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OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: **Isofenphos** [1-Methylethyl 2-{[ethoxy[(1-methylethyl)amino] phosphinothioyl] oxy] benzoate}: Hazard Identification Committee Report.

CASRN: 25311-71-1
PC Code: 109401
Caswell: 447AB

FROM: George Z. Ghali, PhD. G. Z. Ghali 12.22.97
Executive Secretary, Hazard Identification Committee
Health Effects Division (7509C)

Thru: Clark Swentzel Memorandum for C. Swentzel 12/23/97
Chairman, Hazard Identification Committee
Health Effects Division (7509C)

To: Tina Levine, PM 04
Insecticide-Rodenticide Branch
Registration Division (7505C)

The Health Effects Division-Hazard Identification Committee met on October 23, and 30 and on December 10 and 17, 1997 to evaluate the existing and/or recently submitted toxicology data in support of isofenphos re-registration, identify toxicological endpoints and dose levels of concern appropriate for use in risk assessments for different exposure routes and duration, and assess/reassess the reference dose for this chemical.

Material available for review consisted of data evaluation records (DERs) for an acute dermal toxicity study in rats **(81-2)**, an acute inhalation toxicity study in rats **(81-3)**, acute neurotoxicity study in rats **(81-8)**, a subchronic dermal toxicity study in rabbits **(82-3)**, a subchronic neurotoxicity study in rats **(82-7)**, a two-year feeding study in dogs **(83-1b)**, developmental toxicity studies in rats and rabbits **(83-3a and -3b)**, a two-generation reproductive toxicity study in rats **(83-4)**, a subchronic delayed neurotoxicity study in hens **(82-5)**, a metabolism study in rats **(85-1)** and a battery of mutagenicity studies **(84-2)**.

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INDIVIDUALS IN ATTENDANCE

Hazard Identification Committee members present, in at least one of the four meetings, were Karle Baetcke (Senior Science Advisor, HED), William Burnam (Chief, SAB, HED), George Ghali (Executive Secretary, Hazard Identification Committee, HED), Susan Makris, Nancy McCarroll, Melba Morrow, Kathleen Raffaele, John Redden, Jess Rowland, and Clark Swentzel (Chief TB II, Chairman, Hazard Identification Committee, HED). Hazard Identification Committee member(s) in absentia: David Anderson.

In attendance also were Stephen Dapson, Sanjivani Diwan, Pauline Wagner, Nicole Paguette, and Jonathan Becker, HED, as observers.

Scientific reviewer(s) (Committee or non-committee member(s) responsible for data presentation; signature(s) indicate technical accuracy of panel report and concurrence with the hazard identification assessment review unless otherwise stated.

Robert Fricke Robert F. Fricke Jan 6, 1998

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A. Acute Toxicity

I. TOXICOLOGY PROFILE:

A. Carcinogenicity:

The carcinogenicity issue has not been discussed by the Hazard Identification Committee in the meeting of October 23, 1997 since the rat carcinogenicity study was not submitted to the Committee at that time. Subsequently, on October 30, 1997, based on the toxicology data available, the Hazard Identification Committee determined that isofenphos did not alter the spontaneous tumor profile in rats or mice under the testing conditions. Therefore, it was recommended that isofenphos be classified as a **"Group E"**, indicating evidence of non-carcinogenicity for humans; i.e., the chemical is characterized as **"Not Likely"** to be carcinogenic in humans via relevant routes of exposure.

This weight of the evidence judgement is largely based on the absence of significant tumor increases in adequate carcinogenicity studies in rats (MRID No. 000000) and mice (MRID No. 000000). This classification is also supported by the lack of mutagenic activity in several mutagenicity assays (MRID Nos. 41609912, 41008801, 41008802).

It should be noted, however, that designation of an agent as being in **"Group E"** or **"Not Likely"** is based on the available evidence and should not be interpreted as a definitive conclusion that the agent will not be a carcinogen under any circumstances.

B. Neurotoxicity:

In an acute neurotoxicity study (MRID 44285601; Doc. No. 012306), isofenphos (92.5% Purity) was administered by a single gavage dose to fasted Wistar rats at nominal doses of 2, 8, or 15 mg/kg. The NOEL for neurotoxicity was not established. The LOEL of 2 mg/kg was based on inhibition of plasma, RBC, and brain cholinesterase, clinical signs (muscle fasciculation) in females. In addition, at 8 mg/kg, gait abnormalities and involuntary muscle movements were observed. At 15 mg/kg, there was a higher incidence of those findings along with uncoordinated righting reflex, decreased number of rearings, decreased forelimb and hindlimb grip strength, decreased body temperature, and decreased motor and locomotor activities on days 0 and 7 posttreatment. There were no effects on brain weight or indications of neuropathology at any treatment level.

In a 90-day neurotoxicity study in rats (MRID 44236601; Doc. No. 012306), isofenphos (91.6%) was administered to male and female Wistar rats at dietary levels of 1, 25, or 125 ppm (0.06, 1.62, or 8.45 mg/kg/day in males and 0.09, 2.07, or 11.54 mg/kg/day in females). The NOEL was 1 ppm (0.06 mg/kg/day in males and 0.09 mg/kg/day in females). The LOEL was 25 ppm (1.62/2.07 mg/kg/day in M/F), based on inhibition of plasma, RBC, and brain cholinesterase.

In addition, at 125 ppm, the HDT, clinical signs (piloerection, tremors, palmus, and nonspecific behavioral disturbances), decreased body weight gain and food consumption in the first week of study, FOB effects (muscle fasciculation in both sexes and abnormal gait and decreased grip strength in females), and a slow pupillary reflex in five males.

Several acute delayed neurotoxicity studies in hens described in the "toxicology one-liners" did not elicit neurotoxic effects at doses up to 100 mg/kg by gavage.

In a subchronic delayed neurotoxicity study (MRID 00146887, 41074101; Doc. No. 005435, 006808, 007612), hens were administered 92.5% isofenphos once daily by gavage to the crop at doses of 0.25, 1.00, or 2.00 mg/kg/day for 90 days. The study was negative for delayed neurotoxicity at a dose of 2.00 mg/kg/day. The study NOEL was 0.25 mg/kg/day, based on decreased plasma and/or RBC cholinesterase at the LOEL of 1.00 mg/kg/day. Additionally, at 2.00 mg/kg/day, mean body weight was depressed.

A neurotoxic esterase assay submitted to the Agency was declared invalid. The literature, however, indicates that isofenphos inhibits NTE in hens at high doses in vivo (Chow et al., 1986, as cited by Cherniack, 1988). A report of delayed neuropathy in an agricultural worker in the published literature described clinical manifestations, EMG, and nerve conduction assays compatible with a pathology of a distal, mainly axonal, motor neuropathy following accidental ingestion isofenphos.

There were no indications of effects on brain weight, and following processing of tissues without perfusion, no effects on the histopathology of the brain or peripheral nervous system were observed in the 2-year chronic dog study and 90-day rabbit dermal study. No other subchronic or chronic study DERs were provided for Committee review, but the one-liners did not describe findings of this nature.

C. **Reproductive and Developmental Toxicity**:

The following evaluation of the chemical isofenphos is provided to address FQPA considerations on the sensitivity of infants and children.

1. Reproductive Toxicity:

In a two-generation reproduction study in Wistar rats (MRID 41509902; Doc. No. 012311), isofenphos (92.9%) was administered at dietary concentrations of 1, 5, or 25 ppm (0.08-0.16, 0.44-0.69, or 2.21-3.92 mg/kg/day). The parental systemic NOEL was 1 ppm (0.08-0.16 mg/kg/day), based on plasma, RBC, and/or brain cholinesterase inhibition at 5 ppm (0.44-0.69 mg/kg/day), the parental systemic LOEL. In addition, at 25 ppm (2.21-3.92 mg/kg/day), treatment-

related increases in mortality and increases in absolute ovarian weights were observed. The offspring NOEL was 1 ppm (0.08-0.16 mg/kg/day) and the offspring LOEL was 5 ppm (0.44-0.69 mg/kg/day), based on clinical signs of toxicity (small to very small and emaciated pups) and increased pup mortality (observed as reductions in the lactation indices and mean litter sizes). Cholinesterase inhibition was apparently not measured in parental animals or pups.

2. Developmental Toxicity:

In a prenatal developmental toxicity study in Sprague-Dawley rats (MRID 42381201; Doc. No. 009740), 91.4% isofenphos was administered on gestation days 6-15 by gavage in carboxymethylcellulose and Tween 80 at dose levels of 0.05, 0.45, or 4.0 mg/kg/day. Cholinesterase activity was measured in dams (blood and brain) at days 16 and 20 and fetuses (brain only) at gestation day. The maternal NOEL was 0.05 mg/kg/day, and the maternal LOEL was 0.45 mg/kg/day, based on decreased plasma, RBC, and brain cholinesterase at gestation day 16. By gestation day 20, cholinesterase activity was recovered at 0.45 mg/kg/day and only RBC and brain cholinesterase activity was decreased at 4.0 mg/kg/day. No developmental toxicity was observed (developmental NOEL $\geq$4.0 mg/kg/day. Fetal brain cholinesterase activity was not altered. This study was included in the review by Astroff et al, 1996.

In a prenatal developmental toxicity study conducted in New Zealand white rabbits (MRID 42382801; Doc. No. 009896), isofenphos (91.4%) was administered by gavage in carboxymethylcellulose and Tween 80 at doses of 0.25, 1.25, or 7.5 mg/kg/day on gestation days 6-18. Cholinesterase activity was measured in dams (blood and brain) at days 19 and 29; fetal cholinesterase values were apparently not measured. The maternal cholinesterase inhibition NOEL was 0.25 mg/kg/day, based upon plasma cholinesterase inhibition on gestation day 19, and RBC and brain cholinesterase inhibition on gestation day 19 and 29, at the maternal cholinesterase inhibition LOEL of 1.25 mg/kg/day. The maternal systemic NOEL was 1.25 mg/kg/day, and the maternal systemic LOEL was 7.5 mg/kg/day, based upon increased mortality, decreased body weight and body weight gain, and decreased food consumption, in addition to the decreased cholinesterase activity. No developmental effects were observed (developmental NOEL $\geq$7.5 mg/kg/day).

3. Developmental Neurotoxicity:

In developing a weight of evidence for the need for a developmental neurotoxicity study on isofenphos, primary consideration was given to the following:

On one hand, administration of isofenphos, like most other organophosphorus pesticides, to various species results in plasma,

erythrocytes, and brain cholinesterase inhibition. Isofenphos also inhibits NTE in hens at high doses in vivo (Chow et al., 1986, as cited by Cherniack, 1988).

A report of delayed neuropathy in an agricultural worker in the published literature (Catz et al., 1988) described clinical manifestations, EMG, and nerve conduction assays compatible with a pathology of a distal, mainly axonal, motor neuropathy following accidental isofenphos ingestion.

Isofenphos is considered to be relatively acutely toxic, with oral LD_{50} values ranging from 28.8-38.7 mg/kg in 2 studies in the rat and from 91-127 mg/kg in the mouse. The dermal LD_{50} ranged from 70 to 191 mg/kg in rats and 315-1172 in rabbits. The LC_{50} ranged from 0.144 to 0.525 mg/L over 5 separate studies.

On the other hand, no evidence of abnormalities in the development of the fetal nervous system, were observed in the prenatal developmental toxicity studies in either rats or rabbits, at maternally toxic oral doses up to 4.0 or 7.5 mg/kg/day, respectively.

In the prenatal developmental toxicity study in rats, fetal brain cholinesterase was not different from control on gestation day 20, although maternal RBC and brain cholinesterase were inhibited at that time point.

Neither brain weight nor histopathology (nonperfused) of the nervous system were affected in the subchronic and chronic toxicity studies examined.

Acute and subchronic delayed neurotoxicity studies in hens were negative for OPIDN. Acute and subchronic neurotoxicity studies in rats did not indicate brain weight changes or neuropathological lesions.

The Committee determined that <u>a developmental neurotoxicity study in rats should be conducted</u> with isofenphos in order to assess functional development following prenatal exposure. The following information was considered in arriving at this decision.

D. **<u>FQPA Considerations</u>:**

Under the Food Quality Protection Act (FQPA), P.L. 104-170, which was promulgated in 1996 as an amendment to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug and Cosmetic Act (FFDCA), the Agency was directed to "ensure that there is a reasonable certainty that no harm will result to infants and children" from aggregate exposure to a pesticide chemical residue. The law further states that in the case of threshold effects, for purposes of providing this

reasonable certainty of no harm, "an additional tenfold margin of safety for the pesticide chemical residue and other sources of exposure shall be applied for infants and children to take into account potential pre- and post-natal toxicity and completeness of the data with respect to exposure and toxicity to infants and children. Notwithstanding such requirement for an additional margin of safety, the Administrator may use a different margin of safety for the pesticide residue only if, on the basis of reliable data, such margin will be safe for infants and children."

Pursuant to the language and intent of the FQPA directive regarding infants and children, the applicable toxicity database for isofenphos was evaluated by the Hazard Identification Committee. The following conclusions were made:

Adequacy of data: The data base included acceptable two-generation reproduction study in rats and prenatal developmental toxicity studies in rats and rabbits, meeting the FIFRA basic data requirements, as defined for a food-use chemical by 40 CFR Part 158. <u>However, the Committee recommend for a developmental neurotoxicity study in rats to assess functional development following prenatal exposure to isofenphos</u>. This is considered a data gap for the assessment of the effects of isofenphos following *in utero* and/or early postnatal exposure.

Susceptibility issues: In the three-generation reproduction study in rats and the prenatal developmental toxicity studies in rats and rabbits, there was no indication of increased sensitivity of the young animals to pre-and/or postnatal exposure to isofenphos.

Uncertainty factor: The Committee determined that for isofenphos the 10-fold uncertainty factor for the protection of infants and children would be retained because of the lack of a developmental neurotoxicity study in rats to assess functional development following prenatal exposure to isofenphos. This is considered a data gap for the evaluation of hazard to infants and children (see weight of the evidence under developmental neurotoxicity, above).

E. <u>Mutagenicity</u>:

Three acceptable mutagenicity studies were available for review. The following are summaries of the these studies and the Committee's conclusions:

1. Gene Mutations:

<u>Salmonella typhimurium</u> reverse gene mutation assay (MRID No. 41609912, HED Doc. No. 009749): The test was negative in <u>S. typhimurium</u> strains TA1535, TA1537, TA1538, TA98 and TA100 up to

the highest dose tested (10,000 μg/plate +/-S9). Compound precipitation was seen at concentrations ≥3333 μg/plate +/-S9.

2. Chromosomal Aberrations:

In vitro Chinese hamster ovary (CHO) cell chromosome aberration assay (MRID No. 41008801, HED Doc. No. 007192): The test was negative up to cytotoxic concentrations (≥0.04 μL/mL -S9; ≥0.08 μL/mL +S9).

3. Other Mutagenic Mechanisms:

Unscheduled DNA synthesis (UDS) in cultured primary rat hepatocytes assay (MRID No. 41008802; Doc. No. 007192): The test was negative up to cytotoxic doses (≥0.03 μL/mL). Concentrations ≥1.0 μL/mL were insoluble.

4. Other Information:

Open literature information available indicated that isofenphos is not mutagenic in bacteria, clastogenic in vitro in mammalian cells, or genotoxic in cultured primary rat hepatocytes.

The submitted test battery satisfies the pre-1991 mutagenicity initial testing battery guidelines. No further testing is required at this time.

F. Dermal Absorption:

There were no dermal absorption studies appropriate for use for the purpose of risk assessment. The 21-day dermal toxicity study with formulations, and the 90-day dermal with the technical material, were conducted in rabbits. This species is inappropriate to conduct dermal studies with organophosphorus compound requiring activation, i.e, thiophosphates which are normally activated to phosphates. There were no dermal absorption studies conducted in rats, the most sensitive species in this case. Therefore, the default value of 100% will be used for the dermal absorption rate.

II. HAZARD IDENTIFICATION:

Based on comprehensive evaluation of the toxicology data available on isofenphos, toxicology endpoints and dose levels of concern have been identified for use in risk assessments corresponding to the hazard categories indicated below:

Dietary Hazard resulting from ingestion of residues of this particular pesticide when used on agricultural food commodities for pest control purposes or as a food additive and may include **acute and/or chronic exposure,**

Occupational/Residential Hazard resulting from **dermal and/or inhalation** exposure to the chemical and may include **short-, intermediate-, and/or long-term exposure.**

Issues related to the Food Quality Protection Act (FQPA), P.L. 104-170, which was promulgated in 1996 as an amendment to the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug and Cosmetic Act (FFDCA) are also addressed.

Where no appropriate data have been identified for a particular duration or exposure scenario, or if a risk assessment is not warranted, this is noted. Levels of uncertainties associated with intraspecies variability, interspecies extrapolation, route to route conversion, or variable duration extrapolation are also addressed.

Based on the use pattern/exposure profile, the Committee determined that the risk assessments indicated below are required for isofenphos.

Dietary Exposure

A. Acute Dietary Exposure (one day):

Critical Study: Acute Oral Neurotoxicity Study in Rats (81-8), MRID No. 44285601.

Male and female Wistar rats (12/sex/dose, main study; 6/sex/dose, satellite study) were fasted overnight and then orally dosed once with Isofenphos (92.5%) at nominal doses of 0 (vehicle), 2, 8, or 15 mg/kg (analytically confirmed doses: 0, 2.62, 7.86 or 13.82 mg/kg, respectively). Main study animals were evaluated for neurobehavioral effects (FOB and motor activity) on day 0, at the peak time-of-effect (1 hr 50 min (minimum), males; 5 hr (minimum), females) and days 7 and 14; neuropathological examination were carried out at terminal sacrifice (day 14) on 6 animals/sex/dose. The satellite group was used for determination of plasma, RBC and brain

cholinesterase activities at the peak time-of-effect on day 0.

Clinical signs and FOB evaluations were consistent with acute cholinergic toxicity. At the mid-dose level, gait abnormalities and involuntary motor movements were observed in males and females. In high-dose males and females, a higher incidence of these findings was observed along with uncoordinated righting reflex, decreased number of rearings, decreased forelimb and hindlimb grip strength and decreased body temperature. No reaction to the approach response was noted in 4/12 high-dose males. In general, the onset of clinical signs started sooner in males (4 hr) than in females (8 hrs), but did not last as long (day 6, males; day 7, females).

Decreases in mean body weighs and body weight gains were observed in high-dose males and females. Following an overnight fast, high-dose males lost a significant ($p \leq 0.05$, 4%) amount of body weight. At day 7, the body weights of high-dose males and females were 11% and 7% lower, respectively, than the concurrent control values. By day 14, males regained some, but not all, of the lost body weight; the mean body weight was, however, still significantly lower than the concurrent control value. By day 14, the mean body weight of high-dose females was comparable to the control value. Body weight gain from day 0 to day 7 was 38% lower in males and 37% lower in females in the high-dose group. Overall body weight gain (day 0 to 14) for high-dose males was 18% lower for males, while that of high-dose females was comparable to the control value.

High-dose animals had significantly decreased motor (58%, males; 64% females) and locomotor (79%, males; 85%, females) activities on day 0 (peak time-of-effect). The day 7 evaluation of high-dose animals showed a decrease in motor activity of 28% (not significant) in females and decreased locomotor activity of 29% (not significant) in males and 34% ($p \leq 0.05$) in females.

Plasma, RBC and brain cholinesterase activity was statistically significantly ($p \leq 0.01$) decreased in low- mid- and high-dose males and females at the peak time-of-effect on day 0. At the low-dose level, plasma, RBC and brain cholinesterase activities were decreased 59 to 89%, 18 to 55%, and 10 to 21%, respectively. At the mid-dose level, plasma, RBC and brain Chew activities were significantly decreased 85 to 97%, 68 to 89%, and 51 to 69%, respectively. At the high-dose level plasma cholinesterase was inhibited 94 to 98%, RBC cholinesterase, 95 to 98%, and brain cholinesterase, 83 to 85%.

At terminal sacrifice, gross examination did not reveal any treatment-related effects. Terminal body weights of high-dose animals were significantly lower (10%, $p \leq 0.05$) than control values. The body weights of mid- and low dose animals and the absolute and relative brain weights of treated animals were

comparable to controls. Neuropathological findings of treated animals were comparable to control animals.

Based on the results of this study [inhibition of plasma, RBC and brain cholinesterase with clinical signs (muscle fasciculation) in females], the LOEL was established at 2 mg/kg, the lowest dose level tested. The NOEL was not established.

Endpoint and Dose Level Selected for Use in Risk Assessment: The NOEL was not established in this study. The LOEL is 2.0 mg/kg/day based on inhibition of plasma, RBC and brain Chew with clinical signs (muscle fasciculation) in females.

Uncertainty Factor (UF): A UF of 3000 was applied; this includes a UF of 100 to account for both interspecies extrapolation and intraspecies variability, an additional UF of 3 to account for the lack of a NOEL, and an additional UF of 10 for FQPA considerations.

Comments: The findings of this study are supported by the findings of an oral developmental toxicity study in the rat with a parental NOEL of 0.05 mg/kg/day based on cholinesterase inhibition observed at the next higher dose level of 0.45 mg/kg/day.

B. <u>Chronic Dietary Exposure-Reference Dose (RfD)</u>:

Reference Dose (RfD): 0.00008 mg/kg/day

Critical Study: 2-Generation Reproductive Toxicity Study in Rats (83-4), MRID 41609902.

Executive Summary: In this study, SRA 12869 (92.9%) was administered to Bor strain:WISW (SPF Cpb) rats (25/sex/dose) at dietary levels of 0, 1, 5, or 25 ppm (achieved doses of 0, 0.08-0.16, 0.44-0.69, or 2.21-3.92 mg/kg/day). Exposure to F_0 animals began at 5 weeks of age and lasted for 13 weeks prior to mating the first time to produce F_{1a} pups. F_0 animals were mated a second time to produce F_{1b} pups. At 4 weeks of age, F_{1b} pups were selected to become parents of the F_{2a} and F_{2b} generations and were given the same concentration of SPA 12869 in their diets as their dam. The F_{1b} parental animals were given test diets for approximately 12 weeks prior to mating the first time to produce the F_{2a} pups. Exposure of the test material to all animals was continuous in the diet throughout the study.

Parental toxicity was characterized at the mid-dose as reductions in cholinesterase activity in plasma (18.5-31.9%, $p \leq 0.01$, both sexes) and in erythrocytes (7.1%, $p \leq 0.05$, females only). At the high-dose, treatment-related reductions in cholinesterase activity in the brain (27.0%, males; 31.8%,

females; $p \leq 0.01$), plasma (16.5-26.4%, $p \leq 0.01$, both sexes), and RBC (53.7-80.7%, $p \leq 0.01$, both sexes) were noted. In addition at the high-dose, treatment-related increases in mortality (12%, F_0 females) and increases in absolute ovarian weights (F_0, 9%; F_{1b}, 12%; $p \leq 0.05$) were noted.

No treatment-related clinical findings or changes in body weights, body weight gains, food consumption, or reproductive indices were noted in either sex of either generation throughout the study.

The LOEL for systemic toxicity is 5 ppm (0.44-0.69 mg/kg/day) based on reductions in plasma and RBC cholinesterase activities. The systemic NOEL is 1 ppm (0.08-0.16 mg/kg/day).

Reproductive toxicity was demonstrated at 5 ppm as treatment-related increases in the number of litters with small to very small pups (F_{1b}) and emaciated pups (F_{2b}). For the F_{1b} mid-dose litters, treatment-related reductions were noted in the lactation index (34.9% vs. 63.5% for controls, $p \leq 0.01$) and in mean litter sizes for days 14-28 (47%, $p \leq 0.01$). The lactation index was also decreased for the mid-dose F_{2b} litters (71.2% vs. 89.6% in controls, $p \leq 0.01$).

At 25 ppm, treatment-related increases in the numbers of litters with small to very small pups (F_{1a} and F_{1b}), cold pups (F_{1b} and F_{2b}), and emaciated pups (F_{2b}) were observed. For the high-dose F_{1a} and F_{1b} litters, treatment-related increases were noted in the number of deaths between days 5-28 and related reductions were observed in mean litter sizes on days 14-28 (F_{1a}, 47%, $p \leq 0.01$) or 7-28 (F_{1b}, 34-60%, $p \leq 0.01$ or ≤ 0.05), number of pups alive by day 28, and lactational indices (F_{1a}: 47.1% vs. 88.1% for controls, $p \leq 0.01$; F_{1b}: 11.8% vs. 63.5% for controls, $p \leq 0.01$). In addition for the F_{1b} litters, a treatment-related reduction in the viability index was noted (75.8% vs. 96.6% for controls, $p \leq 0.01$). For the high-dose F_{2b} litters, treatment-related reductions in the viability index (91.5% vs. 99.1% for controls, $p \leq 0.01$) and lactation index (70.0% vs. 89.6%, $p \leq 0.01$) were observed. For both generations, the total number of pups born was reduced at the high-dose; this was because of increased mortality of the F_0 dams and their offspring (only 9 F_{1b} females were available for mating) resulting in a smaller number of females which gave birth. A treatment-related reduction in pup body weights during lactation was also noted at the high-dose (F_{1a}, 11-19% $p \leq 0.01$ or 0.05; F_{1b}, 23-29%, $p \leq 0.01$).

The LOEL for reproductive toxicity is 5 ppm (0.44-0.69 mg/kg/day) based on clinical signs of toxicity (small to very small and emaciated pups) and increased pup mortality (reductions in the lactation indices and mean litter sizes). The reproductive NOEL is 1 ppm (0.08-0.16 mg/kg/day).

Endpoint and Dose selected for use in risk assessment: The reproductive NOEL is 1 ppm (0.08-0.16 mg/kg/day), based on clinical signs of toxicity (small to very small and emaciated pups) and increased pup mortality (reductions in the lactation indices and mean litter sizes) observed at 5 ppm (0.44-0.69 mg/kg/day).

Uncertainty Factor (UF): An uncertainty factor of 1000 was applied; this includes a UF of 100 to account for both interspecies extrapolation and intraspecies variability. An additional UF of 10 was recommended for FQPA considerations.

The use of a UF of 100 to account for interspecies extrapolation and intraspecies variability was justified based on the availability of two chronic toxicity studies (in rodent and non-rodent species) and the reproductive toxicity study in rats, in accordance with the rules established by the Agency-IRIS (Integration Risk Information System) Work Group.

Comments and Rationale: The NOEL and the effects observed in this study are supported by similar findings in the chronic dog study (MRID No. 92085016, 43198001).

C. **Short Term Occupational or Residential Exposure (1-7 days):**

Critical Study: Acute Oral Neurotoxicity Study (81-8), MRID No. 44285601.

For more details about this study or the executive summary, see Section II-A, above.

Endpoint and Dose Level selected for use in risk assessment: There NOEL was not established in this study. The LOEL is 2.0 mg/kg/day based on inhibition of plasma, RBC and brain Cholinesterase inhibition with clinical signs (muscle fasciculation) in females.

Uncertainty Factor (UF): A UF of 3000 was applied; this includes a UF of 100 to account for both interspecies extrapolation and intraspecies variability, an additional UF of 3 to account for the lack of a NOEL, and an additional UF of 10 for FQPA considerations.

Comments: Although two 21-day and a 90-day dermal toxicity studies were available on this chemical, and although these studies cover the time points of 1-7 days, the Committee recommended the use of an oral study for this purpose. This conclusion was based on the fact that the 21-say dermal toxicity studies were conducted with isofenphos formulations not with the technical material (in the rabbit), and the 90-day dermal toxicity study, though conducted with the technical material, was

also performed in the rabbit. The rabbit is considered inappropriate to conduct dermal studies with organophosphorus compound requiring metabolic activation, i.e, thiophosphates, phosphorothioates, and phosphorodithioates which are normally activated to the corresponding phosphates by the hepatic microsomal enzymes. These particular organophosphorus compounds, when administered dermally to the rabbit, are metabolically deactivated and lose their anticholinesterase properties via hydrolytic cleavage of the ester bond by esterase enzymes normally present in the blood.

Because of the lack of a dermal absorption study and because of the similarity of toxicity via the oral and dermal routes as evidenced in several acute oral and dermal toxicity studies, the Committee recommended the use of a dermal absorption rate of 100%.

D. **Intermediate Term Occupational or Residential Exposure (one week to several months):**

Critical Study: Subchronic Neurotoxicity Screening Study in Wistar Rats (82-7), MRID No.: 44236601.

Male and female Wistar rats (12/sex/dose) were fed diets containing Isofenphos (91.6%) at 0 (basal diet), 1, 25, or 125 ppm (mg/kg/day equivalents: 0, 0.06, 1.62, or 8.45, males; 0, 0.09, 2.07, or 11.54, females) for at least 13 weeks. Neurobehavioral evaluations, consisting of Functional Observational Battery and motor activity measurements, were performed at pretesting and after 4, 8 and 13 weeks of treatment. Gross pathology (all animals) and neuropathological (6/sex/dose) examinations were carried out at terminal sacrifice. Six animals/sex/dose were selected for determination of plasma and RBC cholinesterase activities at week 4 and plasma, RBC and brain cholinesterase activities at week 14.

Treatment-related, cholinergic signs were observed during the clinical evaluations of high-dose males and females. During the first two to four weeks of treatment, males and females showed piloerection and tremors; high incidences of palmus and non-specific behavioral disturbances (females only) were observed during the entire study. No treatment-related clinical signs were observed in the low and mid-dose groups. All animals survived to terminal sacrifice.

Mean body weights of high-dose males and females were statistically significantly lower than control values during the first six to seven weeks of the study. These decreases appear to be a result of decreased body weight gains of 51% in males and 100% (no weight gain) in females during the first week of the study. The decreased body weight gains appear to be a result of decreased food consumption (g/animals/day) of 19% in males and

35% in females. Excluding the body weight data for the first week of the study, the body weight gains for weeks 1 to 13 were the same as the control value in males and 11% greater than control value in females.

Plasma, RBC and brain cholinesterase activities of mid- and high-dose animals were all significantly decreased. The evaluations at week 4 for mid-dose animals showed significant decreases in plasma (54%, males; 84%, females) and RBC (64%, males; 81%, females) cholinesterase activities. At week 14, mid-dose animals had decreases in plasma, RBC and brain cholinesterase activities of 54%, 63% and 32% in males, respectively and 88%, 66% and 60% in females, respectively. At week 4, high-dose animals had decreases in plasma and RBC cholinesterase activities of 85% and 98%, in males, respectively and 97% and 100% in females, respectively. At week 14, plasma, RBC and brain cholinesterase activities of high-dose animals were decreased 84%, 96%, and 75% in males, respectively and 97%, 97%, and 89% in females, respectively.

Neurobehavioral evaluations revealed treatment-related effects in high-dose males and females, with females being more affected than males. Treatment-related FOB effects consisted in part, of muscle fasciculation in both sexes and abnormal gait and decreased grip strength in females. Motor and locomotor activities were significantly decreased in high-dose females.

Ophthalmological examination at week 13 revealed a slow pupillary reflex in five high-dose females, this is regarded as a treatment-related effect.

The incidences of gross and neuropathological finding of treated animals were comparable to controls.

Based on the results of this study (inhibition of plasma, RBC and brain cholinesterase, the LOEL was established at 25 ppm (1.62 mg/kg/day, males; 2.07 mg/kg/day, females); the NOEL was established at 1 ppm (0.06 mg/kg/day, males; 0.09 mg/kg/day, females).

Endpoint and Dose Level Selected for Use in Risk Assessment: The NOEL of 1 ppm (0.06 mg/kg/day, males; 0.09 mg/kg/day, females), based on inhibition of plasma, RBC and brain Chew observed at the next higher dose level of 25 ppm (1.62 mg/kg/day, males; 2.07 mg/kg/day, females).

Uncertainty Factor: An uncertainty factor of 1000 was applied; this includes a UF of 100 to account for both interspecies extrapolation and intraspecies variability. An additional UF of 10 was recommended for FQPA considerations.

Comments and Rationale: See comments and rationale for Section

II-C, above, for the explanation of why an oral toxicity was used for dermal risk assessment although dermal studies were available covering the range of 1-90 days, and what is the dermal absorption rate to be used for the derivation of the dermal equivalent dose in this case and why.

F. **Inhalation Exposure (variable duration):**

For the purpose of inhalation risk assessment of short and intermediate duration, the Committee recommended that the inhalation exposure be converted from mg/L to the equivalent mg/kg/day dose assuming an inhalation absorption rate of 100%. This dose should be compared to the oral LOEL of 2 mg/kg/day (generated in the acute neurotoxicity study, MRID No. 44236601), in the case of short term and compared to the oral NOEL of 0.06 mg/kg/day (generated in the subchronic neurotoxicity study, MRID No. 44236601) in the case of the intermediate-term risk assessment. Based on the use pattern and exposure profile, the Committee determined that the long-term inhalation risk assessment would not be required.

An Uncertainty Factor of 3000 was recommended for the short-term exposure. This includes a UF of 100 to account for both interspecies extrapolation and intraspecies variability, an additional UF of 3 to account for the lack of a NOEL, and an additional UF of 10 for FQPA considerations.

An Uncertainty Factor of 1000 was recommended for the intermediate-term exposure. This includes a UF of 100 to account for both interspecies extrapolation and intraspecies variability. An additional UF of 10 was recommended for FQPA considerations.

Comments and Rationale: Since there were no appropriate subchronic inhalation studies, but there was concern about potential inhalation exposure, the inhalation exposure was converted to an equivalent oral dose assuming 100% lung absorption. This was added to the dermal exposure (after assuming 100% dermal absorption) and compared to the oral neurotoxicity endpoint of either 2 or 0.06 mg/kg/day depending on the exposure duration.

G. **Aggregate Risk:**

Because of the similarity of the endpoints identified both in the dermal and inhalation exposure, i.e. cholinesterase inhibition, the following equation might be appropriate in expressing the aggregate risk for this chemical.

$$\text{Aggregate Risk} = \text{inverse } 1/MOE_{(dermal)} + 1/MOE_{(inhalation)}$$

III. References:

1. Astroff, B., G.K. Sangha, and J.H. Thyssen. (1996) The relationship between organophosphate-induced maternal cholinesterase inhibition and embryo/fetal effects in the Sprague-Dawley rat. *The Toxicologist* 30(1):191.

2. Catz, A., B. Chen, I. Jutrin, and L. Mendelson. (1988) Late onset isofenphos neurotoxicity. *Journal of Neurology, Neurosurgery, and Psychiatry* 51:1338-1340.

3. Cherniack, M.G. (1988) Toxicological screening for organophosphorus-induced delayed neurotoxicity: complications in toxicity testing. *NeuroToxicology* 9(2):249-272.

cc: Stephanie Irene
Robert Fricke
Clark Swentzel
Michael Metzger
Paula Deschamp
Karen Whitby
Jess Rowland
Amal Mahfouz (OW)
Hazard ID file
Caswell File