visceral malformations. The clinical signs observed after acute exposure to tribufos were primarily neurological. Cholinergic signs were seen in most laboratory animals after acute exposure to tribufos by various routes. Hypothermia was observed in rats, mice and guinea pigs when tribufos was administered by the oral, intraperitoneal and intravenous route (Ray, 1980; Ray and Cunningham, 1985). The investigators suggested a selective action on a central thermogenic control process may be involved. Other research indicates that the hypothermia associated with organophosphates is due to central AChE inhibition because hypothermia is antagonized by centrally active anti-ChE drugs, such as atropine, but not by peripherally active anti-ChE drugs, such as 2-PAM (Kenley *et al.*, 1982).

In general, DPR considers brain ChE inhibition to be indicative of overt toxicity since it is one of the primary functional target sites and more subtle central neurological signs, such as memory and learning losses, may not be easily detected in animals unless they are specifically tested for these effects. The toxicological significance of plasma and RBC ChE inhibition is less certain because the physiological function of ChEs in blood have not been clearly established, although several possible physiological functions have been proposed. As mentioned in the Introduction, plasma ChE, or more specifically butyrylcholinesterase (BuChE), may be involved in the binding/metabolism of certain drugs, such as succinylcholine, which suggests that its inhibition may compromise an organism's ability to defend against subsequent toxic insults (Lockridge and Masson, 2000). BuChE is also the predominant form of ChE in the developing nervous system of birds and mammals (Brimijoin and Koenigsberger, 1999). Due to the expression of AChE in several types of hematopoietic cell lines, it has been proposed that circulating AChE may be important in erythropoiesis (Grisaru *et al.* 1999). ACh analogs and AChE inhibitors have been reported to increase platelet production in mice. U.S. EPA does not consider plasma or RBC ChE inhibition an adverse effect in itself, but does use it as a surrogate for peripheral ChE inhibition (U.S. EPA, 2000b). However, it is unclear how representative plasma or RBC ChE activity is of peripheral ChE activity. Plasma ChE is primarily BuChE which is a different enzyme than the enzyme, AChE, that is involved in neurotransmission. As a result, ChE inhibitors can have different affinities for the active sites of BuChE and AChE. The ChE in RBCs is AChE, but RBCs lack the ability to synthesize new AChE (Brimijoin, 1992). The recovery of RBC ChE activity is dependent on the replacement of RBCs, and, consequently, is much slower than in neurological and neuromuscular tissue. The Joint Meeting on Pesticide Residues of the FAO/WHO concluded only RBC ChE activity at the time of peak effect with acute exposure should be used as a surrogate for peripheral ChE activity (JMPR, 1999). In humans, where brain ChE activity is not available, plasma or RBC ChE inhibition can be used as a regulatory endpoint.

Table 19. Acute Adverse Effects of Tribufos and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL mg/kg	LOEL mg/kg	Ref.[a]
		Inhalation[b]			
Rat	Single, 4-hr, nose only	Death, cholinergic signs, red turbinates, firm zones in lungs	------	254	1*
Rat	Single, 4-hr, nose only	Decreased preening, lethargy	12.3	20.8	2
Rat	13 weeks, 6 hr/day, 5 day/wk	Reduced motility, bradypnea, piloerection, ungroomed coat, vocalization, irregular breathing, increased startle response (onset days 1-3)	2.9	14.3	3*
Hen	Single, 4-hr	Leg weakness, drowsiness, inactivity, breathing disorders	------	43	4
	5 Days, 4-hr	Ataxia	6.8	16	
		Intraperitoneal			
Rat	Single, injection	Hypothermia	------	20	5
		Oral			
Rat	Single, gavage	Cholinergic signs	------	192	6*
Rat[c]	**Single, gavage**	**Reduced motor activity, neurobehavioral effects, plasma (8-36%) and RBC (45-59%) ChE[d] inhibition**	**2**	**20**	**7***
Rat[e]	9 Days, gavage	Excessive salivation (onset day 3)	7	28	8*
Hen	Single, gavage	Death, unspecified toxic effects	50	100	9
Hen	Single, capsule	"Late acute" effects, ataxia	50	100	10
Hen	Single, capsule	Ataxia, peripheral demyelination	------	100	11
		Subcutaneous			
Hen	Single, injection	Ataxia, paralysis	200	1060	12
Hen	Single, injection	Ataxia	220	1010	13
		Dermal			
Rabbit	Single, 24-hr	Cholinergic signs, erythema	------	500	14*
Rabbit	6 hr/day, 5 day/wk, 3 weeks	Muscle fasciculations (onset day 2)	11	29	15*
Hen	Single, 24-hr	Impaired general health, ataxia, paralysis	500	1000	16
Hen	Single	Brain ChE inhibition, ataxia (nerve degeneration at 1,000 mg/kg)	------	400	10
Hen	Single	Ataxia, paralysis	100	250	17
Hen	Single	Ataxia, paralysis, cholinergic signs	100	250	11

a References: 1. Warren, 1990; 2. Thyssen, 1978a; 3. Pauluhn, 1992; 4. Thyssen and Schilde, 1976a; 5. Ray, 1980; 6. Sheets, 1991a; 7. Sheets and Gilmore, 2000; 8. Kowalski *et al.*, 1986; 9. Thyssen, 1976; 10. Abou-Donia *et al.*, 1979a; 11. Abou-Donia *et al.*, 1984; 12. Johnson, 1970a; 13. Johnson, 1970b; 14. Sheets and Phillips, 1991; 15. Sheets *et al.*, 1991; 16. Thyssen and Schilde, 1976b; 17. Abdo *et al.*, 1983a.
b Estimated assuming a respiratory rate of 0.16 and 0.11 m^3/kg/4 hrs for a rat and hen, respectively.
c Neurotoxicity study
d ChE = cholinesterase. Inhibition is expressed as percent of control activity
e Developmental toxicity study: All fetal effects were considered acute effects; however, only maternal effects observed within the first few days of exposure were considered acute effects.
* Acceptable study based on FIFRA guidelines.

Organophosphate-induced delayed neuropathy (OPIDN) was observed in hens in the form of ataxia, paralysis, and nerve degeneration approximately 2-3 weeks after acute exposure to tribufos by inhalation, oral, subcutaneous or dermal routes (Thyssen and Schilde, 1976a; Abou-Donia *et al.*, 1979a & 1984; Johnson, 1970a & b; Thyssen and Schilde, 1976b; Abdo *et al.*, 1983a). There was no evidence of tribufos-induced delayed neuropathy in other species tested; however, rodents generally are less susceptible to OPIDN (Abou-Donia, 1981; Somkuti *et al.*, 1988; De Bleeker *et al.*, 1992). Sensitivity in rodents appears to vary depending on strain and age (Padilla and Veronesi, 1988; Veronesi *et al.*, 1991; Moretto *et al.*, 1992; Inui *et al.*, 1993).

Other effects described as "late acute" effects were also seen in hens; however, the late acute effects were only observed with oral exposure (Abou-Donia *et al.*, 1979a & 1984). Abou-Donia and coworkers attributed these late acute effects to nBM which was found in the excreta of hens after oral exposure, presumably from the hydrolysis of tribufos in the gut. In subsequent studies, it was shown that in hens nBM causes RBC deformation and lysis through the inhibition of glucose-6-phosphate dehydrogenase (Abdo *et al*, 1983b). Clinical signs similar to the late acute effects in hens have not been described in other species administered tribufos, but changes in RBC morphology were seen in rabbits administered tribufos (route not indicated) at 242 mg/kg (Mirakhmedov *et al.*, 1989). In addition, mice, rats, and dogs had reductions in RBC counts, hematocrits and hemoglobin after long-term exposure to tribufos in the diet (Hayes, 1989; Christenson, 1991; Christenson, 1992). Similar hematological changes were also seen at the termination of a 90-day inhalation study in rats, presumably from either degradation of tribufos in the chamber or normal tissue metabolism of tribufos (Pauluhn, 1992).

After acute oral exposure in hens, OPIDN occurred at approximately the same dose levels or higher than the cholinergic and late acute effects. However, the distinction between the cholinergic, late acute, and delayed neurotoxic effects was blurred in some hen studies because some effects such as leg weakness and unsteadiness were common to all syndromes and could only be separated based on their time of onset. There is also some uncertainty about the dose levels at which cholinergic signs occurred because many of the hen studies provided insufficient information about the incidence of cholinergic effects to accurately determine a NOEL. In mammals, cholinergic signs were the primary effects observed after acute exposure to tribufos; however, the dose levels were too high in the standard LD_{50}/LC_{50} tests to establish a NOEL. Acute NOELs were established for tribufos in 4 studies that met FIFRA guidelines (Pauluhn, 1992; Sheets and Gilmore, 2000; Kowalski *et al.*, 1986; Sheets *et al.*, 1991). The lowest NOEL, 2 mg/kg, was observed in the acute neurotoxicity study in rats based on reduced motor activity, neurobehavioral effects observed in the functional observational battery (sluggishness and reduced auditory response), and reduced ChE activity in the plasma (M: 36%; F: 8% of controls) and RBCs (M: 59%; F: 45% of controls) (Sheets and Gilmore, 2000). The acute neurotoxicity study in rats was selected for characterizing the risk for adverse health effects from acute occupational and dietary exposure to tribufos.

Mild dermal and ocular irritation were observed with exposure to technical grade tribufos. However, the formulation caused severe eye irritation and was corrosive to the skin (Crawford and Anderson, 1972a; Sheets and Fuss, 1991; Sheets and Phillips, 1992a). A NOEL for the dermal irritation was estimated to be 8.3 mg formulation/cm^2 by dividing the amount of tribufos applied to the site (0.5 ml per 6 cm^2) by an uncertainty factor of 10 to extrapolate from a LOEL to a NOEL. This critical NOEL was selected for characterizing the risk for local effects from acute dermal exposure to tribufos formulations.

Subchronic Toxicity

The effects of subchronic exposure to tribufos in experimental animals are summarized in Table 20. Included in this summary are some maternal effects observed in the developmental toxicity studies after the first few days of exposure, and all effects observed in the developmental neurotoxicity and reproductive toxicity studies. Not included in this table were two neurotoxicity studies in which hens were given daily intraperitoneal injections at 50 and 100 mg/kg for 5 to 15 days (Casida *et al.*, 1963; Baron and Johnson, 1964). Baron and Johnson (1964) also observed OPIDN in hens administered tribufos by oral gavage at 50-150 mg/kg/day for 5 to 15 days. These two studies were not used because NOELs were not established, and they had major deficiencies including inadequate number of animals and inconsistent exposure periods within and between dose levels. Also, not included was a 3-month feeding study in rats and dogs (Root and Doull, 1966). A NOEL of 5 ppm (0.25 and 0.125 mg/kg/day for rats and dogs, respectively) was reported for both species, but the effects seen at the LOEL were not reported. This study had other major deficiencies such as no summary of body weights, food consumption, hematology, clinical chemistry or pathological lesions.

Signs of OPIDN were the most frequent effects seen in hens and usually occurred at lower doses than the cholinergic signs, regardless of the route of exposure. In mammals, ChE inhibition and cholinergic signs were some of the more sensitive endpoints. Other effects observed in rodents with subchronic exposure include reduced weight gain, reduced food consumption, hematological changes, impaired retinal function, pale retinal fundus, bilateral retinal atrophy, fatty droplets in the adrenal cortex, and increased adrenal weights. Several reproductive effects were observed in a 2-generation rat reproductive toxicity study including a reduction in fertility, birth and viability indices, an increase in gestation length, reduced pup weights, cannibalism of pups, and discolored pup livers. Although the reproductive toxicity of tribufos was only examined with oral exposure, it was assumed that these effects would occur with exposure by any route. Dermal irritation was also observed with dermal exposure.

The lowest subchronic NOEL, 0.1 mg/kg/day, was reported in a neurotoxicity study in hens in which mild ataxia was observed at 0.5 mg/kg/day when tribufos was administered in capsules for 90 days (Abou-Donia *et al.*, 1979b). It is unclear if the ataxia with oral exposure was due to tribufos or nBM which can be formed in the gastrointestinal tract of hens from the hydrolysis of tribufos (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). There was limited histological evidence of OPIDN with oral exposure even at high doses. By contrast, similar doses of tribufos administered by the dermal route produced clear evidence of OPIDN. Hens as 20 mg/kg/day and higher had severe ataxia and paralysis and died within the first few weeks from late effects attributed to nBM. The limited histological evidence of OPIDN with oral exposure, suggests that the ataxia and paralysis is due to nBM rather than tribufos. Abou-Donia and others have reported that nBM causes incoordination, muscle weakness, paralysis, CNS depression and cyanosis in rats and/or hens (Fairchild and Stokinger, 1958; Abou-Donia *et al.*, 1979a). If sufficient amounts of tribufos are degrading to nBM with oral exposure to kill hens at 20 mg/kg/day and higher, then less tribufos should be available to produce OPIDN. Less weight was also given to the Abou-Donia *et al.* (1979b) study because of the lack of detail in the published report regarding the incidence and duration of clinical signs, body weight changes, and histopathological lesions. The study also did not meet FIFRA guidelines with respect to the number and age of animals and analysis of the test article. It is difficult to interpret the findings of this study without more information, especially considering the NOEL is an order of magnitude lower than the NOELs for any other subchronic neurotoxicity study in hens, including one which met FIFRA guidelines (Sheets, 1991b).

Table 20. Subchronic Adverse Effects of Tribufos and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL mg/kg/day	LOEL mg/kg/day	Ref.[a]
		Inhalation[b]			
Rat	6 hr/day, 5 day/wk, 3 weeks, nose only	RBC (73-76%) and brain (73%) ChE[c] inhibition, decreased preening, lethargy, inflammation in lung	1.7	7.7	1
		Plasma ChE inhibition (52-64%)	0.5	1.7	
Rat	6 hr/day, 5 day/wk, 2 weeks, nose only	Cholinergic signs, plasma (13-42%) and brain ChE inhibition (61%)	3.2	15	2
		RBC ChE inhibition (62%)	0.6	3.2	
Rat	6 hr/day, 5 day/wk, 13 weeks, nose only	Cholinergic signs, hematological changes, brain ChE inhibition (60%), impaired retinal function, pale retinal fundus, fatty droplets in adrenals, inc. adrenal wts.	2.9	14.3	3*
		Plasma (60%) and RBC (35-36%) ChE inhibition	0.6	2.9	
Hen	6 hr/day, 5 day/wk, 3 weeks	Dec. preening, lethargy, ataxia, paralysis, nerve degeneration	3.6	14.3	4
		Oral			
Mouse	8 weeks, feed	Brain ChE inhibition (74%)	40	140	5
		RBC ChE inhibition (56-63%)	3.4	40	
		Plasma ChE inhibition (29-36%)	—	3.4	
Rat[d]	**13 weeks, feed**	**Plasma (16-65%), RBC (28-45%) and brain ChE inhibition (92%)**	**0.14**	**2.89**	**6***
Rat[e]	9 days, gavage	Maternal: Red. weight gain and brain ChE inhibition (54%)	7	28	7*
		Plasma (42%) and RBC (29%) ChE inhibition	1	7	
Rat[f]	6 weeks, feed	Maternal: Plasma (34%), RBC (24%) and brain (78%) ChE inhibition	0.4	3.5	8*
		Pup: Clin. signs, delayed develop., red. body wts. & food consump., red. startle resp., plasma (78-79%) & brain (94%) ChE inhibition, red. brain wts. & size	3.5	17.4	
		Pup: RBC ChE inhibition	17.4	—	
Rat[g]	Diet, 2-gen., 10 wk/gen.	Parental: Brain ChE inhibition (F:71%)	0.4	3.0	9*
		Plasma (7-32%) and RBC (88-93%) ChE inhibition	—	0.4	
		Reproductive: Reduced fertility, birth and viability indices, increased gestation length, reduced pup weights, cannibalism of pups, discolored pup livers, plasma (36-64%), RBC (62-77%), and brain (85%) ChE inhibition	3.0	24.7	

Table 20 (cont.). Subchronic Adverse Effects of Tribufos and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL mg/kg/day	LOEL mg/kg/day	Ref.[a]
		Oral (cont.)			
Rabbit[e]	12 days, gavage	Maternal: Reduced weight gain	3	9	10*
		Plasma (60%) & RBC (30%) ChE inhibition	—	1	
Hen	30 days, feed	Focal liquefaction of brain	34	87	11
Hen	30 days, feed	Reduced food consumption, neurohistological lesions	6.1	10.9	12
Hen	90 days, capsule	Ataxia	0.1	0.5	13
Hen	91-97 days, capsule	Death, ataxia, paralysis	5-6	38-40	14
		Dermal			
Rabbit	6 hr/day, 5 day/wk, 3 weeks	Muscle fasciculations, brain ChE inhibition (85-86%), skin lesions	2	11	15*
		Plasma (82-89%) and RBC (80-89%) ChE inhibition	—	2	
Hen	6 hr/day, 5 day/wk, 3 weeks	Ataxia, paralysis	~30	~100	16
Hen	90 days	Ataxia	—	20	13
Hen	91-101 days	Ataxia, dermal irritation	—	6-8	14
Hen	13 weeks, 5 day/wk	Axonal degeneration	2.6	11	17*

a References: 1. Thyssen, 1978b; 2. Pauluhn, 1991; 3. Pauluhn, 1992; 4. Thyssen and Schilde, 1978a; 5. Hayes, 1985; 6. Sheets and Gilmore, 2001; 7. Kowalski *et al.*, 1986; 8. Lake, 2001; 9. Eigenberg, 1991a; 10. Clemens *et al.*, 1987; 11. Harris, 1965; 12. Thyssen *et al.*, 1977; 13. Abou-Donia *et al.*, 1979b; 14. Hansen *et al.*, 1982; 15. Sheets *et al.*, 1991; 16. Thyssen and Schilde, 1978b; 17. Sheets, 1991b.
b Estimated assuming a respiratory rate of 0.24 and 0.17 m^3/kg/6 hrs for a rat and hen, respectively.
c ChE = cholinesterase. Inhibition is expressed as percent of control activity.
d Neurotoxicity study
e Developmental toxicity study: Only maternal effects observed after the first few days of exposure were included.
f Developmental neurotoxicity study.
g Reproductive toxicity study
* Acceptable study based on FIFRA guidelines

The next lowest subchronic NOEL, 0.14 mg/kg/day, was observed in the subchronic neurotoxicity study in rats (Sheets and Gilmore, 2001). Urine stains, reduced motor and locomotor activity and bilateral retinal atrophy were observed at the high dose in this study. Effects observed at the LOEL included reduced ChE activity in the plasma (M: 61-65%; F: 16-22% of controls), RBCs (M: 37-41%; F: 28-45% of controls) and brain (F: 92% of controls). This study was found acceptable to DPR based on FIFRA guidelines. Similar LOELs for brain ChE inhibition were observed in two other rat studies with slightly higher NOELs, a developmental neurotoxicity study and a reproductive toxicity study (Lake, 2001; Eigenberg, 1991a). The LOEL for plasma and RBC ChE inhibition in the reproductive toxicity study was lower than in the developmental toxicity study probably due the longer exposure period. The blood ChE LOEL in the reproductive toxicity study is also lower than in the subchronic neurotoxicity study, but this may be a product of dose selection. There were gender-related differences in ChE activity in the reproductive study which were most pronounced at the terminal sacrifice. The most likely explanation for these gender-related differences was the higher compound consumption in females during lactation. During lactation, the average compound consumption for females in both generations was approximately twice as high as their consumption during premating and

gestation (0.7, 5.5, and 39.2 mg/kg/day at 4, 32, and 260 ppm, respectively). The gender-related differences in ChE activity were not as pronounced in the subchronic neurotoxicity study in rats where the compound consumption was more similar between sexes at the LOEL, 40 ppm (M: 2.89 mg/kg/day; F; 3.54 mg/kg/day). The subchronic neurotoxicity study was selected at the definitive study for evaluating the occupational exposure to tribufos since it had the lowest NOEL in an acceptable guideline study with the least uncertainty about the compound consumption and the most thorough evaluation of the neurotoxic potential of tribufos.

Chronic Toxicity

In all of the available chronic toxicity/oncogenicity studies, tribufos was administered to animals in their feed. Evidence of overt toxicity was seen in all of these chronic toxicity/ oncogenicity studies (Table 21). Reduced weight gain was observed in two rat chronic toxicity studies at 100 ppm (5 mg/kg/day) and 320 ppm (M: 16.8 mg/kg/day; F: 21.1 mg/kg/day) (Root *et al.*, 1967;Christenson, 1992). The mean brain ChE activity was significantly reduced in males of the mouse oncogenicity study at 10 ppm (1.5 mg/kg/day - 91% of controls) and in two rat chronic feeding study at 100 ppm (F: 5 mg/kg/day - 69% of controls) and 320 ppm (M: 16.8 mg/kg/day - 40% of controls; F: 21.1 mg/kg/day - 32% of controls) (Hayes, 1989; Root *et al.*, 1967; Christenson, 1992). The toxicological significance of the reduced brain ChE activity at 10 ppm in male mice is uncertain because no cholinergic signs were observed at 50 ppm where brain ChE activity in males was reduced to 87% of control activity. Only mild signs (loose stools and perianal stains) were observed at 250 ppm where brain ChE activity was reduced in males to 62% of controls. Hematological changes (reduced RBCs, hemoglobin and hematocrits) were seen in female mice at 50 ppm (11.3 mg/kg/day), in rats of both sexes at 40 and 320 ppm (M: 1.8 and 16.8 mg/kg/day; F: 2.3 and 21.1 mg/kg/day, respectively), and in female dogs at 64 ppm (2.0 mg/kg/day) (Hayes, 1989; Christenson, 1991; Christenson, 1992).

Table 21. Chronic Adverse Effects of Tribufos and Their Respective NOELs and LOELs

Species	Exposure	Effect	NOEL mg/kg/day	LOEL mg/kg/day	Ref.[a]
Mouse	Diet, 90 weeks	Plasma (33-35%), RBC (82%), and brain (M:91%) ChE[b] inhibition	---	1.5	1*
Rat	Diet, 2 years	Liver cytoplasmic vacuolation, reduced weight gain, brain ChE inhibition (F:69%)	1.25	5.0	2
		Plasma (65-69%) and RBC (42-53%) inhibition	0.25	1.25	
Rat	Diet, 2 years	Hyperplasia and vacuolar degeneration in small intestine, hematological changes, plasma (40-44%) & RBC ChE inhibition (72-73%)	0.2	1.8	3*
Dog	**Diet, 1 year**	**Hematological changes (F), RBC ChE inhibition (84-87%)**	**0.4**	**2.0**	**4***
		Plasma ChE inhibition (M:67%)	**0.1**	**0.4**	

a References: 1. Hayes, 1989; 2. Root *et al.*, 1967; 3. Christenson, 1992; 4. Christenson, 1991.
b ChE = cholinesterase. Inhibition expressed as percent of control activity.
* Acceptable study based on FIFRA guidelines

Transient hypothermia was also observed in one rat chronic toxicity study at 40 and 320 ppm (M: 1.8 and 16.8 mg/kg/day; F: 2.3 and 21.1 mg/kg/day, respectively) (Christenson, 1992).

There were dose-related increases in microscopic lesions in several studies. In a mouse oncogenicity study, an increase in non-neoplastic lesions in the gastrointestinal tract (small intestine vacuolar degeneration, dilated/distended small intestine and cecum, and rectal necrosis/ulceration), liver (hypertrophy), adrenal glands (degeneration/ pigmentation), and spleen (hematopoiesis) was observed at 250 ppm (M: 48.1 mg/kg/day; F: 63.1 mg/kg/day) (Hayes, 1989). The incidence of small intestine vacuolar degeneration and spleen hematopoiesis was also significantly higher in mice at 50 ppm (M: 8.4 mg/kg/day; F: 11.3 mg/kg/day). Increases in several pre-neoplastic lesions in the small intestine of both sexes (mucosal hyperplasia and focal atypia) and the lungs of females (epithelialization and focal hyperplasia) were also observed at 250 ppm (M: 48.1 mg/kg/day; F: 63.1 mg/kg/day). Liver cytoplasmic vacuolation was observed in a rat chronic feeding study at 100 ppm (5 mg/kg/day) (Root *et al.*, 1967). Numerous ocular effects were seen in another rat chronic feeding at 320 ppm (M: 16.8 mg/kg/day; F: 21.1 mg/kg/day) including corneal opacity, lens opacity, cataracts, corneal neovascularization, iritis, uveitis, bilateral flat ERG responses, bilateral retinal atrophy, and optical nerve atrophy (Christenson, 1992). In addition, increased adrenal weights and adrenal vacuolar degeneration were observed at 320 ppm. Vacuolar degeneration of the small intestine was seen in animals exposed for one or two years at both 40 and 320 ppm (M: 1.8 and 16.8 mg/kg/day; F: 2.3 and 21.1 mg/kg/day, respectively). Mucosal hyperplasia was also observed at 40 and 320 ppm, but only in animals exposed for two years.

Plasma and/or RBC ChE inhibition was often observed at lower dose levels than the other endpoints that were more clearly indicative of overt toxicity. The lowest LOEL established for plasma ChE inhibition was 4 ppm (0.2 mg/kg/day) in the acceptable 2-year rat study (Christenson, 1992). However, the plasma ChE activity was reduced by less than 20% in both sexes (M: 84%: F: 94% of controls). Even taking into consideration the possible role of plasma ChE in the protection against subsequent toxic insults this minor reduction was not considered to be biologically significant. At 40 ppm (1.8 mg/kg/day), the reduction in plasma ChE activity was more pronounced (M: 44%; F: 40% of controls). RBC ChE activity was also significantly reduced (M: 73%; F: 72% of controls) at this dose level. The LOEL for blood ChE inhibition in this rat study is similar to the LOEL for blood ChE inhibition in the mouse oncogenicity study when expressed as mg/kg/day (1.5 mg/kg/day or 10 ppm) with similar levels of inhibition. A slightly lower LOEL for plasma ChE inhibition (0.4 mg/kg/day) was observed in the 1-year dog study with a reduction to 67% of controls. The plasma inhibition at this dose level is of sufficient magnitude to raise concern about defense against subsequent toxic insult.

One of the more sensitive endpoints with chronic oral exposure to tribufos appears to be the hematological effects. There is evidence in hens that these hematological effects may be due to nBM which inhibits glucose-6-phosphate dehydrogenase (Abdo *et al.*, 1983b). In previous experiments, these investigators isolated nBM in the plasma and excreta of hens and proposed that it is the product of hydrolysis of tribufos in the gut (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). These investigators also found nBM in the plasma of hens administered tribufos dermally, although the concentration was an order of magnitude lower than when the same dose was given orally (Abou-Donia *et al.*, 1979a). Although the hematological changes may be more prevalent with the oral route of exposure, they do not appear to be unique to this route of exposure. nBM is an anticipated metabolite of tribufos through its normal metabolism in the liver of animals. Also, tribufos readily degrades to nBM in the environment. Slight reductions in RBC counts, hematocrits, and hemoglobin values were also seen in a 90-day inhalation study in rats (Pauluhn, 1992). The significance of these hematological effects in assessing the long-term human health risks from occupational exposure to tribufos is uncertain

because exposure is clearly seasonal and these hematological effects appear to be reversible. In the chronic feeding study in rats, some of the hematological values began to return to normal even with continued exposure (Christenson, 1992). In fact, the RBC count, hemoglobin and hematocrit values were higher in the high-dose animals than controls by the end of the 2-year exposure period.

Since occupational exposure to tribufos is only seasonal, the relevance of some of the chronic effects is questionable. The lesions in the small intestine, liver and spleen observed in animals after chronic oral exposure to tribufos were not observed in any of the subchronic studies, including the acceptable guideline studies like the 13-week inhalation study in rats, the subchronic neurotoxicity study in rats, the developmental neurotoxicity in rats, the reproductive toxicity study in rats, and the 3-week dermal toxicity study in rabbits (Pauluhn, 1992; Sheets and Gilmore, 2001; Lake, 2001; Eigenberg, 1991a; Sheets *et al.*, 1991). The lack of concordance in the histological findings between the subchronic and chronic studies could be due to the difference in either the duration or the route of exposure. Some of these histological lesions, like the vacuolar degeneration and mucosal hyperplasia in the small intestine, could be due to irritation from the degradation product of tribufos, nBM, which is known to form in the gut through hydrolysis. Consequently, it may only be relevant for the oral route of exposure. Other effects in the chronic studies, such as the hematological changes and ChE inhibition, appeared to be reversible, and will be addressed in evaluating seasonal occupational exposure.

Chronic dietary exposure to tribufos may be expected due to year-round consumption of cottonseed oil, meal or milk and meat containing secondary residues from livestock consuming cottonseed products. The lowest chronic NOEL was observed in an acceptable 1-year dog study based on reduced plasma ChE activity in males (67% of controls) (Christenson, 1991). The NOEL of 0.1 mg/kg/day from the 1-year dog study was similar to the NOEL from the subchronic neurotoxicity study in rats of 0.14 mg/kg/day (Sheets and Gilmore., 2001) based on plasma, RBC and brain ChE inhibition. The 1-year dog study was selected as the definitive study for evaluating the chronic dietary exposure to tribufos.

Weight of Evidence for Oncogenicity

There was no evidence of oncogenicity in a rat study where tribufos was administered in the feed for 2 years (Christenson, 1992). However, an increase in adenocarcinomas of the small intestine (both sexes), liver hemangiosarcomas (males only), and alveolar/bronchiolar adenomas (females only) was seen in mice fed tribufos for 90 weeks (Tables 9 and 10) (Hayes, 1989). The adenocarcinomas were often associated with vacuolar degeneration, mucosal hyperplasia and/or focal atypia of the small intestine. The liver hemangiosarcomas were often associated with hemorrhage and necrosis. The increase in alveolar/bronchiolar adenomas was often associated with epithelization and focal hyperplasia. Both oncogenicity studies met FIFRA guidelines.

In the mouse oncogenicity study, there was a high incidence of marked anemia, fluid-filled or dilated intestines, degenerative lesions in the adrenal gland and gastrointestinal tract, and increased non-oncogenic mortality (females) at 250 ppm suggesting that the maximum tolerated dose (MTD) was exceeded. This excessive toxicity might be due to saturation of metabolic pathways which could lead to an increase in a tumor incidence through the accumulation of or formation of more reactive metabolites (Carr and Kolbye, 1991). Increased cell proliferation due to cytotoxicity can result in the promotion of endogenous DNA damage by decreasing the time available to repair DNA damage (Swenberg, 1995). Other non-genotoxic mechanisms could be responsible for the increase in tumor incidences including immunosuppression or an alteration in hormone levels (MacDonald *et al.*, 1994). Theoretically,

a biological threshold could exist for the oncogenic response if there is evidence that the metabolic pathways are saturated or the endocrine or immune systems are dysfunctional at doses where there is an increased tumor incidence.

There was no evidence of genotoxicity in the four available studies for tribufos (an Ames assay, an *in vitro* chromosomal aberrations assay, an *in vitro* sister chromatid exchange assay, and an unscheduled DNA synthesis assay). All of these genotoxicity studies met FIFRA guidelines, except the sister chromatid exchange assay which was a published report.

Quantitative Assessment of Oncogenic Effects

Although the increase in small intestine adenocarcinomas and alveolar/ bronchiolar adenomas only occurred on at the highest dose level where there was evidence of excessive toxicity, there was insufficient data for tribufos to indicate whether any threshold mechanisms might be responsible for the oncogenic response. Moreover, multiple tumor sites were involved, one of which is a rare tumor type (small intestine adenocarcinoma) with a reported historical control range for this laboratory of 0% in both sexes. Consequently, it was assumed there was no threshold and the potential oncogenic risk to humans was evaluated using a linear, low dose extrapolation model to estimate potency.

It was not possible to accurately estimate the oncogenic potency (Q_1) or upper bound (Q_1*) on the slope for small intestine adenocarcinomas and alveolar/bronchiolar adenomas because the slope estimate (Q_1) is zero when the tumor incidence is only increased at the high dose. Therefore, the incidence of liver hemangiosarcomas in male mice was used to calculate the oncogenic potency of tribufos. Due to the reduced survival of mice at the highest dose tested, 250 ppm, the oncogenic potency of tribufos was estimated using the multistage-Weibull time-to-tumor model, MULTI-WEIB. The dosages for male mice (0, 1.5, 8.4 or 48.1 mg/kg/day) were converted to human equivalent dosages multiplying by an interspecies scaling factor of body weight to the 3/4 power $[(BWt_A/BWt_H)^{0.25} = (0.030 \text{ kg}/70 \text{ kg})^{0.25} = 0.144]$. The estimated oncogenic potency ranged from 3.3×10^{-2} (maximum likelihood estimate or MLE) to 5.9×10^{-2} (95% upper bound or 95% UB) $(\text{mg/kg/day})^{-1}$.

n-Butyl Mercaptan

Only limited animal toxicity data were available for nBM. Effects observed in a battery of acute toxicity tests (intraperitoneal LD50, oral LD50, inhalation LD50, ocular irritation) were indicative of CNS depression including incoordination, muscular weakness, paralysis, lethargy, sedation, respiratory depression, cyanosis, and coma (Fairchild and Stokinger, 1958). Other effects included restlessness, increased respiration, diarrhea (oral exposure), sneezing (inhalation exposure), and ocular irritation. Liver damage (lymphatic infiltration and necrotic foci with small hemorrhages) and kidney damage (cloudy swelling of the tubules and hyaline casts in the lumina) were observed with all routes of exposure. With inhalation exposure, hyperemia of the trachea and lungs, capillary engorgement, edema and occasional hemorrhage were seen. There was insufficient information available in the published report by Fairchild and Stokinger (1958) to establish a NOEL by any of the routes tested. An inhalation developmental toxicity study was available in which mice and rats were exposed to vapors of nBM for 6 hrs/day on gestation days 6-16 and 6-19, respectively (Thomas *et al.*, 1987). No maternal or developmental effects were seen in rats. The NOEL for the maternal and developmental effects in mice was 10 ppm (17 mg/kg/day) based on increased mortalities, reduced body weight gain, unkempt appearance, lethargy, red/brown perianal stains, increased post-implantation losses, and fetal malformations.

As mentioned earlier, the “late acute” effects seen in hens with oral exposure were attributed to nBM which is probably formed in the gut from the hydrolysis of tribufos (Abou-Donia *et al.*, 1979a; Abou-Donia *et al.*, 1984). These investigators tested this theory by administering nBM to hens and found they developed signs similar to those described as late acute effects (malaise, leg or general weakness, loss of balance, diarrhea, loss of appetite, disorientation, tremors, loss of breath, and dark and droopy comb just prior to death), except the onset of signs was earlier (6-12 hrs after administration). Hens administered nBM did not respond to atropine therapy, did not have any inhibition of brain or plasma ChE activity, and did not develop degenerative changes in peripheral nerves. A NOEL of 100 mg/kg was observed based on clinical signs in hens administered nBM. Abdo *et al*. (1983b) observed RBC deformation and lysis in hens 24-48 hrs after administering nBM at 500 mg/kg. Methemoglobin levels were elevated while RBC counts, hematocrit, hemoglobin levels and G-6-PD activity were reduced. Because the time course of the hematological changes and the late acute effects were similar, the investigators proposed that the inhibition of G-6-PD was responsible for the hematological changes. G-6-PD is involved in the regeneration of NADPH which in turn is needed for the reduction of glutathione. A reduction in glutathione levels could lead to the formation of methemoglobin and Heinz bodies, coagulation of surface proteins on RBCs, leading to deformation and eventual cell lysis. Since only one dose was administered in this study, a NOEL was not established for the hematological changes.

Residents of agricultural communities in cotton-growing regions have complained of eye and throat irritation, rhinitis, wheezing, coughing, shortness of breath, nausea and diarrhea during the time of cotton defoliation with tribufos (Maddy and Peoples, 1977; Scarborough, 1989). Based on the acute effects seen in animals exposed to nBM, it appears that nBM may be responsible for the ocular and respiratory irritation. Some of these complaints may also be due to the strong skunk-like odor. The an odor threshold of nBM in humans between 0.01 and 1.0 ppb (Santodonato *et al.*, 1985). Offensive odors may trigger symptoms in humans, such as nausea and headache, by indirect physiologic mechanisms including exacerbating an underlying medical condition, innate odor aversion, odor-related aversive conditioning, stress-induced illness, and possible innate pheromonal reaction (Shusterman, 1992). Ames and Stratton (1991) analyzed health effects reported by residents living near a potato field that had been treated with ethoprop which breaks down to n-propyl mercaptan. They found that symptoms more closely correlated with odor perception than with distance from the potato field.

B. EXPOSURE ASSESSMENT

Occupational Exposure

Exposure estimates for handlers involved in the aerial or ground application of tribufos to cotton are summarized in Table 22. The total daily exposure by the dermal and inhalation routes are the geometric means from the data presented in Table 3 of the revised Exposure Assessment Document for tribufos which comes from a study conducted by Eberhart (1993), except for flaggers (Formoli, 2000). A weighted average of the exposure data from both the Eberhart (1993) and Peoples *et al.* (1981) studies were used for flaggers. The exposure data for mixer/loaders and pilots from the Peoples *et al.* (1981) study was not used because of the inconsistent use of protective clothing. The dermal exposure represented more than 95% of the total daily exposure for handlers. To address the risk for severe dermal irritation in handlers from the tribufos formulation, the average hand exposure (µg/person/day) was divided by the concentration of tribufos in the formulation (70%) and the surface area of the hand (990 cm^2). The hand exposure ranged from 0.23 µg formulation/cm^2 for ground applicators to 5.48 µg

Table 22. Estimated Exposure Dosages in Pesticide Workers for Tribufos Use on Cotton[a]

Job Categories	Hand Exposure[b]	ADD[c]	SADD[d]	LADD[e]
	µg Tribufos EC/cm^2	µg/kg/day		
Handlers				
Mixer/Loader (aerial)	1.84	4.6	2.1	0.15
Pilot	5.48	5.1	2.4	0.17
Flagger	0.79	4.4	2.1	0.14
Mixer/Loader (ground)	5.01	8.5	4.0	0.28
Applicator (ground)	0.23	0.7	0.3	0.02
Field Workers				
Irrigators/weeders (4 days)	------	25.5	11.9	0.84
Irrigators/weeders (7 days)	------	11.3	5.3	0.37
Picker Operator	------	5.0	2.3	0.17
Module Builder Operator	------	1.9	0.9	0.06
Raker	------	3.4	1.6	0.11
Tramper	------	8.3	3.9	0.27

[a] Exposure estimates for handlers are from Eberhart (1993), except for flaggers which is the weighted average of exposure estimates from both Peoples *et al.* (1981) and Eberhart (1993). A 7-hour workday was assumed for aerial application and 8-hour workday for ground application. Exposure estimates for harvesters were calculated using dermal transfer factors and the average residue level on cotton bolls at 7 days after application derived from a study conducted by Eberhart and Ellisor (1993). Inhalation exposure was assumed to be negligible for harvesters.

[b] The hand exposure (µg/person/day) for handlers was converted to µg of tribufos emulsifiable concentrated (EC) per cm^2 by dividing by the concentration of tribufos in the formulation (70%) and the surface area of the hand (990 cm^2).

[c] ADD = Absorbed Daily Dosage assuming 7.1% dermal absorption, 50% respiratory uptake of tribufos as a vapor with occupational exposure, an inhalation rate of 14 L/minute, a body weight of 75.9 kg and 8- hour workday. The value represents the geometric mean for handlers and the arithmetic mean for harvesters based on the distribution of the data.

[d] SADD = Seasonal Average Daily Dosage assuming workers are exposed 21 days in a 45-day season

[e] LADD = Lifetime Average Daily Dosage assuming an exposure over 40 years of a 70-year lifespan.

formulation/ cm^2 for pilots. The Absorbed Daily Dosage (ADD) was the geometric mean of the total daily exposure by the dermal and inhalation routes assuming 1) an average worker weighs 75.9 kg, 2) dermal absorption is 7.1%, 3) respiratory uptake is 50% with tribufos as a vapor during occupational exposure, and 4) a workday is 8 hours. The ADD was highest among handlers for mixer/loaders involved in ground application of tribufos (8.5 µg/kg/day). The ground applicators had the lowest exposure (0.7 µg/kg/day). The Seasonal Average Daily Dosage (SADD) was calculated from the ADD assuming 21 days of exposure in a 45-day use season for cotton. The SADDs for handlers ranged from 0.3 to 4.0 µg/kg/day. Because occupational exposure was limited to a 45-day use season per year, a chronic exposure dosage (i.e., Annual Average Daily Dosage) was not calculated. The Lifetime Average Daily Dosage (LADD) is used to calculate the oncogenic risk for workers. The LADDs for handlers ranged from 0.02 to 0.28 µg/kg/day for handlers.

The tribufos label allows for field workers to reenter cotton fields 7 days after application for activities that may involved some contact with foliage, such as irrigation and weeding. There was no chemical specific or crop specific exposure data for irrigators or weeders. Therefore, a uniform foliar deposition value of 10.6 µg/cm^2 was assumed based on a maximum application rate of 1.9 lb a.i./acre. Assuming the residues degrade at a similar rate to that of cotton boll residues, the foliar residues at 4 and 7 days would be 25% and 11%, respectively, of the initial residue value. The estimated foliar residues at 4 and 7 days were 2.65 and 1.17 µg/cm^2, respectively. A dermal transfer factor of 1,288 cm^2/hr was assumed for irrigation and weeding based the dermal transfer factor derived for cotton scouts in a methyl parathion study. The dermal exposure was then estimated by multiplying the foliar residues by the dermal transfer factor. The ADD was then calculated by multiplying the dermal exposure (µg/hr) by the number of hours worked (8 hrs) and the dermal absorption (7.1%) and dividing by the body weight (75.9 kg). The ADDs for irrigators and weeders were 25.5 (4 days) and 11.3 (7 days) µg/kg/day. The SADDs were 11.9 (4 days) and 5.3 (7 days) µg/kg/day. The LADDs were 0.84 (4 days) and 0.37 (7 days) µg/kg/day.

The exposure estimates for workers harvesting cotton treated with tribufos are also summarized in Table 22. The exposure estimates were derived using dermal transfer factors derived from a study conducted by Eberhart and Ellisor (1993) in which cotton treated with tribufos was harvested 15 to 20 days after application. The arithmetic mean of the dermal exposure was used in calculating the dermal transfer factors. The estimated dermal transfer factors ranged from 250 g/hr for module builder operators to 1108 g/hr for trampers. The dermal transfer factors were then used to estimate exposure if the cotton had been harvested at the minimum pre-harvest interval of 7 days after application using residue data that was also collected in this same study. Predicted residue levels were estimated from observed levels using log-quadratic regression analysis. The predicted residues for day 7 with aerial and ground application were 0.216 and 0.363 µg/g cotton boll, respectively. The higher residue with ground application was used to estimate dermal exposure for harvesters. The dermal exposure and ADDs for harvesters was calculated assuming an 8-hour workday, a dermal absorption of 7.1% and a body weight of 75.9 kg. The ADDs ranged from 1.9 µg/kg/day for module builder operators to 8.3 µg/kg/day for trampers. The SADDs ranged from 0.9 to 3.9 µg/kg/day. The LADDs for harvesters ranged from 0.06 to 0.27 µg/kg/day. Hand exposure to the tribufos formulation was not calculated for field workers because it was assumed that the inert ingredients were volatile and had dissipated by 7 days. Consequently, these field workers were exposed primarily to technical grade tribufos which only caused mild dermal irritation.

Dietary Exposure

DPR evaluates the risk of exposure to an active ingredient in the diet using two processes: (1) use of residue levels detected in foods to evaluate the risk from total exposure, and (2) use of tolerance levels to evaluate the risk from exposure to individual commodities (see Tolerance Assessment section). For the evaluation of risk to detected residue levels, the total exposure in the diet is determined for all label-approved raw agricultural commodities, processed forms, and animal products (meat and milk) that have established U.S. EPA tolerances. The potential exposure from residues in the water is also assessed in some cases. Tolerances may be established for the parent compound and associated metabolites. DPR considers metabolites and other degradation products that may be of toxicological concern in the dietary assessment.

The only registered use for tribufos is on cotton defoliation. This use can result in human dietary exposure to tribufos through primary residues in cottonseed oil and meal and secondary residues in meat and milk products through use of cottonseed products in animal

feed. The environmental fate data suggests that tribufos is not likely to be a ground water contaminant; therefore, it was assumed there were no tribufos residues in drinking water.

Residue Data

The sources of residue data for dietary exposure assessment include DPR and federal monitoring programs, field trials, and survey studies. Residue data obtained from the monitoring programs are preferred because they represent a realistic estimate of potential exposure. In the absence of data, surrogate data from the same crop group as defined by U.S. EPA or theoretical residues equal to U.S. EPA tolerances. Residue levels that exceed established tolerances (over-tolerance) are not utilized in the dietary exposure assessment because over-tolerance incidents are investigated by the DPR Pesticide Enforcement Branch and are relatively infrequent. DPR evaluates the potential risk from consuming commodities with residues over tolerance levels using an expedited acute risk assessment.

DPR has two major sampling programs: priority pesticide and marketplace surveillance. The priority pesticide program focuses on pesticides of health concern as determined by DPR Enforcement and Medical Toxicology Branches. Samples are collected from fields known to have been treated with the specific pesticides. For the marketplace surveillance program, samples are collected at the wholesale and retail outlets, and at the point of entry for imported foods. The sampling strategies for both priority pesticide and marketplace surveillance are similar and are weighted toward such factors as pattern of pesticide use; relative number and volume of pesticides typically used to produce a commodity; relative dietary importance of the commodity; past monitoring results; and extent of local pesticide use.

The U.S. Food and Drug Administration (FDA) has three programs for determining examining residues in food: (1) regulatory monitoring, (2) total diet study, and (3) incidence/level monitoring. For the surveillance monitoring, surveillance samples are collected from individual lots of domestic and imported foods at the source of production or at the wholesale level. In contrast to the regulatory monitoring program, the total diet study monitors residue levels in the form that a commodity is commonly eaten or found in a prepared meal. The incidence /level monitoring program is designed to address specific concerns about pesticide residues in particular foods.

The U.S. Department of Agriculture (USDA) is responsible for the Pesticide Data Program (PDP), a nationwide cooperative monitoring program. The PDP is designed to collect objective, comprehensive pesticide residue data for risk assessments. Several states, including California, collect samples at produce markets and chain store distribution centers close to the consumer level. The pesticide and produce combinations are selected based on the toxicity of the pesticide as well as the need for residue data to determine exposure. In addition, USDA is responsible for the National Residue Program which provides data on potential pesticide residues in meat and poultry. These residues in farm animals can occur from direct application or consumption of commodities or by-products in their feed.

Primary Residues

DPR added tribufos to the multi-residue screen in its marketplace surveillance and priority pesticide programs for 1991. No tribufos residues in cottonseed products have been detected since its inclusion in the multi-residue screen, but this is not surprising since neither cottonseed meal nor oil products are sampled by DPR. The USDA Pesticide Data Program and FDA monitoring program also do not monitor for tribufos residues in cottonseed products.

There were 36 field studies of cottonseed samples submitted to DPR by the registrants (Chemagro Corp, 1965a & 1969). Fifty percent of these studies were not suitable for consideration in the dietary exposure analysis because the field study application rates and/or the pre-harvest intervals did not approximate those on the current labels for tribufos. The residue values for cottonseed were generated from 18 registrant studies where tribufos was applied at 30-42 oz/acre and samples were collected at 7 days post application (Chemagro, 1965a&b, 1969). The maximum and mean residue level of these 18 samples was 2.60 and 0.87 ppm, respectively. The MDL for all of these studies was 0.1 ppm.

Anticipated residue levels of tribufos in processed cottonseed products were derived by multiplying the level in whole cottonseed by various processing factors (Table 23). The processing factors for refined cottonseed hulls, meal, oil, and gin trash were 1.24, 0.04, 0.29, and 6.76, respectively, based on some limited residue data submitted by the registrant for tribufos in processed cottonseed (Chemagro Corp., 1965b & 1969). All cottonseed oil prepared for human consumption undergoes an additional processing step (deodorization) to remove aromatics and low boiling point constituents. Approximately 99% of the tribufos was removed from cottonseed oil in a simulation of this process in which the oil was steam stripped (Thornton, 1968). However, no residue data were submitted for deodorized cottonseed oil after going through the normal deodorization process; therefore, the residue levels in cottonseed oil were not adjusted for this additional processing step.

Table 23. Processing Factors and Anticipated Residue Levels of Tribufos in Processed Cottonseed

		Anticipated Tribufos Residues	
Product	Processing Factor[a]	Maximum (ppm)	Mean (ppm)
Whole Cottonseed[b]	----	2.60	0.87
Cottonseed Hulls	1.24	3.22	1.08
Cottonseed Meal	0.04	0.10	0.03
Refined Cottonseed Oil	0.29	0.75	0.25
Cotton Gin Trash	6.76	17.58	5.88

[a] The processing factors were based on tribufos residues in a few samples of processed cottonseed (Chemagro Corp., 1965b & 1969).

[b] The tribufos residue levels in whole cottonseed were based on field studies conducted by registrant (Chemagro Corp., 1965a).

Drinking water exposure to tribufos was not considered a likely scenario since tribufos is not considered a groundwater contaminant by DPR and no residues have been detected in the well monitoring that has been conducted (DPR, 2002a). Furthermore, only 2 of 342 samples had detectable residues right at the limit of quantitation (0.01 ppb) in the surface water monitoring (DPR, 2002b).

Secondary Residues

The registrant provided residue studies for cattle, goats and poultry (Chemagro Corp., 1968a&b; Sahali, 1991; Hall, 1991). Tribufos was administered to cattle at 84.5 mg/1000 lb/day

(0.19 mg/kg/day) in capsules for 28 days (Chemagro Corp., 1968a&b). This dose level was theoretically equivalent to that which the animal would receive if it consumed 3% of its body weight in feed that was 50% cottonseed hulls containing tribufos at 12.4 ppm. The theoretical residue level of the hulls in the animal feed was based on the highest residue level detected in whole cottonseed, 9.06 ppm, in the field studies submitted by the registrant and adjusting for the higher concentration of tribufos in the hull. However, the highest residue level in the whole cottonseed occurred when tribufos was applied at twice the maximum application rate. To obtain more realistic tribufos residue levels in cattle tissues and milk, the relationship between the administered dose and the tissue level was assumed to be proportional and the tissue levels were adjusted by a distribution factor (Table 24). The distribution factor is simply the ratio of the residue level in the tissue to the level in the diet. The amount of processed cottonseed products in feed was assumed to be 10, 25, 20, and 30% for meal, seeds, hulls, and gin trash, respectively, based on the U.S. EPA guidelines for residue studies (U.S. EPA, 1994). The maximum theoretical residue in animal feed from the various cottonseed products was estimated to be 6.58 ppm. The mean theoretical residue in animal feed was 2.20 ppm.

Table 24. Distribution Factors and Anticipated Tribufos Residues in Cattle Tissues and Milk

Tissue	Distribution Factor[a] (%)	Anticipated Tribufos Residues[b]	
		Maximum (ppm)	Mean (ppm)
Brain	0.37	0.02[c]	0.008
Heart	0.51	0.02[c]	0.011
Liver	0.44	0.02[c]	0.010
Kidney	0.55	0.02[c]	0.012
Muscle	0.25	0.016	0.006
Fat	0.32	0.02[c]	0.007
Milk	0.05	0.002[c,d]	0.0008[d]

[a] The distribution factors were based on the residue levels found in cattle tissues after administering tribufos at 84.5 mg/1000 lb/day (0.19 mg/kg/day) for 28 days (Chemagro Corp., 1968a&b). The distribution factor is the ratio of the residue level in the tissue to the level in the diet.

[b] The maximum and mean theoretical residue level in feed for beef cattle was estimated to be 6.59 and 2.20 ppm, respectively, assuming the feed contained meal, seeds, hulls, and gin trash at 10, 25, 20 and 30%, respectively (U.S. EPA, 1994). The maximum or mean theoretical residue levels in tissues were estimated by multiplying the distribution factor by the maximum or mean theoretical residue level in the feed (e.g., the mean residue in cattle brain = 0.37% x 2.20 ppm = 0.008 ppm).

[c] Based on the maximum theoretical residue in feed of 6.59 ppm, the maximum anticipated residue in brain, heart, liver, kidney, fat, and milk exceeded the tolerance; therefore, the residue levels were set at the tolerance.

[d] The maximum and mean theoretical residue level in feed for dairy cattle was estimated to be 4.66 and 1.56 ppm, respectively, assuming the feed contained meal, seeds, hulls, and gin trash at 15, 25, 15 and 20%, respectively (U.S. EPA, 1994).

Two lactating goats were administered tribufos in capsules at 0.82 and 0.85 mg/kg/day on 3 consecutive days (Sahali, 1991). These dosages were theoretically equivalent to residue levels of 109 and 113 ppm, respectively, for whole cottonseed if 25% of the feed is from cottonseed products and goats consume 3% of their bodyweight in feed daily. These theoretical residue levels are approximately 25 times the tolerance level for whole cottonseed. As with cattle, more realistic tribufos residue levels in goat tissues and milk were derived by

assuming the tissue levels were proportional to dose and adjusting by a distribution factor (Table 25). Although the total radioactivity in tissues was measured, only the residues of the parent compound were used in the estimation of dietary exposure. Tribufos represented 36, 5, and <1% of the total radioactive residues in fat, milk, and other tissues, respectively. The other radioactive components in the tissues did not match the reference standards for various known degradation products of tribufos including nBM, S,S-dibutyl phosphorodithioate, and S-butyl phosphorothioate. The majority of the radioactivity was found in the protein and fatty acid fraction of the tissues. The percentage of cottonseed products in the feed of goats was assumed to be identical to cattle (i.e., 10% meal, 25% seeds, 20% hulls, and 30% gin trash).

Table 25. Distribution Factors and Anticipated Tribufos Residues in Goat Tissues and Milk

Tissue	Distribution Factor[a] (%)	Anticipated Tribufos Residues[b]	
		Maximum (ppm)	Mean (ppm)
Liver	0.126	0.0083	0.0028
Kidney	0.014	0.0009	0.0003
Fat	0.245	0.0161	0.0054
Muscle	0.002	0.00013	0.00004
Milk	0.022	0.0010[c]	0.0003[c]

[a] The distribution factors were based on the residue levels found in goat tissues after administering tribufos at 0.82-0.85 mg/kg/day for 3 days (Sahali, 1991). The distribution factor is the ratio of the residue level in the tissue to the level in the diet.

[b] The maximum and mean theoretical residue in feed for goat was estimated to be 6.58 and 2.20 ppm, respectively, assuming the feed is similar to beef cattle which contained meal, seeds, hulls, and gin trash at 10, 25, 20, and 30%, respectively. The maximum or mean theoretical residue levels in tissues were estimated by multiplying the distribution factor by the maximum or mean theoretical residue level in the feed (e.g., the mean residue in goat liver = 0.126% x 2.20 ppm = 0.0028 ppm).

[c] The maximum and mean theoretical residue in feed for lactating goats was estimated to be 4.66 and 1.56 ppm, respectively, assuming the feed is similar to dairy cattle which contained meal, seeds, hulls, and gin trash at 15, 25, 15, and 20%, respectively.

The distribution factors for goat tissue and milk were significantly lower than those for cattle. Most likely this is due to the shorter exposure period (3 days vs. 28 days) in the goat study; however, differences in methodology and metabolism may also be involved. Since species differences in metabolism are likely and could not be eliminated as a possible cause, different distribution factors were used for goat and cattle tissues. No residue studies were available for sheep tissues; therefore, the distribution factors for goat were used for sheep.

Currently, there are no tolerances for poultry or eggs, so the residue levels from the metabolism study with laying hens was not incorporated into the dietary exposure (Hall, 1991).

Consumption Database and Dietary Exposure Software

The United States Department of Agriculture (USDA) directs the Continuing Survey of Food Intakes by Individuals (CSFII). The purpose of the CSFII is to analyze food intake every few years to provide up-to-date information on the adequacy of the diets of various population groups and early indications of dietary changes. Individual intake data are collected using both

a 1-day recall and a 2-day record protocol. The most recent CSFII survey data, collected from January 1994 to February 1997 (referred to as 1994-96) and from December 1997 to December 1998 (referred to as 1998), were used in this dietary exposure assessment. The surveys were conducted in all months of the year. In each year, approximately 5,500 participants in 62 geographical areas were surveyed. The 1994-96 data included all population subgroups, including 4,253 children, ages 0 to 9 years old. The 1998 CSFII data included an additional 5,559 children of the same age to increase the database for dietary patterns of infants and children in response to the Food Quality Protection Act of 1996.

The acute and chronic dietary exposure analyses were conducted using the Dietary Exposure Evaluation Model (DEEM™, version 7.74) software program developed by Novigen Sciences, Inc. DEEM calculates acute and chronic exposure estimates for 18 different population subgroups, including nursing or non-nursing infants less than 1 year old, children ages 1-6 years old or 7-12 years old, pregnant or nursing women, and seniors 55 years and older. The Acute Analysis program also allows for calculation of exposure for custom populations, such as workers, ages 16 years and older. The Acute Analysis program estimates the distribution of exposure per user-day (i.e., the percentile exposure for only individuals that consume at least one commodity on which the pesticide of concern is used on that survey day). The Acute Analysis estimates exposure either using a deterministic approach (i.e., a single residue value or point estimate for each commodity) or a probabilistic approach (i.e., Monte Carlo method where residue and consumption values are randomly selected from different distribution curves for each commodity). Since the probabilistic approach is more time consuming, it is only used if the margins of exposure are inadequate using the deterministic approach and/or there is sufficient residue data to describe the distributions. The Chronic Analysis estimates the annual average exposure per capita using the average residue values. The residue values for both acute and chronic exposure can be adjusted by percent crop treated; however, DPR generally only adjusts the acute values if the Monte Carlo method is used. DPR did not adjust the chronic residue values for percent crop treated in this dietary exposure assessment because the dietary residues were theoretical and further refinement did not seem warranted without additional direct measurement of these residues in cottonseed products or their secondary residues in cattle tissue or milk. Critical commodity contributions were calculated for both the acute and chronic exposure analysis to determine which commodities were contributing the most to exposure.

Acute Dietary Exposure

Estimates of potential acute dietary exposure used the highest measured residue values at or below the tolerance for each commodity. The processing and distribution factors described in Tables 23-25 were used to derive the anticipated maximum residue levels for processed cottonseed products, animal tissues, and milk from the maximum residue level for whole cottonseed. The following assumptions were used to estimate potential acute dietary exposure from measured residue values: a) the residue level does not change over time and b) all foods that are consumed will contain the highest residue anticipated. Based on the 95th percentile of user-days exposures for all specific population subgroups, the potential acute dietary exposures to tribufos from all labeled uses (i.e., cotton defoliation) ranged from 52 to 224 ng/kg/day (Table 26). Children, ages 1 to 6, had the highest potential acute exposure to tribufos.

Chronic Dietary Exposure

The anticipated mean tribufos residue levels in processed cottonseed products, animal tissues, and milk are given in Tables 23-25. The following assumptions were used to estimate

Table 26. Estimated Exposure Dosages for Selected Population Subgroups Potentially Exposed to Tribufos in the Diet Alone or in Combination with Ambient Air[a]

Population Subgroup	ADD[b] (ng/kg)		AADD[c] (ng/kg/day)	
	Diet[d]	Diet+Air[e]	Diet[f]	Diet+Air[g]
U.S. Population - All Seasons	110	220	13	20
Western Region	120	230	14	21
All Infants	174	478	11	31
Nursing Infants (< 1 yr)	96	400	3	23
Non-nursing Infants (< 1 yr)	187	491	14	34
Children (1-6 yrs)	224	528	37	57
Children (7-12 yrs)	143	447	22	42
Females (13+ yrs/pregnant/not nursing)	75	169	11	17
Females (13+ yrs/nursing)	90	184	14	20
Females (13-19 yrs/not pregnant or nursing)	77	171	11	17
Females (20+ yrs/not pregnant or nursing)	57	151	8	14
Females (13-50 yrs)	66	160	9	15
Males (13-19 yrs)	104	230	15	23
Males (20+ yrs)	69	195	10	18
Seniors (55+ yrs)	52	162	8	15
Workers (M & F, 16+ yrs)	66	176	---	---

[a] Potential dietary sources of tribufos include cottonseed oil, meal, and secondary residues in meat and milk.
[b] ADD = Absorbed Daily Dosage assuming 70% oral absorption and 100% respiratory uptake and absorption of tribufos as a particulate in ambient air.
[c] AADD = Annual Average Daily Dosage, assuming 70% oral absorption and 100% respiratory uptake and absorption of tribufos as a particulate in ambient air (See Lewis, 1998b).
[d] Based on 95th exposure percentile for all user-day population subgroups.
[e] Based on the upper 95% of ambient air concentration just offsite in the community with the highest detected air concentrations of tribufos. The estimated exposure for children (304 ng/kg) was used for all infants and children subgroups. The estimated exposure for adult male (126 ng/kg) and adult female (94 ng/kg) were used for the respective adult male and female subgroups. The exposure dosage for adult males and females (110 ng/kg) was averaged for the U.S. population, western region, seniors, and workers subgroup.
[f] Based on the annual average daily dosage for all population subgroups.
[g] Based on the mean ambient air concentration during use, assuming 60 days of exposure per year. The estimated exposure for children (20 ng/kg/day) was used for all infant and children subgroups. The estimated exposure for adult males (8 ng/kg/day) and adult females (6 ng/kg/day) were used for the respective adult male and female subgroups. The exposure dosages for adult males and females were averaged (7 ng/kg/day) for the U.S. population, western region, seniors, and workers subgroup.

potential chronic dietary exposure from measured residue values: a) the residue level does not change over time, b) individuals will consume foods that contain the average reported residue, and c) exposures to a commodity at all reported residue levels do occur (i.e., a commodity with the average calculated residue is consumed every day at an annual average level). No adjustment was made for percent crop treated because the dietary residues were theoretical and further refinement did not seem warranted without additional direct measurement of these residues in cottonseed products or their secondary residues in cattle tissue or milk. The mean

potential chronic dietary exposure for all population subgroups ranged from 3 to 37 ng/kg/day (Table 26). The population subgroup with the highest potential exposure was children, 1 to 6 years old.

Aggregate Exposure

The combined exposure to tribufos from occupational, dietary and ambient air exposure was also evaluated. The estimated exposure dosages to tribufos in ambient air were based on the document prepared to evaluate tribufos as a potential toxic air contaminant (Lewis, 1998b). The highest estimated exposure in ambient air was just offsite. For workers, the estimated acute, seasonal, and chronic ambient air exposure were 110, 44, and 7 ng/kg/day, respectively, based on the average offsite exposure for adult males and females in the rural community with the highest air concentrations of tribufos. It was assumed tribufos was in particulate form in ambient air; therefore, 100% respiratory uptake and absorption was used. The dietary and ambient air contribution to the total exposure for most pesticide workers was minor when compared to their occupational exposure (0.7 to 8.4% for acute exposure, 0.4 to 5.1% for seasonal exposure, and 0.9 to 11.7% for chronic exposure). The dietary and ambient air contribution was greatest among the ground applicators whose occupational exposure was lowest (19.6,12.8 and 28% for acute, seasonal and chronic exposure, respectively). Due to the minor contribution to the total exposure in workers with the highest exposure, no further evaluation of the combined occupational, dietary and ambient air exposure was conducted.

The combined exposure to tribufos from diet and ambient air was evaluated for the general population using the estimated exposure for adults and children from the document prepared to evaluate tribufos as a potential toxic air contaminant (Lewis, 1998b). The highest estimated acute ambient air exposure dosages for children, adult males and adult females were 304, 126, and 94 ng/kg, respectively. The combined acute exposure to tribufos from diet and ambient air ranged from 151 ng/kg/day for non-pregnant, non-nursing females (20 years and older) to 528 ng/kg/day for children ages 1 to 6 years old. The highest estimated chronic ambient air exposure dosages for children, adult males and adult females were 20, 8, and 6 ng/kg/day, respectively. The combined chronic exposure to tribufos from diet and ambient air ranged from 14 ng/kg/day for nursing females, 13 years and older, to 57 ng/kg/day for children 1 to 6 years old.

C. RISK CHARACTERIZATION

The risk for non-oncogenic health effects in humans is expressed as a margin of exposure (MOE). The MOE is the ratio of the NOEL from experimental animal studies to the human exposure dosage.

$$\textit{Margin of Exposure} = \frac{\textit{NOEL}}{\textit{Exposure Dosage}}$$

Acute Toxicity

The MOEs for dermal irritation were calculated for handlers using the NOEL for dermal irritation for the formulation (8.3 mg formulation/cm^2) and the estimated hand exposure to the formulation in Table 22. The MOEs for dermal irritation ranged from 1,500 for pilots to 36,000 for ground applicators (Table 27).

Table 27. Estimated Margins of Exposure for Pesticide Workers for Potential Acute and Seasonal Exposure to Tribufos[a]

	Acute		
Workers	Dermal[b]	Systemic[c]	Seasonal[d]
Handlers			
Mixer/Loader (aerial)	4,500	300	57
Pilot	1,500	270	50
Flagger	11,000	320	57
Mixer/Loader (ground)	1,700	160	30
Applicator (ground)	36,000	2,000	400
Field Workers			
Irrigators/Weeders (4-day REI)	------	55	10
Irrigators/Weeders (7-day REI)	------	120	23
Picker Operator	------	280	52
Module Builder Operator	------	740	133
Raker	------	412	75
Tramper	------	170	31

[a] Margin of Exposure = NOEL / Exposure Dosage. See Table 22 for exposure dosages for pesticide workers.
[b] The NOEL for dermal irritation was 8.4 mg formulation/cm^2 (rabbits).
[c] The adjusted acute NOEL for systemic effects was 1.4 mg/kg (rats - reduced motor activity, neurobehavioral effects, plasma and RBC ChE inhibition).
[d] The adjusted subchronic NOEL was 0.10 mg/kg/day (rats - plasma, RBC and brain ChE inhibition).

Since occupational exposure dosages are expressed as absorbed dosages, the critical NOEL was adjusted to an absorbed dosage of 1.4 mg/kg for evaluating occupational exposure based on an oral absorption of 70%. Since dietary and ambient air exposure to tribufos was minor when compared to the occupational exposure, the MOEs for systemic effects in pesticide workers were calculated using only the ADDs in Table 22 for occupational exposure and the adjusted acute NOEL. The acute MOEs ranged from 55 for irrigators and weeders with a 4-day reentry interval to 2,000 for ground applicators (Table 27).

For dietary exposure, the MOEs were calculated for the various population subgroups using the adjusted acute oral NOEL (1.4 mg/kg/day) and the acute dietary exposure dosages in Table 26. The MOEs ranged from 6,300 for children, 1 to 6 years old, to 27,000 for seniors 55 years and older (Table 28). For combined dietary and ambient air exposure, the adjusted acute oral NOEL (1.4 mg/kg/day) and combined acute exposure dosages in Table 26 were used to calculate the MOEs. The acute MOEs for combined exposure ranged from 2,700 for children 1 to 6 years old to 9,300 for non-pregnant, non-nursing females, 20 years and older (Table 28).

Table 28. Estimated Margins of Exposure for Selected Population Subgroups for Potential Acute and Chronic Exposure to Tribufos in the Diet Alone or in Combination with Ambient Air[a]

Population Subgroup	Acute[b]		Chronic[c]	
	Diet	Diet + Air	Diet	Diet + Air
U.S. Population	13,000	6,400	5,300	3,500
Western Region	12,000	6,100	4,900	3,300
All Infants	8,000	2,900	6,300	2,300
Nursing Infants (<1 yr old)	15,000	3,500	20,000	3,000
Non-Nursing Infants (<1 yr old)	7,500	2,900	5,000	2,100
Children (1-6 yrs)	6,300	2,700	1,900	1,200
Children (7-12)	9,800	3,100	3,100	1,700
Females (13+ yrs/pregnant/not nursing)	19,000	8,300	6,100	4,100
Females (13+ yrs/nursing)	16,000	7,600	5,100	3,500
Females (13-19 yrs/not pregnant/not nursing)	18,000	8,200	6,200	4,100
Females (20+ yrs/not pregnant/not nursing)	25,000	9,300	8,600	5,000
Females (13-50 yrs)	21,000	8,800	7,600	4,700
Males (13-19 yrs)	14,000	6,100	4,600	3,000
Males (20+ yrs)	20,000	7,200	6,900	3,900
Seniors (55+ yrs)	27,000	8,600	8,800	4,700
Workers (M & F, 16+ yrs)	21,000	8,000	------	------

[a] Margin of Exposure = Adjusted NOEL / Exposure Dosage. Values are rounded to two significant figures. Potential dietary sources of tribufos include cottonseed oil, meal, and secondary residues in meat and milk. See Table 26 for exposure dosages.

[b] The adjusted acute NOEL was 1.4 mg/kg (rats - reduced motor activity, neurobehavioral effects, plasma and RBC ChE inhibition).

[c] The adjusted chronic NOEL was 0.07 mg/kg (dogs - plasma ChE inhibition).

Subchronic Toxicity

The MOEs for seasonal occupational exposure for pesticide workers were calculated using the SADD (Table 22) and the adjusted subchronic NOEL (0.10 mg/kg/day). The MOEs ranged from 10 for irrigators and weeders with a 4-day reentry interval to 400 for ground applicators (Table 27).

Since exposure to tribufos does not appear to vary significantly from season to season, the seasonal dietary exposure in the general population was assumed to be the same as the chronic dietary exposure.

Chronic Toxicity

The MOEs for chronic dietary exposure to tribufos were calculated for the various population subgroups using the adjusted chronic NOEL (0.07 mg/kg/day) and the chronic dietary exposure dosages in Table 26. The MOEs ranged from 1,900 for children, 1 to 6 years old, to 20,000 for nursing infants less than 1 year old (Table 28).

For combined dietary and ambient air exposure, the adjusted chronic oral NOEL (0.07 mg/kg/day) and combined chronic exposure dosages in Table 26 were used to calculate the MOEs. The chronic MOEs from combined exposure ranged from 1,200 for children 1 to 6 years old to 5,000 for non-pregnant, non-nursing females 20 years and older (Table 28).

Oncogenicity

The risk for oncogenic effects was calculated by multiplying the oncogenic potency by the exposure dosage.

$$\textit{Oncogenic Risk} = \textit{Oncogenic Potency x Exposure Dosage}$$

The oncogenic risk for pesticide workers was calculated using the LADDs in Table 22. The estimated oncogenic potency of tribufos based on the incidence of liver hemangiosarcomas in male mice ranged from 4.7×10^{-2} (MLE) to 8.4×10^{-2} (95% UB) $(mg/kg/day)^{-1}$ after adjusting for oral absorption (70%). The estimated oncogenic risk for pesticide workers using the MLE for oncogenic potency ranged 9.4×10^{-7} to 3.9×10^{-5} (Table 29). When the 95% UB for oncogenic potency was used, the estimated oncogenic risk for workers ranged from 1.7×10^{-6} to 7.1×10^{-5}. Irrigators and weeders with a 4-day reentry interval had the highest oncogenic risk estimates based on both the MLE and the 95% UB.

The estimated oncogenic risk from dietary exposure alone was calculated using the chronic exposure for the U.S. population (13 ng/kg/day) and the adjusted oncogenic potency. The estimated oncogenic risk from dietary exposure to tribufos ranged from 6.3×10^{-7} (MLE) to 1.1×10^{-6} (95% UB).

The oncogenic risk from combined dietary and ambient air exposure was estimated using the adjusted chronic dietary exposure for the U.S. population (13 ng/kg/day) and the average chronic offsite air exposure for adult males and females (7 ng/kg/day). The estimated oncogenic risk from combined exposure to tribufos in the diet and ambient air ranged from 9.4×10^{-7} (MLE) to 1.7×10^{-6} (95% UB).

Table 29. The Estimated Oncogenic Risk for Pesticide Workers for Potential Lifetime Exposure to Tribufos[a]

Workers	Maximum Likelihood Estimate	95% Upper Bound
Handlers		
Mixer/Loader (aerial)	7.1×10^{-6}	1.3×10^{-5}
Pilot	8.0×10^{-6}	1.4×10^{-5}
Flagger	6.6×10^{-6}	1.2×10^{-5}
Mixer/Loader (ground)	1.3×10^{-5}	2.4×10^{-5}
Applicator (ground)	9.4×10^{-7}	1.7×10^{-6}
Field Workers		
Irrigators/Weeders (4-day REI)	3.9×10^{-5}	7.1×10^{-5}
Irrigators/Weeders (7-day REI)	1.7×10^{-5}	3.1×10^{-5}
Picker Operator	8.0×10^{-6}	1.4×10^{-5}
Module Builder Operator	2.8×10^{-6}	5.0×10^{-6}
Raker	5.2×10^{-6}	9.2×10^{-6}
Tramper	1.3×10^{-5}	2.3×10^{-5}

[a] Oncogenic Risk = Oncogenic Potency x Exposure Dosage. The exposure dosage was the LADD in Table 18. The maximum likelihood estimate for oncogenic potency after adjusting for oral adsorption (70%) was 4.7×10^{-2}. The 95% upper bound estimate for oncogenic potency was 8.4×10^{-2}.

V. RISK APPRAISAL

A. Introduction

Risk assessment is the process used to evaluate the potential for human exposure and the likelihood that the adverse effects observed in toxicity studies with laboratory animals will occur in humans under the specific exposure conditions. Every risk assessment has inherent limitations on the application of existing data to estimate the potential risk to human health. Therefore, certain assumptions and extrapolations are incorporated into the hazard identification, dose-response assessment, and exposure assessment processes. These, in turn, result in uncertainty in the risk characterization which integrates all the information from the previous three processes. Qualitatively, risk assessments for all chemicals have similar uncertainties. However, the degree or magnitude of the uncertainty can vary depending on the availability and quality of the data, and the types of exposure scenarios being assessed. Specific areas of uncertainty associated with this risk assessment for tribufos are delineated in the following discussion.

B. Hazard Identification

The metabolism of tribufos by the various routes of exposure is uncertain since only a few metabolites have been identified. Tribufos sulfoxide and S,S-dibutyl-S-1-hydroxybutyl phosphorotrithioate were identified in rat urine after intraperitoneal injection of tribufos (Hur *et al.*, 1992). A number of metabolites were detected in the urine and feces of several species (rat, goat, chicken) after oral administration of tribufos; however, only one metabolite, butyl-gamma-glutamylcysteinylglycine, was identified in rat urine (Kao *et al.*, 1991; Hall, 1991; Sahali, 1991). These investigators suggested that most of the parent compound had been extensively metabolized into natural constituents, such as fatty acids and proteins. nBM was also identified in the excreta of hens administered tribufos orally (Abou-Donia, 1979; Abou-Donia *et al.*, 1979a&b). These investigators proposed that tribufos was hydrolyzed to nBM in the gut causing the late acute effects which were only observed with oral administration of tribufos. The hydrolysis of tribufos in the gut could be due to either simple degradation or microbial metabolism. Due to the differences in the gastrointestinal tract between birds and mammals, it is unknown if tribufos is also easily hydrolyzed to nBM in the gut of mammals. Clinical signs similar to late acute effects in hens have not been observed in mammals; however, similar hematological effects have been observed in a subchronic inhalation study in rats and in chronic feeding studies in mice, rats, and dogs. These hematological changes and the gastrointestinal lesions observed in the chronic feeding studies may be route-specific effects due to nBM rather than tribufos. Consequently, these endpoints may not be relevant for occupational exposure in humans which occurs primarily by the dermal route. Although metabolic pathways were proposed based on these few metabolites, the metabolism of tribufos by the various routes is still highly speculative.

The physiological role of AChE in the nervous system is well known; however, there is some uncertainty regarding the toxicological significance of brain ChE inhibition because of the poor correlation between the severity of cholinergic signs and the level of ChE inhibition in the brain (U.S. EPA, 1988b). Several factors probably contribute to the poor correlation. One of these factors is that ChE inhibitors produce different degrees of inhibition in the various regions of the brain (Nieminen *et al.*, 1990). Certain cholinergic signs may be due to inhibition in specific regions of the brain. The level of brain ChE inhibition required to produce these effects may not be representative if the activity is measured in the whole brain or regions of the brain

that are insensitive to ChE inhibitors. Another factor is that some cholinergic signs may be due to peripheral rather than central inhibition of AChE (Murphy, 1986). For example, some of the respiratory effects may be due to peripheral inhibition of AChE in the diaphragm resulting in paralysis. In addition, brain ChE activity is usually measured at the end of the study whereas the cholinergic signs may be observed at various time points during the study. Often cholinergic signs are observed only at the beginning of the study and then the animals appear to develop a "tolerance" to the ChE inhibitor. This adaptation or "tolerance" may be due to several possible mechanisms including down-regulation of post-synaptic receptors (Costa *et al.*, 1982). Finally, clinical observation in animal studies is a very crude and subjective measurement. Some mild cholinergic symptoms, such as headaches and anxiety, cannot readily be detected in animals. The clinical signs in animals can also be missed because of the timing of the observations, especially with reversible ChE inhibitors. Rodents are nocturnal and generally eat and drink at night. If a chemical is a reversible inhibitor, some of the cholinergic signs could be missed because the signs occurred shortly after the animals had eaten during the night. There may also be other subtle changes in neurological function that will only be detected if the animal is stressed or required to perform certain tasks (Nagymajtényi *et al.*, 1988; Raffaele and Rees, 1990). It is possible that some level of brain ChE inhibition can occur without any untoward effect on neurological function, overt or subtle. However, the only way to be certain of this is through rigorous behavioral and neurophysiological testing in animals or humans after acute and long-term exposure. Although some neurobehavioral testing was conducted (FOB and motor activity) with acute and subchronic exposure to tribufos, no tests for memory or learning deficits were performed. Nor were there any tests for subtle neurological effects with chronic exposure to tribufos. Therefore, the assumption was made that since there was a statistically significant inhibition of brain ChE inhibition, there was probably some deleterious effect to the neurological system.

The most thorough investigation of the neurological effects in laboratory animals after acute exposure to tribufos was an acute neurotoxicity study in rats (Sheets and Gilmore, 2000). The NOEL in this study was 2 mg/kg based on clinical signs (perianal and urine staining), FOB effects (reduced auditory response -1F, reduced activity in the open field - 1M), reduced motor activity and reduced ChE activity in plasma (8-36% of controls) and RBCs (45-59% of controls). The reduced motor activity in absence of significant brain ChE inhibition suggests that the some neurobehavioral effects are due to peripheral ChE inhibition. This places greater importance on the need for a surrogate for peripheral ChE inhibition in evaluating tribufos. Therefore, greater weight should be given to the RBC ChE inhibition as a surrogate for peripheral ChE inhibition. The NOEL for this study is slightly lower than the NOEL from the rat developmental toxicity study (7 mg/kg) that was used in the previous RCD for tribufos. It is unclear if the differences in the NOELs in these two studies are the result of a more thorough evaluation of the neurotoxicity or simply a product of the dose selection since the LOELs are similar (20 mg/kg vs. 28 mg/kg). Because the acute neurotoxicity study had the most thorough evaluation of the acute neurotoxic potential of tribufos and had the lowest acute NOEL in an acceptable guideline study, it was selected as the definitive study for evaluating acute occupational and dietary exposure to tribufos. If the NOEL from the rat developmental toxicity study had still been used the acute MOEs would be 3.5 times higher than estimated.

The most thorough evaluation of the neurotoxic potential of tribufos in laboratory animals after subchronic exposure was a subchronic neurotoxicity study in rats (Sheets and Gilmore, 2001). The NOEL in this study was 0.14 mg/kg/day based on reduced ChE activity in the plasma (16-65%), RBCs (28-45%) and brain (92%). Unlike the acute neurotoxicity study, there were no neurobehavioral effects at dose levels that produced significant brain ChE inhibition. It is possible that with repeated exposure the ChE activity was gradually reduced without causing neurobehavioral effects due to tolerance. The NOEL in the subchronic neurotoxicity study in

rats was selected because it had the lowest NOEL in an acceptable guideline study. The NOEL from the subchronic neurotoxicity study in rats is significantly lower than the NOEL from a rabbit 3-week dermal toxicity study (2 mg/kg/day) that used in the previous RCD for tribufos (Sheets *et al.*, 1991). The NOEL in the rabbit study was based on muscle fasciculations, reduced brain ChE activity (~85% of controls) and skin lesions. The NOEL for plasma and RBC ChE inhibition was less than 2 mg/kg/day in this study. At the time the previous RCD had been prepared, neither plasma or RBC ChE inhibition were considered adverse by themselves. If blood ChE inhibition had been considered an adverse effect and the NOEL for blood ChE inhibition was estimated by dividing by an uncertainty factor of 10, the MOE using the rabbit study would still have been higher than that estimated from using the rat subchronic neurotoxicity study. Species differences in sensitivity, route of exposure and duration of exposure may have contributed to the higher NOEL in the rabbit study. A NOEL of 0.1 mg/kg/day was observed in a 90-day oral neurotoxicity study in hens (Abou-Donia *et al.*, 1979b). This study was not used for a variety of reasons as previously discussed under the Hazard Identification section, the most significant being the uncertainty about the relevance of the mild ataxia observed at the LOEL because of the route of exposure. It is very possible that the mild ataxia is due to nBM (which also causes incoordination) rather than tribufos since unequivocal evidence of OPIDN (paralysis and nerve degeneration) were not observed in the hens until 20 mg/kg/day. These same investigators had proposed that tribufos is hydrolyzed in the gastrointestinal tract to nBM. If the ataxia is caused by nBM, then this effect is not necessarily relevant to occupational exposure to tribufos in humans which is primarily dermal exposure. However, if this study had been used for seasonal occupational exposure, the MOEs would be approximately 30% lower than estimated.

The lowest NOEL for overt toxicity in a chronic study for tribufos was in the 2-year rat study (Christenson, 1992). A NOEL of 0.2 mg/kg/day was observed for hyperplasia and vacuolar degeneration in the small intestine and hematological changes in this study. This NOEL was selected for evaluating chronic exposure in the previous RCD for tribufos. However, if ChE inhibition in plasma and RBCs were also taken into consideration, the lowest NOEL in any chronic toxicity study for tribufos was in the 1-year dog study (Christenson, 1991). The NOEL for plasma ChE inhibition (M: 67% of controls) in this study was 0.1 mg/kg/day. It is unclear if the dogs are more sensitive than rats or the lower NOEL in the dog study is a result of the dose selection. However, without more data the assumption was made that the dogs were more sensitive to plasma ChE inhibition and the 1-year dog study was selected as the definitive study for evaluating chronic dietary exposure to tribufos. If the NOEL from the rat study had been selected, the MOEs would be 2 times greater than estimated.

MOEs were not calculated for chronic occupational exposure because potential long-term health effects (ChE inhibition) were either already addressed under subchronic toxicity or they were not considered relevant to occupational exposure to tribufos due to differences in duration or route of exposure (hematological changes, lesions in the small intestine, liver and spleen). The hematological changes and gastrointestinal lesions may be due to the degradation product, nBM, which is readily formed in the gut through the hydrolysis of tribufos. Lesions in the adrenal glands and hematological changes were observed in rats after subchronic inhalation exposure, but were not reported in the subchronic neurotoxicity study since no hematology was done in this study and only neurological tissue was examined microscopically. However, it was assumed that the NOEL for these endpoints was not lower than the NOEL for ChE inhibition in the subchronic neurotoxicity study based on the NOELs for these endpoints in the subchronic inhalation study. The bilateral retinal degeneration observed in the chronic rat study was also observed in both the subchronic inhalation and the subchronic neurotoxicity study in rats. Even if chronic MOEs had been calculated for occupational exposure using the chronic NOEL, 0.1 mg/kg/day, they would still be larger than the seasonal

MOEs. Although the chronic NOEL was slightly lower than the subchronic NOEL, the chronic exposure was 8 times lower than the seasonal exposure.

There was a significant increase in the incidence of adenocarcinomas in the small intestine of both sexes, in liver hemangiosarcomas in males, and alveolar/bronchiolar adenomas in females in a mouse study; however, there was no evidence of an oncogenic effect in a rat oncogenicity study and the genotoxicity data was negative. Among the tumors seen in mice only the liver hemangiosarcomas in males had an increase in the incidence at doses below the highest dose tested. There was a significant increase in non-neoplastic lesions especially in the small intestine at the high dose suggesting the MTD had been exceeded. At or above the MTD, normal physiology, metabolism and/or repair mechanisms may be overwhelmed, resulting in the initiation or promotion of tumors (Carr and Kolbye, 1991; Swenberg, 1995). Increased cell proliferation due to cytotoxicity can result in the promotion of tumors by decreasing the time available to repair DNA damage. Other nongenotoxic mechanisms, such as immunosuppression or endocrine disruption, could also be responsible for the increase in tumors (MacDonald *et al.*, 1994). If a threshold mechanism, such as increased cell proliferation was involved, the use of a linearized multistage model to estimate oncogenic risk would exaggerate the risk since it assumes there is no biological threshold.

Very little is known about the toxicity of nBM. Only a few studies were available describing the effects in laboratory animals after acute exposure. Some effects observed in animals were indicative of CNS depression including incoordination, muscular weakness, paralysis, lethargy, sedation, respiratory depression, cyanosis, and coma (Fairchild and Stokinger, 1958). Other effects included restlessness, increased respiration, diarrhea, ocular irritation, liver and kidney damage. Evidence of respiratory irritation was seen with inhalation exposure, including sneezing, hyperemia of the trachea and lungs, capillary engorgement, edema and occasional hemorrhage in the lungs. There was insufficient information available in the published report by Fairchild and Stokinger (1958) to establish a NOEL by any of the routes tested.

Abou-Donia and coworkers (1979a & 1984) administered single doses of nBM to hens and observed various clinical including malaise, leg or general weakness, loss of balance, diarrhea, loss of appetite, disorientation, tremors, loss of breath, and just prior to death, a dark and droopy comb. The NOEL was 100 mg/kg based on these clinical signs. Abdo *et al*. (1983b) found that hens administered nBM had elevated methemoglobin levels and reduced RBC counts, hematocrit, hemoglobin levels and G-6-PD activity. Because the time course of the hematological changes and the clinical signs were similar, the investigators proposed that the inhibition of G-6-PD was responsible for the hematological changes. A NOEL was not established for the hematological effects in any of the toxicity studies for nBM.

An acute NOEL of 10 ppm (17 mg/kg/day) was established in a developmental toxicity study based on increased mortalities, reduced body weight gain, unkempt appearance, lethargy, red/brown stains, increased post-implantation losses and fetal malformations in mice. Complaints of nausea, eye and respiratory irritation among residents of communities in cotton-growing regions have been attributed to nBM, which has a strong skunk-like odor (Maddy and Peoples, 1977; Scarborough, 1989). It is not clear if ocular and respiratory irritation were evaluated in the developmental toxicity study in mice. It also does not appear that the mice were evaluated for hematological changes. Therefore, it is possible the acute NOEL for nBM would be lower based on these endpoints.

There were no studies available in which animals were exposed to nBM on a subchronic or chronic basis. Consequently, the potential long-term health effects in humans from seasonal

or chronic exposure to nBM are unknown. The long-term health effects from nBM are of particular concern since there is evidence of oncogenicity in mice administered tribufos orally. If tribufos is significantly hydrolyzed to nBM in the gut of mice as it is in chickens, it is possible that the oncogenicity may be due to the nBM rather than tribufos. Additionally, no genotoxicity data were available for nBM either.

C. Exposure Assessment

1. Occupational Exposure

With acute exposure, it is preferable to use a high-end estimate such as the 95th percentile. Insufficient information was available in the study selected for harvesters to calculate the 95th percentile. Consequently, the geometric mean or arithmetic mean were used for estimating a single day exposure. Therefore, the estimated MOEs for acute occupational exposure would not cover those people at the upper end of the exposure distribution curve. The exposure for the harvesters may have also been underestimated slightly because it was calculated from predicted cotton boll residues on day 7 and dermal transfer factors estimated from the study conducted by Eberhart and Ellisor (1993) and did not take into consideration inhalation exposure. However, based on the dermal and inhalation exposures on day 15 and 20 of this study, the inhalation exposure represented approximately 5% of the absorbed dose for harvesters.

The occupational exposure for handlers and harvesters was estimated by extrapolating from a 4-hour monitoring period to a 7-hour or 8-hour work day, respectively. It has been demonstrated that the accumulation of residues on clothing and hands reaches a plateau after the first few hours, so that extrapolating an 8-hour exposure from a 4-hour monitoring period may overestimate exposure by 20-40% (Spencer *et al.*, 1995). Residues on hands, in particular, remained virtually constant over the work day.

2. Dietary Exposure

The dietary exposure was based entirely on anticipated residues that were estimated from whole cottonseed by using processing and distribution factors derived from limited data on the residues in processed cottonseed products, cattle tissues and milk. The anticipated residues in cottonseed oil are the least certain since the registrant provided evidence which suggests that most of the tribufos residues are removed in a deodorization process. However, no data was provided from samples that had undergone the normal deodorization process; therefore, the anticipated residues in undeodorized oil were used. Elimination of the exposure from cottonseed oil would result in a reduction of the chronic dietary exposure by approximately 75%. Several other assumptions, probably overestimated the chronic dietary exposure for tribufos. It was assumed that cattle ate cotton by-products at the maximum level allowed by U.S. EPA (85%) on a long-term basis. Furthermore, only residue levels where tribufos was applied at or near the maximum application rate and collected at the shortest allowable preharvest interval were used, although tribufos may be applied at a lower rate to cotton and can be harvested from 7 to 21 days post application. Finally, the percent of crop treated was also not factored into the chronic exposure by DPR, although U.S. EPA assumed that 35% of the crop was treated in their dietary exposure assessment for tribufos (Travaglini, 1999). These factors were not included in the calculations because the dietary residues were theoretical and further refinement did not seem warranted without additional direct measurement of these residues in cottonseed products or their secondary residues in cattle tissue or milk.

3. Combined Exposure

The ambient air exposure dosages were based on air monitoring data from one rural site near Fresno that was less than ½ mile from a cotton field. This site was selected for evaluating ambient air exposure just offsite because it had the highest air concentrations of four sites monitored in the Fresno area. Therefore, these exposure estimates represent a worst case scenario. If the ambient air exposure had been based on air monitoring data from six rural locations in Kern County that were further from the application sites, the exposure dosages would be approximately 80% lower than estimated.

D. Risk Characterization

Generally, an MOE of at least 100 is considered sufficiently protective of human health when the NOEL for an adverse systemic effect is derived from an animal study. The MOE of 100 allows for humans being 10 times more sensitive than animals and for the most sensitive human being 10 times more sensitive than the average human. The MOEs for acute dermal effects were greater than 1,000 for all pesticide workers. The MOEs for acute systemic effects were greater than 100 for all workers, except for irrigators and weeders with a 4-day REI. However, with a 7-day REI the acute MOE for irrigators and weeders is greater than 100. The MOEs for seasonal occupational exposure were less than 100 for all handlers, except ground applicators and all field workers, except for module builder operators. The acute and chronic MOEs for dietary exposure are all greater than 1,000. The acute and chronic MOEs for combined dietary and ambient air exposure were also all greater than 1,000.

An oncogenic risk level less than 10^{-6} is generally considered negligible. The oncogenic risk estimates for most pesticide workers were between 10^{-5} and 10^{-6}. Estimated dietary oncogenic risk for the U.S. population was between 10^{-6} and 10^{-7}. However, the oncogenic risk has probably been overestimated for chronic dietary exposure because of the conservative assumptions made regarding the residue levels in cottonseed oil, the amount of cotton by-products consumed by cattle and the percent crop treated. The estimated oncogenic risk from combined dietary and ambient air exposure were also between 10^{-6} and 10^{-7}. The oncogenic risk from combined exposure has also been overestimated not only because of conservative assumptions made in the dietary exposure, but also because the ambient air exposure was based on the air monitoring data from one location with the highest air concentrations of tribufos.

MOEs were not calculated for nBM because of lack of reliable toxicity data for nBM and no air monitoring data for nBM in workers. The American Conference of Government Industrial Hygienists (ACGIH) threshold limit value (TLV) for nBM is 0.5 ppm (ACGIH, 1986). The TLV is based on a study with ethyl mercaptan in which human volunteers were exposed for 3 hours daily. No complaints were recorded at 1 mg/m^3 (0.4 ppm). A reference exposure level for nBM could also be estimated by dividing the NOEL of 10 ppm (17 mg/kg/day) from the inhalation developmental toxicity study in mice by an uncertainty factor of 100 for interspecies and intraspecies variation in susceptibility. The estimated reference exposure level for nBM is 250 µg/m^3 or 67.8 ppb, assuming a 24-hr respiratory rate of 0.68 m^3/kg/day for a 6-year-old child. The highest daily average air concentration for nBM in ambient air (28.6 µg/m^3 or 7.75 ppb) was reported in the CDFA (1981) study. Therefore, the ambient air concentration is more than 8-fold below the estimated reference exposure level for nBM. However, this ambient air concentration is above the reported odor threshold (0.01 to 1.0 ppb) for nBM (Santodonato *et al.*, 1985). Offensive odors may trigger symptoms in humans, such as nausea and headache, by indirect physiologic mechanisms including exacerbating an underlying medical condition, innate odor

aversion, odor-related aversive conditioning, stress-induced illness, and possible innate pheromonal reaction (Shusterman, 1992). Ames and Stratton (1991) found that symptoms more closely correlated with odor perception than distance from a potato field treated with ethoprop which breaks down to n-propyl mercaptan. Theoretically, workers could be exposed to higher air concentrations of nBM during application due to their close proximity to the source. However, it is unclear if the degradation of nBM would be sufficiently rapid that the amounts generated during application would be a health concern for workers.

E. U.S. EPA's Reregistration Eligibility Document for Tribufos

U.S. EPA completed a Human Health Risk Assessment for tribufos in September 1999 (Travaglini, 1999) . U.S. EPA evaluated both occupational and dietary exposure to tribufos using route-specific NOELs. They evaluated inhalation exposure in workers using the 90-day inhalation study conducted by Pauluhn (1992) with a short-term and intermediate term NOEL of 0.9 mg/kg/day. U.S. EPA selected the 21-day dermal toxicity study in rabbits for evaluating short-term and intermediate-term dermal exposure to tribufos in workers with an estimated NOEL of 0.2 mg/kg/day based on plasma and RBC ChE inhibition (Sheets *et al*, 1991). DPR preferred to use the acute and subchronic neurotoxicity study in rats (Sheets and Gilmore, 2000 & 2001) to evaluate acute and seasonal occupational exposure to tribufos since they had the most thorough evaluation of the neurotoxic potential of tribufos. If DPR had used U.S. EPA's dermal NOEL in evaluating the acute occupational exposure, the MOEs would be approximately 15-fold lower than estimated. If this NOEL had been used in evaluating seasonal occupational exposure to tribufos, the MOEs would be approximately the same.

U.S. EPA selected a NOEL of 1 mg/kg/day from the developmental toxicity study in rats to evaluate acute dietary exposure to tribufos based on plasma and RBC ChE inhibition (Kowalski *et al.*, 1986). DPR selected the NOEL of 2 mg/kg from the acute neurotoxicity study to evaluate acute dietary exposure based on reduced motor activity, neurobehavioral effects and reduced plasma and RBC ChE activity (Sheets and Gilmore, 2000). If DPR has used U.S. EPA's NOEL in evaluating acute dietary exposure, the MOEs would be 2-fold lower after adjusting for oral absorption (70%). U.S. EPA and DPR used the same NOEL of 0.1 mg/kg/day to evaluate chronic dietary exposure based on plasma ChE inhibition in the 1-year dog study (Christenson, 1991).

U.S. EPA had previously used the Abou-Donia *et al.* (1979b) study to calculate an RfD for tribufos when many of the acceptable registrant studies were not available. In this Human Health Risk Assessment document, the RfD is no longer estimated using the Abou-Donia *et al.* (1979b) study. U.S. EPA identified a NOEL of 11 mg/kg/day for delayed neurotoxicity study in the 90-day neurotoxicity study in hens submitted by the registrant (Sheets, 1991b). This NOEL is higher than the NOEL of 2.6 mg/kg/day that DPR identified for this study based on delayed neuropathy. DPR made the health protective assumption that the slight increase in equivocal lesions at 11 mg/kg/day was treatment-related, even though it was not statistically significant.

As part of the Food Quality Protection Act (FQPA), U.S. EPA evaluated the developmental and reproductive toxicity studies for tribufos and concluded, as did DPR, that there was no evidence for increased pre- or post-natal sensitivity. However, they recommended that the 10X uncertainty factor for children be retained because of data gaps. At that time, the registrant had not submitted the acute, subchronic and developmental neurotoxicity studies in rats. Although none of these studies are required under the Birth Defect Prevention Act (SB 950) to register pesticides in California, the registrant has submitted them to DPR, too.

U.S. EPA classified tribufos as a Likely High Dose/Not Likely Low Dose carcinogen under its new carcinogenicity classification system. Their justification for this classification was that tumors were only increased at the highest dose level where severe toxicity occurred. Although not explicitly stated, this classification treats tribufos is a threshold carcinogen because they use an MOE approach to protect for oncogenicity, rather than calculate an oncogenic potency factor. The NOEL they selected to calculate the MOEs for oncogenicity was 0.1 mg/kg/day based on plasma ChE inhibition in dogs, the most sensitive endpoint for chronic exposure (i.e, the same NOEL used for evaluating the chronic dietary exposure). If DPR had used this approach in evaluating oncogenic risk from occupational exposure to tribufos, the MOEs for oncogenicity would have ranged from 120 to 5,000. The MOEs for oncogenicity from dietary exposure to tribufos would be the same as those calculated for chronic toxicity.

In their 1999 risk assessment, U.S. EPA calculated dietary exposure using the DEEM program and the 1989-1992 CSFII data. For acute exposure they did a probabilistic analysis using the anticipated residues from field trial studies, processing factors, and percent crop treated. Residues in meat and milk were estimated from livestock metabolism and feeding studies. No PDP or FDA monitoring data was available since neither of these programs monitor for tribufos. A direct comparison of the dietary risk estimates from U.S. EPA and DPR is not feasible because U.S. EPA does not calculate MOEs for dietary exposure, but rather a percentage of the Population Adjusted Dose. The dietary risk estimates at the 99.9th percentile were 3-9% of acute PAD (0.001 mg/kg/day) and 1-6% of the chronic PAD (0.0001 mg/kg/day) in the most sensitive subpopulations, indicating there is no great acute or chronic dietary concern. U.S. EPA was not concerned about residues of tribufos in groundwater because it binds to soil and appears immobile. U.S. EPA determined by use of modeling (PRIZM/EXAM II) that tribufos could potentially contaminate surface waters. The maximum concentration was assumed to be 14 ppb and the annual chronic average was 1.66 ppb. DPR assumed there was no drinking water exposure to tribufos based on its high soil absorption and minimal surface water residues (only 2 of 342 samples with residues at 0.01 ppb). U.S. EPA had no concerns regarding the acute or chronic exposure to tribufos through drinking water.

U.S. EPA calculated the occupational exposure for handlers using their Pesticide Handlers Exposure Database (PHED). DPR used a chemical-specific study for handlers conducted by Eberhart (1993). For post-application occupational exposure, U.S. EPA used the same chemical-specific study used by DPR (Eberhar and Ellisor, 1993). A direct comparison of U.S. EPA's and DPR's occupational exposure estimates is complicated because U.S. EPA uses route-specific exposure estimates for dermal and inhalation exposure and compares these with route-specific NOELs whereas DPR combines these exposures after adjusting for absorption and compares this with a NOEL that has been adjusted for absorption. However, in their 1999 risk assessment, U.S. EPA estimated the dermal MOEs were less than their target of 1,000 for all of the handler occupational exposure scenarios despite mitigation measures. The dermal MOEs for post-application exposure scenarios were greater than 1,000 only after increasing the reentry intervals to 20-30 days. The inhalation MOEs were greater than the taget MOE of 100 for all of the occupational exposure scenarios with some personal protective equipment and/or engineering controls.

U.S. EPA completed an Interim Reregistration Eligibility Document (IRED) in September 2000 (U.S. EPA, 2000a). The studies selected for evaluated acute and chronic dietary exposure did not change, although U.S. EPA had not received the acute, subchronic and developmental neurotoxicity studies for tribufos when the IRED was completed. Consequently, they still applied an additional 10-fold FQPA factor to the dietary exposure due to data gaps. They also continued to use the 1989-1992 CSFII consumption data with the DEEM software. Therefore, their dietary risk estimates did not change from their 1999 risk assessment. The

studies selected for evaluating short-term and intermediate-term occupational exposure also did not change; however, the uncertainty factor used to derive the NOEL for the 21-day dermal toxicity study was reduced from 10 to 3. In addition, the IRED took into consideration the new dermal absorption study in monkeys and applied a conversion factor of 7 (which represents the ratio of the estimated dermal absorption in rats, 48%, to the dermal absorption in monkeys, 7%) to the LOEL for the dermal toxicity study in rabbits before calculating the MOE. Taking these two changes into consideration, their estimated dermal NOEL was approximately 4.7 mg/kg/day. Despite these changes, U.S. EPA remained concerned about dermal exposure for several handler scenarios for aerial application: flaggers, mixers, loaders and applicators because they were below their target MOE of 300. However, they were no longer concerned about dermal exposure from mixing, loading and application by groundboom. U.S. EPA still had risk concerns for 3 post-application exposure scenarios (rakers, trampers and pickers) at the current application rate and 7-day REI. However, there were no risk concerns for these exposure scenarios at the lower proposed application rate of 1.125 lbs a.i./acre with a 7-day REI.

F. Issues Related to the Food Quality Protection Act

The Food Quality Protection Act of 1996 mandated U.S. EPA to "upgrade its risk assessment process as part of the tolerance setting procedures" (U.S. EPA, 1997a and b). The improvements to risk assessment were based on the recommendations from the 1993 National Academy of Sciences report, "Pesticides in the Diets of Infants and Children" (NAS, 1993). The Act required an explicit finding that tolerances are safe for children. U.S. EPA was required to use an extra 10-fold safety factor to take into account potential pre- and post-natal developmental toxicity and the completeness of the data unless U.S. EPA determined, based on reliable data, that a different margin would be safe. In addition, U.S. EPA must consider available information on: 1) aggregate exposure from all non-occupational sources; 2) effects of cumulative exposure to the pesticide and other substances with common mechanisms of toxicity; 3) the effects of *in utero* exposure; and 4) the potential for endocrine disrupting effects.

1. Pre- and Post-natal Sensitivity

Developmental toxicity studies in rats and rabbits and reproductive toxicity studies in rats were considered in assessing the potential for higher sensitivity in infants and children than adults. Two developmental toxicity studies were conducted in which tribufos was administered by oral gavage, one in rats and the other in rabbits (Kowalski *et al.*, 1986; Clemens *et al.*, 1987). Both studies met FIFRA guidelines. No treatment-related increases in embryotoxicity, fetal malformations or variations were observed in rats and rabbits. Maternal effects included brain ChE inhibition and reduced body weight gain. In rats, the maternal brain ChE activity was reduced (54% of controls) at 28 mg/kg/day on day 20 of gestation; however, fetal brain ChE activity was unaffected. Reductions in the average maternal body weight gain were observed in rats and rabbits at 28 and 9 mg/kg/day, respectively, without corresponding reductions in fetal body weights. These findings in rats and rabbits suggest there is no increased prenatal sensitivity to tribufos.

One reproductive toxicity study was available in which tribufos was administered in the feed to rats (Eigenberg, 1991a). The study met FIFRA guidelines. Several reproductive effects were seen in this study. The reproductive effects included reductions in the fertility, birth, and viability indices, increased gestation length, reduced pup weight, clinical signs in pups, brain ChE inhibition and gross pathological lesions in pups. The reproductive NOEL was 32 ppm (3.0 mg/kg/day). Other non-reproductive effects in the adults included brain ChE inhibition and body

weight reductions. A reduction in mean brain ChE activity was observed in females (71% of controls) at 32 ppm on day 21 of lactation. No reduction in brain ChE activity was observed in the 21-day-old pups at 32 ppm. At 260 ppm, the reduction in the mean brain ChE activity was significantly greater in adult females (F_0&F_1:19% of controls) than the 21-day-old pups (F_{2a}:85% of controls). The mean body weight reductions in the adult females (24%) was similar to the reductions in the 21-day-old pups (25%) at 260 ppm. Based on these findings in rats, there does not appear to be any increased postnatal sensitivity to tribufos.

A developmental neurotoxicity study in rats was also conducted for tribufos (Lake, 2001). The pregnant rats were fed tribufos in the diet from gestation day 0 to lactation day 21. Dams were sacrificed on lactation day 21, but the pups were kept on untreated feed from day 21 to day 75. Effects observed in the dams included tremors, reduced maternal body weights during lactation and ChE inhibition. The maternal NOEL was 4 ppm (0.4 mg/kg/day) based on plasma, RBC and brain ChE inhibition. Effects were observed in the pups at 200 ppm, including clinical signs during lactation (weakness, wound or cut and no milk in stomach), delayed developmental landmarks (surface righting and preputial separation), reduced body weights, reduced acoustic startle response, plasma and brain ChE inhibition and reduced brain size and weights. The pup NOEL was 40 ppm for all of these effects. The pup NOEL for RBC ChE inhibition was equal to or greater than 200 ppm, the highest dose tested. The developmental neurotoxicity study is the most definitive study indicating no evidence for increased pre- or postnatal sensitivity to tribufos. Therefore, DPR recommends no additional uncertainty factor be applied for tribufos for infants and children.

2. Endocrine Effects

The Food Quality Protection Act (FQPA) of 1996 required U.S. EPA to develop a screening program to determine the endocrine disruption potential of pesticides. In 1997, the Risk Assessment Forum of the U.S. EPA published a report that reviewed the current state of science relative to environmental endocrine disruption (U.S. EPA, 1997c). U.S. EPA formed the Endocrine Disrupter Screening and Testing Advisory Committee (EDSTAC) to develop a strategy for screening and testing of pesticides for their potential to produce endocrine disruption. The EDSTAC members include various stakeholders and scientific experts. This screening and testing process is expected to be implemented by August of 1999 as required by FQPA.

Environmental chemicals can interact with the endocrine system, resulting in cancer, reproductive and/or developmental anomalies (EDSTAC, 1998). It may produce these effects by affecting hormonal production and synthesis, binding directly to hormone receptors or interfering with the breakdown of hormones (U.S. EPA, 1997c). The interim science policy stated in U.S. EPA's 1997 report is that "*the Agency does not consider endocrine disruption to be an adverse endpoint per se, but rather to be a mode or mechanism of action leading to other outcomes*." Possible endocrine-related effects were seen in several studies for tribufos, including fatty droplets in the adrenal cortex and elevated absolute and relative adrenal gland weights in the subchronic inhalation study in rats (Pauluhn, 1992), degeneration and pigmentation of the adrenal gland in the 90-week mouse study (Hayes, 1989), vacuolar degeneration in the adrenal gland in the 2-year chronic toxicity/oncogenicity study in rats (Christenson, 1992), and reductions in fertility, birth and viability indices and increased gestation length in the reproductive toxicity study in rats (Eigenberg, 1991a). It is unclear from these data if these effects are mediated through endocrine disruption, ChE inhibition or some other mechanism.

3. Cumulative Toxicity

There is a potential for cumulative toxicity between tribufos and other organophosphates (OPs) because they have a common mechanism of toxicity, inhibition of AChE. However, until recently, a scientific defensible approach to quantitatively evaluate the potential for cumulative toxicity was not available. An elaborate methodology was recently developed by U.S. EPA to assess the exposure to multiple chemicals with a common mechanism of action (U.S. EPA, 2002a). Because the OPs were assigned priority for tolerance reassessment, they were the first to be considered as a "common mechanism group" for cumulative risk assessments. The U.S. EPA recently completed a preliminary cumulative risk assessment for the OPs (U.S. EPA, 2001). The assessment estimated the potential risk from exposure to multiple OPs by multiple pathways. A total of 31 OP pesticides were included in the risk assessment. These OPs were selected based on their detection in the USDA's PDP, as well as their potential for human exposure through residential, non-occupational uses and drinking water. The assessment utilized data from three exposure pathways: food, drinking water and residential/non-occupational exposure to OPs (air, soil, grass, indoor surfaces). Tribufos was one of the evaluated OPs in the food and drinking water exposure pathways.

U.S. EPA employed the relative potency factor (RPF) method to determine the combined exposure to the OPs. RPF was defined as the ratio of the toxic potency of a compound to that of an index chemical. Methamidophos was selected as the index chemical, because of the quality and extensive availability of its dose-response data for all routes of exposure. The toxic potencies for the OPs were based on the common endpoint of the inhibition of the brain ChE activity in female rats for 21 days or longer. Both, the point of comparison among the chemicals and the point of departure (POD) for the index chemical was based on the BMD_{10}, the benchmark response of 10% reduction of the ChE activity. In this analysis, U.S. EPA considered the exposure to OP residues in foods as uniform across the U.S. Twelve regional assessments were conducted for drinking water and residential exposures. The uniform food exposure estimate was combined with region-specific exposures from residential uses and drinking water. In Region 7, which included California, the use of tribufos on cotton was considered in the drinking water exposure modeling for the south central valley.

The conclusions from the preliminary OP cumulative risk assessment were that the drinking water is not a major contributor to the total risk. The exposures from OPs in food at percentiles above the 95^{th} percentile for all population subgroups were at least one order of magnitude higher than water. U.S. EPA indicated that additional sensitivity analysis is needed on the upper percentiles of the food exposure assessments before any risk management decisions can be made. U.S. EPA is in the process of developing guidelines for the application of the FQPA factor for pre and post-natal sensitivity in the cumulative risk assessments for chemicals with a common mechanism of toxicity (U.S. EPA, 2002b).

Inhibition of carboxylesterase by tribufos has resulted in the potentiation of organophosphate pesticides such as malathion that contain a carboxylic ester group (Murphy *et al.*, 1976). However, tribufos also markedly potentiated the toxicity of azinphos-methyl which does not contain any carboxylic ester groups (Gaughan *et al.*, 1980). Inhibition of other detoxification enzymes may be involved. Another mechanism could be competition for non-vital binding sites which may act as a buffer, thereby protecting AChE.

4. Aggregate Exposure

Combined dietary and ambient air exposure in the general population have been addressed in this document under the Exposure Assessment section in Risk Analysis section.

The combined dietary, occupational and ambient air exposure in workers was not evaluated further because the contribution of the dietary and ambient air exposure to the total exposure for most workers was minor compared to their occupational exposure (0.7% to 12%). The contribution was the greatest in ground applicators (20-28%) who had the lowest occupational exposure.

VI. TOLERANCE ASSESSMENT

A. BACKGROUND

A tolerance is the maximum, legal amount of a pesticide residue that is allowed on a raw or processed agricultural commodity, or in an animal tissue used for human consumption. The U.S. EPA tolerance program was developed as an enforcement mechanism to identify illegal residue concentrations resulting from potential noncompliance with the product label requirements (e.g. improper application rates or methods, inadequate preharvest intervals, direct or indirect application to unapproved commodities). Tolerances are enforced by the FDA, USDA, and state enforcement agencies (e.g., Pesticide Enforcement Branch of DPR).

Current pesticide tolerances are generally set at levels that are not expected to produce deleterious health effects in humans from chronic dietary exposure. The data requirements for establishing a specific tolerance include: 1) toxicology data for the parent compound, major metabolites, degradation products and impurities, 2) product chemistry, 3) analytical method(s) that are readily available, accurate and precise, 4) measured residues in crops used for animals feeds, 5) measured residues in animal tissues (e.g., meat, milk, eggs) from direct or indirect (feed) applications, and 6) measured residue levels from field studies. The minimum requirements for the field study include: 1) an application rate at or above the highest rate on the product label, 2) the greatest number of allowable repeat applications, and 3) the shortest pre-harvest interval listed on the product label. Generally, the registrant of the pesticide requests a commodity-specific tolerance, which is equal to the highest measured residue, or some multiple of that value, from the field trial using the specific pesticide.

Assembly Bill 2161 (Bronzan and Jones, 1989) requires the DPR to "conduct an assessment of dietary risks associated with the consumption of produce and processed food treated with pesticides." In the situation where "any pesticide use represents a dietary risk that is deleterious to the health of humans, the DPR shall prohibit or take action to modify that use or modify the tolerance . . . " As part of the tolerance assessment, a theoretical dietary exposure for a specific commodity and specific population subgroups can be calculated from the product of the tolerance and the daily consumption rate.

EPA has established tolerances for tribufos on cottonseed and cottonseed hulls at 4 and 6 ppm, respectively. Because secondary residues of tribufos can occur from feeding cottonseed products to ruminants as roughage, a tolerance level of 0.02 ppm was set for tribufos in the meat, meat by-products and fat of cattle, goats and sheep. The tolerance level for milk was set at 0.002 ppm.

B. ACUTE EXPOSURE

An acute exposure assessment using the residue level equal to the tolerance was conducted for each individual label-approved commodity. The DEEM software and the 1994-98 CSFII data were used in this assessment. Tolerances were not established for tribufos on cottonseed oil and meal; therefore, the tolerance for whole cottonseed, 4.0 ppm, was used for these two commodities. Initially, the tolerance assessment was conducted considering only the primary residues in cottonseed oil and meal. If the MOEs were larger than 1,000 based on the tolerances for the primary residues, no further analysis of the tolerances for secondary residues was conducted since the amount they would contribute to the total dietary exposure would be negligible compared to the tolerances for primary residues. Using the 95th percentile for acute exposure, the theoretical maximum residue contribution (TMRC) for tribufos after adjustment for

Table 30. Margins of Exposure from Acute Dietary Exposure to Tribufos Based on Tolerances

Population Subgroup	Exposure Dosage[a] (ng/kg)	Margin of Exposure[b]
U.S. Population	280	5,000
Western Region	330	4,200
All Infants	938	1,500
Nursing Infants (<1 yr old)	673	2,100
Non-Nursing Infants (<1 yr old)	995	1,400
Children (1-6 yrs)	656	2,100
Children (7-12)	413	3,400
Females (13+ yrs/pregnant/not nursing)	164	8,600
Females (13+ yrs/nursing)	391	3,600
Females (13-19 yrs/not pregnant/not nursing)	233	6,000
Females (20+ yrs/not pregnant/not nursing)	168	8,300
Females (13-50 yrs)	191	7,300
Males (13-19 yrs)	321	4,400
Males (20+ yrs)	175	8,000
Seniors (55+ yrs)	148	9,500
Workers (M & F, 16+ yrs)	179	7,800

[a] Based on the 95th exposure percentile for all user-day population subgroups, after adjusting for oral absorption (70%).

[b] Margin of Exposure = Adjusted Acute NOEL (1.4 mg/kg) / Dietary Exposure. Values are rounded to two significant figures.

oral absorption ranged from 148 mg/kg/day for seniors 55 years and older to 995 ng/kg/day for non-nursing infants, less than 1 year old (Table 30). The resultant MOEs ranged from 1,400 for non-nursing infants less than 1 year old to 9,500 for seniors 55 years and older. Since the MOEs were greater than 1,000 for all population groups, no further analysis of secondary residues was warranted. Based on these MOEs, the tolerances for tribufos appear to be adequately protective for all population subgroups with regards to acute toxicity.

C. CHRONIC EXPOSURE

A chronic exposure assessment using residues equal to the established tolerances for individual or combinations of commodities has not been conducted because it is highly improbable that an individual would chronically consume single or multiple commodities with pesticide residues at the tolerance levels. Support for this conclusion comes from the DPR pesticide monitoring programs which indicate that less than one percent of all sampled commodities have residue levels at or above the established tolerance (DPR, 1996).

VII. CONCLUSIONS

The risks for potential adverse human health effects with occupational and dietary exposure to tribufos were evaluated. The MOEs for acute dermal effects were greater than 100 for all pesticide workers. The MOEs for acute systemic effects was greater than 100 for all handlers involved in application of tribufos. The acute MOEs for systemic effects were also greater than 100 for all field workers, except with irrigators and weeders with a 4-day REI. The MOEs for seasonal occupational exposure were less than 100 for all pesticide workers, except ground applicators and module builder operators. The estimated oncogenic risk for pesticide workers ranged from approximately 10^{-5} to 10^{-6}. The acute and chronic MOEs for dietary exposure based on anticipated residues in cottonseed products were greater than 1,000 for all population subgroups. The acute and chronic MOEs for combined dietary and ambient air exposure were also greater than 1,000. The estimated oncogenic risk from dietary exposure in the U.S. population was estimated to be between 10^{-6} and 10^{-7}. The estimated oncogenic risk from combined dietary and ambient air exposure were also between 10^{-6} and 10^{-7}. The acute dietary MOEs based on the tolerance for tribufos residues on cottonseed were greater than 1,000 for all population subgroups.

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APPENDICES

APPENDIX A Equations for Inhalation Studies

APPENDIX B Oncogenicity Computer Model Printout

APPENDIX C DEEM Acute and Chronic Dietary Analyses Printouts

APPENDIX A - EQUATIONS FOR INHALATION STUDIES

1. Dose estimation for animals from an inhalation study when exposure level is in mg/m^3:

$$dose\ (mg/kg/day) = mg/m^3 \times RR_a \times \frac{hours/day}{24\ hours} \times \frac{days/week}{7\ days} \times AF$$

2. Dose estimation for animals from an inhalation study when exposure level is in ppm:

$$dose\ (mg/kg/day) = ppm \times \frac{M.Wt.}{M.Vol.} \times RR_a \times \frac{hours/day}{24\ hours} \times \frac{days/week}{7\ days} \times AF$$

NOTE: 1 mg/m^3 = 1 µg/liter
1 ppm = 1 µg/ml
M.Wt. = molecular weight in grams
M.Vol. = molecular volume which is 24.45 liters at 25°C
RR = respiratory rate in m^3/kg/day where a is for animal and h is for human.
AF = respiratory retention/absorption factor

APPENDIX B

Oncogenicity Computer Model Printout

DATE: 04-05-96 TIME: 09:09:17

MULTI-WEIB (MAR 1985)

K.S. CRUMP & COMPANY, INC.
1201 GAINES STREET
RUSTON, LA 71270
(318) 255-4800

Liver Hemangiosarcomas in Males - Term. Sac. Nonfatal

THE 16 OBSERVATIONS AT LEVEL 1 WITH A DOSE OF .000000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
25.0	1	0	51.0	1	0
53.0	1	0	61.0	1	0
72.0	1	0	76.0	1	0
78.0	1	0	80.0	1	0
84.0	1	0	84.0	1	3
85.0	1	0	87.0	2	0
88.0	1	0	89.0	1	0
90.0	1	0	91.0	34	0

THE 14 OBSERVATIONS AT LEVEL 2 WITH A DOSE OF .220000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
49.0	1	0	51.0	1	0
55.0	1	0	57.0	1	0
60.0	1	0	65.0	1	0
72.0	1	0	79.0	2	0
81.0	1	0	82.0	1	0
83.0	1	0	90.0	2	0
91.0	35	0	91.0	1	2

THE 19 OBSERVATIONS AT LEVEL 3 WITH A DOSE OF 1.21000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
44.0	1	0	47.0	1	0
53.0	1	0	59.0	1	3
64.0	1	0	64.0	1	3
68.0	1	0	69.0	1	0
70.0	1	0	75.0	1	0
80.0	1	0	81.0	1	3
81.0	1	0	83.0	1	0

87.0	3	0	88.0	3	0
89.0	1	0	91.0	28	0
91.0	1	2			

THE 26 OBSERVATIONS AT LEVEL 4 WITH A DOSE OF 6.91000

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
25.0	1	0	32.0	1	0
47.0	1	0	52.0	1	0
64.0	1	0	69.0	2	0
70.0	1	0	72.0	1	0
73.0	1	0	74.0	1	0
75.0	2	3	75.0	3	0
76.0	1	0	77.0	1	0
77.0	1	3	78.0	1	0
79.0	2	3	83.0	1	0
85.0	1	0	85.0	1	3
87.0	2	0	88.0	1	0
89.0	1	0	90.0	1	0
91.0	19	0	91.0	1	2

FORM OF PROBABILITY FUNCTION:

$$P(DOSE) = 1 - \exp((-Q0 - Q1 * D - Q2 * D^2 - Q3 * D^3) * (T - T0)^J)$$

THE MAXIMUM LIKELIHOOD ESTIMATION OF:

PROBABILITY FUNCTION COEFFICIENTS

Q(0)= .143009876262E-09
Q(1)= .169936984452E-09
Q(2)= .000000000000
Q(3)= .000000000000

TIME FUNCTION COEFFICIENTS

T0 = 24.9999090000
J = 4.55852108337

THE MAXIMUM LIKELIHOOD IS -31.4923915518

MAXIMUM LIKELIHOOD ESTIMATES OF EXTRA RISK

****

WEIBULL LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK
****

RISK	MLE DOSE	LOWER BOUND ON DOSE	UPPER BOUND ON RISK	CONFIDENCE LIMIT INTERVAL	TIME
1.000000E-06	2.987309E-05	1.666645E-05	1.792408E-06	95.0%	91.0000

WEIBULL UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE
****

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT INTERVAL	TIME
1.00000	3.292087E-02	5.901792E-02	95.0%	91.0000

NORMAL COMPLETION!

APPENDIX C

DEEM Acute and Chronic Dietary Analyses Printouts

California Department of Pesticide Regulation Ver. 7.76
DEEM Acute analysis for Tribufos
Residue file name: H:\MyFiles\DEEM Files\DEF\DEF acute.rs7
Analysis Date 06-03-2002 Residue file dated: 06-03-2002/15:58:05/14
Reference dose (NOEL) = 1.4 mg/kg bw/day

Food Code	Crop Grp	Food Name	Def Res (ppm)	Adj.Factors #1	#2	Comment
290	O	Cottonseed-oil	0.750000	1.000	0.700	Residu
		Full comment: Residue studies + processing factor for oil				
291	O	Cottonseed-meal	0.100000	1.000	0.700	Residu
		Full comment: Residue studies + processing factor for meal				
318	D	Milk-nonfat solids	0.002000	1.000	0.700	Tolera
		Full comment: Tolerance for milk				
319	D	Milk-fat solids	0.002000	1.000	0.700	Tolera
		Full comment: Tolerance for milk				
320	D	Milk sugar (lactose)	0.002000	1.000	0.700	Tolera
		Full comment: Tolerance for milk				
321	M	Beef-meat byproducts	0.020000	1.000	0.700	Tolera
		Full comment: Tolerance for beef				
322	M	Beef-other organ meats	0.020000	1.000	0.700	Tolera
		Full comment: Tolerance for beef				
323	M	Beef-dried	0.016000	1.920	0.700	Residu
		Full comment: Residue studies + distribution factor for muscle				
324	M	Beef-fat w/o bones	0.020000	1.000	0.700	Tolera
		Full comment: Tolerance for beef				
325	M	Beef-kidney	0.020000	1.000	0.700	Tolera
		Full comment: Tolerance for beef				
326	M	Beef-liver	0.020000	1.000	0.700	Tolera
		Full comment: Tolerance for beef				
327	M	Beef-lean (fat/free) w/o bones	0.016000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for muscle				
328	M	Goat-meat byproducts	0.083000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
329	M	Goat-other organ meats	0.083000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
330	M	Goat-fat w/o bone	0.016100	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for fat				
331	M	Goat-kidney	0.000900	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for kidney				
332	M	Goat-liver	0.083000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
333	M	Goat-lean (fat/free) w/o bone	0.000130	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for muscle				
336	M	Sheep-meat byproducts	0.083000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
337	M	Sheep-other organ meats	0.083000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
338	M	Sheep-fat w/o bone	0.016100	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for fat				
339	M	Sheep-kidney	0.000900	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for kidney				
340	M	Sheep-liver	0.083000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
341	M	Sheep-lean (fat free) w/o bone	0.000130	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for muscle				
398	D	Milk-based water	0.002000	1.000	0.700	Tolera
		Full comment: Tolerance for milk				
424	M	Veal-fat w/o bones	0.020000	1.000	0.700	Tolera
		Full comment: Tolerance for beef				

425	M	Veal-lean (fat free) w/o bones	0.016000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for muscle				
426	M	Veal-kidney	0.020000	1.000	0.700	Tolera
		Full comment: Tolerance for beef				
427	M	Veal-liver	0.020000	1.000	0.700	Tolera
		Full comment: Tolerance for beef				
428	M	Veal-other organ meats	0.020000	1.000	0.700	Tolera
		Full comment: Tolerance for beef				
429	M	Veal-dried	0.016000	1.920	0.700	Residu
		Full comment: Residue studies + distribution factor for muscle				
430	M	Veal-meat byproducts	0.020000	1.000	0.700	Tolera
		Full comment: Tolerance for beef				

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

U.S. Population — Daily Exposure Analysis /a (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000037	0.000037
Standard Deviation	0.000043	0.000043
Standard Error of mean	0.000000	0.000000
Margin of Exposure 2/	38,034	37,621

Percent of Person-Days that are User-Days = 98.91%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000007	205,521	90.00	0.000079	17,772
20.00	0.000011	122,216	95.00	0.000110	12,682
30.00	0.000016	89,260	97.50	0.000144	9,738
40.00	0.000020	68,870	99.00	0.000198	7,081
50.00	0.000026	54,753	99.50	0.000252	5,549
60.00	0.000031	44,577	99.75	0.000330	4,246
70.00	0.000040	35,403	99.90	0.000453	3,089
80.00	0.000053	26,650			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000006	223,630	90.00	0.000078	17,892
20.00	0.000011	126,553	95.00	0.000110	12,736
30.00	0.000015	91,151	97.50	0.000143	9,769
40.00	0.000020	69,981	99.00	0.000197	7,120
50.00	0.000025	55,442	99.50	0.000251	5,567
60.00	0.000031	44,991	99.75	0.000329	4,258
70.00	0.000039	35,675	99.90	0.000451	3,102
80.00	0.000052	26,847			

a/ Analysis based on all two-day participant records in CSFII 1994-98 survey.
2/ Margin of Exposure = NOEL/ Dietary Exposure.

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Western region

Daily Exposure Analysis (mg/kg body-weight/day)	per Capita	per User
Mean	0.000039	0.000040
Standard Deviation	0.000048	0.000049
Standard Error of mean	0.000000	0.000000
Margin of Exposure	35,740	35,235

Percent of Person-Days that are User-Days = 98.59%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000007	211,330	90.00	0.000085	16,566
20.00	0.000012	120,125	95.00	0.000120	11,699
30.00	0.000016	86,014	97.50	0.000155	9,022
40.00	0.000021	66,585	99.00	0.000226	6,184
50.00	0.000027	52,599	99.50	0.000289	4,851
60.00	0.000033	42,060	99.75	0.000361	3,883
70.00	0.000042	33,400	99.90	0.000522	2,680
80.00	0.000055	25,305			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000006	235,332	90.00	0.000084	16,663
20.00	0.000011	125,920	95.00	0.000119	11,750
30.00	0.000016	88,707	97.50	0.000155	9,047
40.00	0.000021	67,720	99.00	0.000225	6,232
50.00	0.000026	53,412	99.50	0.000288	4,859
60.00	0.000033	42,666	99.75	0.000360	3,890
70.00	0.000041	33,790	99.90	0.000521	2,689
80.00	0.000055	25,543			

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DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

All infants

Daily Exposure Analysis (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000030	0.000045
Standard Deviation	0.000059	0.000068
Standard Error of mean	0.000001	0.000002
Margin of Exposure	47,060	31,435

Percent of Person-Days that are User-Days = 66.80%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000006	248,732	90.00	0.000116	12,077
20.00	0.000010	138,670	95.00	0.000174	8,030
30.00	0.000014	99,278	97.50	0.000240	5,826
40.00	0.000017	80,520	99.00	0.000334	4,187
50.00	0.000021	65,797	99.50	0.000405	3,452
60.00	0.000026	53,388	99.75	0.000479	2,924
70.00	0.000035	40,245	99.90	0.000570	2,456
80.00	0.000056	25,208			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000000	>1,000,000	90.00	0.000078	18,013
20.00	0.000000	>1,000,000	95.00	0.000140	10,009
30.00	0.000000	>1,000,000	97.50	0.000194	7,198
40.00	0.000006	245,484	99.00	0.000289	4,848
50.00	0.000012	115,719	99.50	0.000381	3,674
60.00	0.000017	80,341	99.75	0.000472	2,966
70.00	0.000023	60,288	99.90	0.000520	2,690
80.00	0.000035	40,155			

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DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Nursing infants (<1 yr old)

Daily Exposure Analysis (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000009	0.000023
Standard Deviation	0.000027	0.000038
Standard Error of mean	0.000001	0.000002
Margin of Exposure	152,235	60,896

Percent of Person-Days that are User-Days = 40.00%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000001	>1,000,000	90.00	0.000055	25,556
20.00	0.000003	434,994	95.00	0.000096	14,646
30.00	0.000006	239,852	97.50	0.000163	8,615
40.00	0.000008	175,635	99.00	0.000205	6,817
50.00	0.000011	126,386	99.50	0.000225	6,214
60.00	0.000015	95,665	99.75	0.000266	5,265
70.00	0.000019	72,869	99.90	0.000331	4,226
80.00	0.000027	51,547			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000000	>1,000,000	90.00	0.000022	62,235
20.00	0.000000	>1,000,000	95.00	0.000045	31,063
30.00	0.000000	>1,000,000	97.50	0.000082	17,015
40.00	0.000000	>1,000,000	99.00	0.000163	8,615
50.00	0.000000	>1,000,000	99.50	0.000190	7,381
60.00	0.000000	>1,000,000	99.75	0.000225	6,233
70.00	0.000005	289,529	99.90	0.000266	5,265
80.00	0.000011	126,383			

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DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Non-nursing infants (<1 yr old) Daily Exposure Analysis
(mg/kg body-weight/day)

	per Capita	per User
Mean	0.000038	0.000049
Standard Deviation	0.000066	0.000071
Standard Error of mean	0.000001	0.000002
Margin of Exposure	37,282	28,696

Percent of Person-Days that are User-Days = 76.97%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000008	175,479	90.00	0.000125	11,194
20.00	0.000012	112,910	95.00	0.000187	7,488
30.00	0.000016	86,759	97.50	0.000261	5,373
40.00	0.000019	73,481	99.00	0.000379	3,693
50.00	0.000023	61,041	99.50	0.000434	3,226
60.00	0.000029	48,125	99.75	0.000492	2,843
70.00	0.000039	36,140	99.90	0.000573	2,443
80.00	0.000064	22,000			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000000	>1,000,000	90.00	0.000104	13,483
20.00	0.000000	>1,000,000	95.00	0.000166	8,429
30.00	0.000008	184,252	97.50	0.000232	6,046
40.00	0.000013	106,962	99.00	0.000333	4,206
50.00	0.000017	80,079	99.50	0.000405	3,455
60.00	0.000022	63,364	99.75	0.000476	2,938
70.00	0.000030	46,626	99.90	0.000570	2,456
80.00	0.000045	30,891			

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Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Children 1-6 yrs

Daily Exposure Analysis (mg/kg body-weight/day)	per Capita	per User
Mean	0.000100	0.000100
Standard Deviation	0.000077	0.000076
Standard Error of mean	0.000001	0.000001
Margin of Exposure	13,990	13,948

Percent of Person-Days that are User-Days = 99.69%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000035	39,711	90.00	0.000175	7,996
20.00	0.000049	28,304	95.00	0.000224	6,256
30.00	0.000061	22,782	97.50	0.000287	4,883
40.00	0.000072	19,327	99.00	0.000386	3,622
50.00	0.000084	16,600	99.50	0.000490	2,857
60.00	0.000097	14,378	99.75	0.000609	2,297
70.00	0.000113	12,399	99.90	0.000717	1,952
80.00	0.000135	10,343			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000035	40,206	90.00	0.000175	8,004
20.00	0.000049	28,492	95.00	0.000224	6,263
30.00	0.000061	22,877	97.50	0.000286	4,888
40.00	0.000072	19,376	99.00	0.000386	3,625
50.00	0.000084	16,641	99.50	0.000490	2,859
60.00	0.000097	14,403	99.75	0.000608	2,303
70.00	0.000113	12,418	99.90	0.000717	1,953
80.00	0.000135	10,357			

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Children 7-12 yrs

Daily Exposure Analysis (mg/kg body-weight/day)	per Capita	per User
Mean	0.000062	0.000062
Standard Deviation	0.000050	0.000050
Standard Error of mean	0.000001	0.000001
Margin of Exposure	22,764	22,728

Percent of Person-Days that are User-Days = 99.84%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000020	71,455	90.00	0.000111	12,577
20.00	0.000029	48,029	95.00	0.000143	9,816
30.00	0.000036	39,056	97.50	0.000181	7,740
40.00	0.000043	32,262	99.00	0.000249	5,615
50.00	0.000051	27,365	99.50	0.000327	4,285
60.00	0.000060	23,371	99.75	0.000412	3,399
70.00	0.000070	20,066	99.90	0.000548	2,556
80.00	0.000082	17,075			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000019	71,816	90.00	0.000111	12,586
20.00	0.000029	48,159	95.00	0.000143	9,822
30.00	0.000036	39,154	97.50	0.000181	7,746
40.00	0.000043	32,301	99.00	0.000249	5,617
50.00	0.000051	27,397	99.50	0.000327	4,287
60.00	0.000060	23,383	99.75	0.000412	3,400
70.00	0.000070	20,078	99.90	0.000548	2,556
80.00	0.000082	17,082			

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
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Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Females 13+ (preg/not nursing)

Daily Exposure Analysis (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000031	0.000032
Standard Deviation	0.000021	0.000021
Standard Error of mean	0.000002	0.000002
Margin of Exposure	45,468	44,317

Percent of Person-Days that are User-Days = 97.47%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000009	160,673	90.00	0.000057	24,650
20.00	0.000015	94,693	95.00	0.000075	18,546
30.00	0.000018	76,194	97.50	0.000081	17,191
40.00	0.000025	56,171	99.00	0.000089	15,706
50.00	0.000027	51,622	99.50	0.000131	10,672
60.00	0.000032	43,964	99.75	0.000132	10,641
70.00	0.000039	35,868	99.90	0.000132	10,623
80.00	0.000047	30,068			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000007	206,059	90.00	0.000057	24,674
20.00	0.000013	109,651	95.00	0.000075	18,560
30.00	0.000018	79,460	97.50	0.000081	17,201
40.00	0.000025	56,715	99.00	0.000089	15,714
50.00	0.000027	52,096	99.50	0.000131	10,674
60.00	0.000031	44,962	99.75	0.000132	10,642
70.00	0.000037	38,264	99.90	0.000132	10,623
80.00	0.000046	30,477			

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Females 13+ (nursing)

Daily Exposure Analysis (mg/kg body-weight/day)	per Capita	per User
Mean	0.000039	0.000039
Standard Deviation	0.000032	0.000032
Standard Error of mean	0.000003	0.000003
Margin of Exposure	36,249	36,249

Percent of Person-Days that are User-Days =100.00%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000009	157,759	90.00	0.000073	19,089
20.00	0.000015	94,246	95.00	0.000090	15,525
30.00	0.000018	77,213	97.50	0.000148	9,455
40.00	0.000024	57,842	99.00	0.000148	9,445
50.00	0.000031	45,460	99.50	0.000148	9,442
60.00	0.000037	37,449	99.75	0.000148	9,441
70.00	0.000046	30,579	99.90	0.000148	9,440
80.00	0.000058	24,308			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000009	157,759	90.00	0.000073	19,089
20.00	0.000015	94,246	95.00	0.000090	15,525
30.00	0.000018	77,213	97.50	0.000148	9,455
40.00	0.000024	57,842	99.00	0.000148	9,445
50.00	0.000031	45,460	99.50	0.000148	9,442
60.00	0.000037	37,449	99.75	0.000148	9,441
70.00	0.000046	30,579	99.90	0.000148	9,440
80.00	0.000058	24,308			

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Females 13-19 (not preg or nursing) Daily Exposure Analysis
(mg/kg body-weight/day)

	per Capita	per User
Mean	0.000032	0.000032
Standard Deviation	0.000026	0.000026
Standard Error of mean	0.000001	0.000001
Margin of Exposure	44,140	43,551

Percent of Person-Days that are User-Days = 98.67%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000008	177,215	90.00	0.000062	22,700
20.00	0.000012	115,045	95.00	0.000077	18,286
30.00	0.000016	84,956	97.50	0.000095	14,730
40.00	0.000021	65,661	99.00	0.000137	10,214
50.00	0.000026	53,253	99.50	0.000158	8,876
60.00	0.000032	44,113	99.75	0.000195	7,180
70.00	0.000038	36,425	99.90	0.000203	6,903
80.00	0.000047	29,967			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000007	199,763	90.00	0.000061	22,926
20.00	0.000012	121,449	95.00	0.000076	18,417
30.00	0.000016	87,036	97.50	0.000095	14,786
40.00	0.000021	66,624	99.00	0.000137	10,221
50.00	0.000026	53,650	99.50	0.000158	8,881
60.00	0.000031	44,625	99.75	0.000195	7,181
70.00	0.000038	36,566	99.90	0.000203	6,905
80.00	0.000046	30,150			

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Females 20+ (not preg or nursing) Daily Exposure Analysis (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000023	0.000023
Standard Deviation	0.000022	0.000022
Standard Error of mean	0.000000	0.000000
Margin of Exposure	61,855	61,440

Percent of Person-Days that are User-Days = 99.33%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000005	271,086	90.00	0.000044	31,837
20.00	0.000008	165,928	95.00	0.000057	24,747
30.00	0.000012	121,418	97.50	0.000072	19,391
40.00	0.000015	95,703	99.00	0.000103	13,648
50.00	0.000018	77,710	99.50	0.000135	10,398
60.00	0.000022	64,420	99.75	0.000155	9,057
70.00	0.000027	52,533	99.90	0.000192	7,293
80.00	0.000033	42,964			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000005	282,228	90.00	0.000044	31,962
20.00	0.000008	169,848	95.00	0.000056	24,789
30.00	0.000011	122,822	97.50	0.000072	19,428
40.00	0.000015	96,456	99.00	0.000102	13,691
50.00	0.000018	78,182	99.50	0.000134	10,409
60.00	0.000022	64,707	99.75	0.000155	9,060
70.00	0.000027	52,740	99.90	0.000192	7,296
80.00	0.000033	43,075			

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Females 13-50 yrs

Daily Exposure Analysis (mg/kg body-weight/day)	per Capita	per User
Mean	0.000026	0.000026
Standard Deviation	0.000024	0.000024
Standard Error of mean	0.000000	0.000000
Margin of Exposure	54,703	54,208

Percent of Person-Days that are User-Days = 99.10%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000005	256,368	90.00	0.000051	27,529
20.00	0.000009	153,362	95.00	0.000066	21,286
30.00	0.000013	110,709	97.50	0.000081	17,291
40.00	0.000016	87,380	99.00	0.000127	11,013
50.00	0.000020	68,962	99.50	0.000144	9,730
60.00	0.000025	56,338	99.75	0.000182	7,679
70.00	0.000030	46,756	99.90	0.000196	7,134
80.00	0.000037	37,486			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000005	271,742	90.00	0.000051	27,644
20.00	0.000009	158,182	95.00	0.000066	21,359
30.00	0.000012	113,072	97.50	0.000081	17,331
40.00	0.000016	88,265	99.00	0.000127	11,064
50.00	0.000020	69,561	99.50	0.000143	9,792
60.00	0.000025	56,888	99.75	0.000182	7,693
70.00	0.000030	47,019	99.90	0.000196	7,135
80.00	0.000037	37,620			

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Males 13-19 yrs

Daily Exposure Analysis (mg/kg body-weight/day)	per Capita	per User
Mean	0.000042	0.000043
Standard Deviation	0.000039	0.000039
Standard Error of mean	0.000001	0.000001
Margin of Exposure	33,058	32,859

Percent of Person-Days that are User-Days = 99.40%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000012	119,922	90.00	0.000081	17,226
20.00	0.000017	80,912	95.00	0.000104	13,521
30.00	0.000023	59,981	97.50	0.000125	11,178
40.00	0.000028	49,170	99.00	0.000183	7,645
50.00	0.000034	41,064	99.50	0.000232	6,034
60.00	0.000040	34,949	99.75	0.000355	3,940
70.00	0.000048	29,333	99.90	0.000539	2,597
80.00	0.000060	23,452			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000011	123,310	90.00	0.000081	17,250
20.00	0.000017	81,738	95.00	0.000103	13,528
30.00	0.000023	60,693	97.50	0.000125	11,193
40.00	0.000028	49,458	99.00	0.000183	7,646
50.00	0.000034	41,252	99.50	0.000232	6,036
60.00	0.000040	35,064	99.75	0.000355	3,940
70.00	0.000048	29,416	99.90	0.000539	2,597
80.00	0.000059	23,649			

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Males 20+ yrs

Daily Exposure Analysis (mg/kg body-weight/day)	per Capita	per User
Mean	0.000028	0.000028
Standard Deviation	0.000023	0.000023
Standard Error of mean	0.000000	0.000000
Margin of Exposure	50,016	49,706

Percent of Person-Days that are User-Days = 99.38%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000006	216,843	90.00	0.000054	25,967
20.00	0.000011	127,491	95.00	0.000069	20,260
30.00	0.000015	93,875	97.50	0.000089	15,772
40.00	0.000019	73,789	99.00	0.000114	12,323
50.00	0.000023	60,244	99.50	0.000130	10,782
60.00	0.000028	50,595	99.75	0.000145	9,672
70.00	0.000033	42,127	99.90	0.000204	6,852
80.00	0.000041	34,430			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000006	227,164	90.00	0.000054	26,009
20.00	0.000011	129,635	95.00	0.000069	20,303
30.00	0.000015	94,909	97.50	0.000089	15,796
40.00	0.000019	74,404	99.00	0.000113	12,354
50.00	0.000023	60,577	99.50	0.000130	10,784
60.00	0.000028	50,847	99.75	0.000145	9,677
70.00	0.000033	42,331	99.90	0.000204	6,856
80.00	0.000041	34,511			

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Seniors 55+

Daily Exposure Analysis (mg/kg body-weight/day)

	per Capita	per User
Mean	0.000022	0.000022
Standard Deviation	0.000018	0.000018
Standard Error of mean	0.000000	0.000000
Margin of Exposure	63,815	63,580

Percent of Person-Days that are User-Days = 99.63%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000006	250,585	90.00	0.000042	33,631
20.00	0.000009	156,557	95.00	0.000052	26,852
30.00	0.000012	115,875	97.50	0.000066	21,298
40.00	0.000015	93,952	99.00	0.000089	15,691
50.00	0.000018	77,459	99.50	0.000111	12,609
60.00	0.000021	65,234	99.75	0.000144	9,742
70.00	0.000026	54,025	99.90	0.000165	8,507
80.00	0.000032	44,227			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000005	255,168	90.00	0.000042	33,674
20.00	0.000009	158,051	95.00	0.000052	26,875
30.00	0.000012	116,519	97.50	0.000066	21,339
40.00	0.000015	94,412	99.00	0.000089	15,706
50.00	0.000018	77,714	99.50	0.000111	12,613
60.00	0.000021	65,404	99.75	0.000144	9,746
70.00	0.000026	54,157	99.90	0.000165	8,508
80.00	0.000032	44,297			

California Department of Pesticide Regulation Ver. 7.76
DEEM ACUTE Analysis for Tribufos (1994-98 data)
Residue file: DEF acute.rs7 Adjustment factor #2 used.
Analysis Date: 06-03-2002/16:00:45 Residue file dated: 06-03-2002/15:58:05/14
NOEL (Acute) = 1.400000 mg/kg body-wt/day
Daily totals for food and foodform consumption used.
Run Comment: ""

Custom demographics 1: Workers, 16+ yrs
All Seasons
All Regions
Sex: M/F-all/
All Races
Age-Low: 16 yrs High: 99 yrs

	Daily Exposure Analysis (mg/kg body-weight/day) per Capita	per User
Mean	0.000026	0.000026
Standard Deviation	0.000024	0.000024
Standard Error of mean	0.000000	0.000000
Margin of Exposure	53,752	53,374

Percent of Person-Days that are User-Days = 99.30%

Estimated percentile of user-days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000006	241,484	90.00	0.000051	27,494
20.00	0.000010	144,298	95.00	0.000066	21,158
30.00	0.000013	105,537	97.50	0.000083	16,781
40.00	0.000017	82,845	99.00	0.000116	12,072
50.00	0.000021	67,044	99.50	0.000134	10,467
60.00	0.000025	54,963	99.75	0.000155	9,028
70.00	0.000031	45,772	99.90	0.000206	6,810
80.00	0.000038	36,883			

Estimated percentile of per-capita days falling below calculated exposure in mg/kg body-wt/day with Margin of Exposure (MOE)

Percentile	Exposure	MOE	Percentile	Exposure	MOE
10.00	0.000006	253,186	90.00	0.000051	27,593
20.00	0.000009	148,105	95.00	0.000066	21,227
30.00	0.000013	107,130	97.50	0.000083	16,811
40.00	0.000017	83,769	99.00	0.000116	12,093
50.00	0.000021	67,483	99.50	0.000134	10,483
60.00	0.000025	55,277	99.75	0.000155	9,034
70.00	0.000030	45,972	99.90	0.000205	6,820
80.00	0.000038	37,030			

California Department of Pesticide Regulation Ver. 7.76
DEEM Chronic analysis for Tribufos 1994-98 data
Residue file: H:\MyFiles\DEEM Files\DEF\DEF chronic.rs7 Adjust. #2 used
Analysis Date 06-03-2002 Residue file dated: 06-03-2002/16:02:04/14
Reference dose (NOEL) = 0.07 mg/kg bw/day

Food Code	Crop Grp	Food Name	RESIDUE (ppm)	Adj.Factors #1	Adj.Factors #2	Comment
290	O	Cottonseed-oil	0.250000	1.000	0.700	Residu
		Full comment: Residue studies + processing factor for oil				
291	O	Cottonseed-meal	0.030000	1.000	0.700	Residu
		Full comment: Residue studies + processing factor for meal				
318	D	Milk-nonfat solids	0.000800	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for milk				
319	D	Milk-fat solids	0.000800	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for milk				
320	D	Milk sugar (lactose)	0.000800	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for milk				
321	M	Beef-meat byproducts	0.012000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for kidney				
322	M	Beef-other organ meats	0.012000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for kidney				
323	M	Beef-dried	0.006000	1.920	0.700	Residu
		Full comment: Residue studies + distribution factor for muscle				
324	M	Beef-fat w/o bones	0.007000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for fat				
325	M	Beef-kidney	0.012000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for kidney				
326	M	Beef-liver	0.010000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
327	M	Beef-lean (fat/free) w/o bones	0.006000	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for muscle				
328	M	Goat-meat byproducts	0.002800	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
329	M	Goat-other organ meats	0.002800	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
330	M	Goat-fat w/o bone	0.005400	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for fat				
331	M	Goat-kidney	0.000300	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for kidney				
332	M	Goat-liver	0.002800	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
333	M	Goat-lean (fat/free) w/o bone	0.000040	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for muscle				
336	M	Sheep-meat byproducts	0.002800	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
337	M	Sheep-other organ meats	0.002800	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
338	M	Sheep-fat w/o bone	0.005400	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for fat				
339	M	Sheep-kidney	0.000300	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for kidney				
340	M	Sheep-liver	0.002800	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for liver				
341	M	Sheep-lean (fat free) w/o bone	0.000040	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for muscle				
398	D	Milk-based water	0.000800	1.000	0.700	Residu
		Full comment: Residue studies + distribution factor for milk				
424	M	Veal-fat w/o bones	0.007000	1.000	0.700	Residu

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 Full comment: Residue studies + distribution factor for fat
425 M    Veal-lean (fat free) w/o bones     0.006000       1.000  0.700 Residu
 Full comment: Residue studies + distribution factor for muscle
426 M    Veal-kidney                        0.012000       1.000  0.700 Residu
 Full comment: Residue studies + distribution factor for kidney
427 M    Veal-liver                         0.010000       1.000  0.700 Residu
 Full comment: Residue studies + distribution factor for liver
428 M    Veal-other organ meats             0.012000       1.000  0.700 Residu
 Full comment: Residue studies + distribution factor for kidney
429 M    Veal-dried                         0.006000       1.920  0.700 Residu
 Full comment: Residue studies + distribution factor for muscle
430 M    Veal-meat byproducts               0.012000       1.000  0.700 Residu
 Full comment: Residue studies + distribution factor for kidney
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California Department of Pesticide Regulation Ver. 7.76
DEEM Chronic analysis for Tribufos (1994-98 data)
Residue file name: H:\MyFiles\DEEM Files\DEF\DEF chronic.rs7
Adjustment factor #2 used.
Analysis Date 06-03-2002/16:02:48 Residue file dated: 06-03-2002/16:02:04/14
NOEL (Chronic) = .07 mg/kg bw/day

Total exposure by population subgroup

Population Subgroup	Total Exposure mg/kg body wt/day	Total Exposure Percent of NOEL	Total Exposure Margin of Exposr 1/
U.S. Population (total)	0.000013	0.02%	5,251
U.S. Population (spring season)	0.000013	0.02%	5,247
U.S. Population (summer season)	0.000013	0.02%	5,411
U.S. Population (autumn season)	0.000014	0.02%	5,110
U.S. Population (winter season)	0.000013	0.02%	5,249
Northeast region	0.000013	0.02%	5,498
Midwest region	0.000014	0.02%	5,007
Southern region	0.000013	0.02%	5,515
Western region	0.000014	0.02%	4,934
Hispanics	0.000016	0.02%	4,487
Non-hispanic whites	0.000013	0.02%	5,524
Non-hispanic blacks	0.000014	0.02%	4,909
Non-hisp/non-white/non-black	0.000016	0.02%	4,353
All infants (< 1 year)	0.000011	0.02%	6,260
Nursing infants	0.000003	0.00%	20,370
Non-nursing infants	0.000014	0.02%	4,957
Children 1-6 yrs	0.000037	0.05%	1,907
Children 7-12 yrs	0.000022	0.03%	3,140
Females 13-19 (not preg or nursing)	0.000011	0.02%	6,146
Females 20+ (not preg or nursing)	0.000008	0.01%	8,617
Females 13-50 yrs	0.000009	0.01%	7,628
Females 13+ (preg/not nursing)	0.000011	0.02%	6,212
Females 13+ (nursing)	0.000014	0.02%	5,070
Males 13-19 yrs	0.000015	0.02%	4,584
Males 20+ yrs	0.000010	0.01%	6,940
Seniors 55+	0.000008	0.01%	8,838

California Department of Pesticide Regulation Ver. 7.76
DEEM Chronic analysis for Tribufos (1994-98 data)
Residue file name: H:\MyFiles\DEEM Files\DEF\DEF chronic.rs7
Adjustment factor #2 used.
Analysis Date 06-04-2002/11:27:35 Residue file dated: 06-04-2002/11:26:12/14
Q1 = 0.047

Total exposure by population subgroup

Population Subgroup	Total Exposure mg/kg body wt/day	Total Exposure Lifetime risk (Q1 = .047)
U.S. Population (total)	0.000013	6.27E-07
U.S. Population (spring season)	0.000013	6.27E-07
U.S. Population (summer season)	0.000013	6.08E-07
U.S. Population (autumn season)	0.000014	6.44E-07
U.S. Population (winter season)	0.000013	6.27E-07
Northeast region	0.000013	5.98E-07
Midwest region	0.000014	6.57E-07
Southern region	0.000013	5.97E-07
Western region	0.000014	6.67E-07
Hispanics	0.000016	7.33E-07
Non-hispanic whites	0.000013	5.96E-07
Non-hispanic blacks	0.000014	6.70E-07
Non-hisp/non-white/non-black	0.000016	7.56E-07
All infants (< 1 year)	0.000011	5.26E-07
Nursing infants	0.000003	1.62E-07
Non-nursing infants	0.000014	6.64E-07
Children 1-6 yrs	0.000037	1.73E-06
Children 7-12 yrs	0.000022	1.05E-06
Females 13-19 (not preg or nursing)	0.000011	5.35E-07
Females 20+ (not preg or nursing)	0.000008	3.82E-07
Females 13-50 yrs	0.000009	4.31E-07
Females 13+ (preg/not nursing)	0.000011	5.30E-07
Females 13+ (nursing)	0.000014	6.49E-07
Males 13-19 yrs	0.000015	7.18E-07
Males 20+ yrs	0.000010	4.74E-07
Seniors 55+	0.000008	3.72E-07

California Department of Pesticide Regulation Ver. 7.76
DEEM Chronic analysis for Tribufos (1994-98 data)
Residue file name: H:\MyFiles\DEEM Files\DEF\DEF chronic.rs7
Adjustment factor #2 used.
Analysis Date 06-04-2002/11:27:08 Residue file dated: 06-04-2002/11:26:12/14
Q1* = 0.084

Total exposure by population subgroup

Population Subgroup	Total Exposure mg/kg body wt/day	Total Exposure Lifetime risk (Q1*= .084)
U.S. Population (total)	0.000013	1.12E-06
U.S. Population (spring season)	0.000013	1.12E-06
U.S. Population (summer season)	0.000013	1.09E-06
U.S. Population (autumn season)	0.000014	1.15E-06
U.S. Population (winter season)	0.000013	1.12E-06
Northeast region	0.000013	1.07E-06
Midwest region	0.000014	1.17E-06
Southern region	0.000013	1.07E-06
Western region	0.000014	1.19E-06
Hispanics	0.000016	1.31E-06
Non-hispanic whites	0.000013	1.06E-06
Non-hispanic blacks	0.000014	1.20E-06
Non-hisp/non-white/non-black	0.000016	1.35E-06
All infants (< 1 year)	0.000011	9.39E-07
Nursing infants	0.000003	2.89E-07
Non-nursing infants	0.000014	1.19E-06
Children 1-6 yrs	0.000037	3.08E-06
Children 7-12 yrs	0.000022	1.87E-06
Females 13-19 (not preg or nursing)	0.000011	9.57E-07
Females 20+ (not preg or nursing)	0.000008	6.82E-07
Females 13-50 yrs	0.000009	7.71E-07
Females 13+ (preg/not nursing)	0.000011	9.47E-07
Females 13+ (nursing)	0.000014	1.16E-06
Males 13-19 yrs	0.000015	1.28E-06
Males 20+ yrs	0.000010	8.47E-07
Seniors 55+	0.000008	6.65E-07