A Comparison of Oral and Inhalation Toxicities of Four Insecticides to Mice and Rats

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Knowledge of inhalation toxicity of pesticides applied by the ultra low volume (ULV) method is becoming increasingly important in assessing possible hazards to field workers. These aerosols include significant amounts of particles (3 μm or less) within the respirable range (LEWIS, et al., 1948). Because these particles reach the alveoli, there exists a potential for rapid translocation of chemicals into the blood stream.

We determined respiratory LD50 values to rodents in mg/kg body-weight for certain insecticides and compared such values with oral LD50 values. Four insecticide formulations were examined; three cholinergic organophosphorus compounds, chlorpyrifos [0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate], malathion [diethyl mercaptosuccinate,S-ester with 0,0-dimethyl phosphorodithioate], and naled [1,2-dibromo-2,2-dichloroethyl dimethyl phosphate], and one pyrethroid, resmethrin [(5-benzyl-3-furyl)methyl (±)-cis, trans-chrysanthemate].

MATERIALS AND METHODS

Aerosols of the formulations given in Table 1, with mass median diameter (MMD) 1.5-2.0 μm (geometric standard deviation 2.0) were generated from Wells type refluxing atomizers. The aerosol was drawn into a modified Henderson chamber containing eight rats or 16 mice. Details of the exposure method, sample sizing and determination of aerosol concentration have been reported elsewhere (BERTEAU and BIERMANN, 1977; ANDERSEN, 1958). Because of stress imposed on animals confined in head-only exposure units (which would influence the toxicity values), whole-body exposure was used to determine the reported LD50 values. Mature virgin female rats and mice were used. Mice were 30-35 g weight NAMRU strain and rats were Sprague-Dawley* strain weighing 225-350 g. After animals were exposed, the number dead were noted

*Charles River Breeding Laboratories, Wilmington, Mass.

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and the remainder of the animals were observed for 14 days to determine total mortality. Exposure was repeated several times, the dose being varied by varying the exposure time. The dose (mg/kg body-weight) was calculated from the following formula*:

$$\text{Dose (mg/kg)} = \frac{ctVdf}{100}$$

where:

c = particle concentration in aerosol (mg/l)
t = animal exposure time (min)
V = animal respiratory minute volume (ml/min/g)
d = fractional whole body deposition
f = percentage w/w of pesticide chemical in the formulation atomized.

Oral toxicity was determined by intubation of 0.1 ml (mice) or 0.5 ml (rats) of the pesticide formulation in soya-bean oil using a blunt-ended syringe needle (GAINES, 1960). Intraperitoneal LD50 values to rats were determined for naled using 0.5 ml of a solution in 1,2-propylene glycol to avoid the formation of fatty emboli.

When mice were exposed to the three organophosphorus insecticides, acetylcholinesterase was determined in plasma before and after exposure using the method of MICHEL (1949) as adapted by WOLFSIE and WINTER (1952). Plasma cholinesterase measurements were made at intervals after animals were exposed to determine recuperation time.

LD50 values and confidence intervals were determined by the method of LITCHFIELD and WILCOXON (1949).

RESULTS

Table 1 lists exposure conditions and the number of exposures used to determine inhalation LD50 values. The MMD of the aerosol particles did not differ enough between formulations (1.5 - 2.0 μm) to justify including them separately. With non-volatile materials (e.g., soya-bean oil) the concentration of particles in the chamber agreed to within 95% of the amount calculated to be in the chamber on the basis of measured atomizer output and the air flow rate through the chamber (18.3 l/min). With volatile materials such as xylene (used for formulating chlorpyrifos) some material was lost as vapor and not collected on the filter; we assumed that the vapor phase contained negligible quantities of relatively non-volatile chlorpyrifos.

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*See also footnote a to Table 2.

TABLE 1.

Conditions of inhalation exposure of mice and rats to four insecticide formulations

Pesticide Chemical	Formulation	No. of exposures to determine LD50	No. of animals per exposure	Duration of exposure (range)(min)	Aerosol Concentration (range)(mg/l)
chlorpyrifos[a]	65% xylene	6	16	27-50	6.7-7.9
malathion[a]	95% technical grade	1	16	300	6.9
naled[a]	10% or 20% Dibrom 14 conc. in soya-bean oil	8	10	72-111	5.6-6.1
resmethrin[a]	40% in xylene	2	16	96-177	6.8
chlorpyrifos[b]	65% in xylene	5	8	60-180	5.9-7.5
naled[b]	10% Dibrom 14 Conc. in soya-bean oil	5	8	48-61	4.7-5.5

[a] Administration to mice

[b] Administration to rats

TABLE 2.

Inhalation and oral toxicities of four pesticides to mice and rats

Pesticide[b] chemical	Inhalation toxicity[a] mg/kg LD50	95% confidence intervals	Oral toxicity, mg/kg LD50	95% confidence intervals	Potency Ratio Oral LD50 / inhal LD50
chlorpyrifos[c]	94	83 - 106	152	143 - 162	1.62
malathion[c]	>759	---	1680	1631 - 1730	--
naled[c]	156	141 - 174	222	209 - 235	1.42
resmethrin[c]	99 - 243	---	1390	1135 - 1703	--
chlorpyrifos[d]	78	57 - 108	169	146 - 196	2.17
naled[d,e]	7.7	7.2 - 8.4	160	131 - 195	20.8

[a] Based upon respiratory minute volumes of 1.25 ml/min/g (mouse) or 0.65 ml/min/g (rat) (GUYTON, 1947) and whole-body deposition of 84% (mouse) or 28% (rat) of the amount inhaled, with soya-bean oil (BERTEAU and BIERMANN, 1977), or 31% (mouse) and 16% (rat) with xylene (unpublished observation).

[b] See TABLE 1 for formulations.

[c] Administration to mice.

[d] Administration to rats.

[e] Intraperitoneal LD50 35.0 mg/kg (31.8 - 38.5).

Table 2 compares inhalation and oral LD50 values for chlorpyrifos and naled based upon whole body deposition. With malathion and resmethrin, we could not administer a sufficiently high dosage to allow computation of inhalation LD50 values. In a five-hour exposure of mice to 95% malathion, there were no deaths; with resmethrin, 2/16 mice died after a five-hour exposure whereas 16/16 died after a five-hour exposure to pure Panasol. Although there was some temporary sedation, no mice died after a two-hour exposure to pure xylene, although 4/8 rats and 13/16 mice died after a three-hour exposure. All of 16 mice survived a five-hour exposure to a soya-bean oil aerosol.

Fig. 1 gives the relationship between the inhalation dosage and cholinesterase inhibition in plasma for three organophosphorus pesticides in mice. Dynamics of recovery of plasma cholinesterase after the animals inhaled given doses of the three organophosphorus insecticides are shown in Fig. 2.

Typical cholinergic symptoms were observed only in rats exposed to naled aerosols. Mice became subdued, listless, and eventually died after exhibiting only flaccid paralysis.

Preliminary gross and histological observations indicate that, in addition to its effect on cholinesterase, naled induces cellular necrosis of the lung alveolar wall and centrolobular necrosis of the liver.

DISCUSSION

On the basis of whole-body deposition, naled formulated in soya-bean oil is approximately 21 times more toxic to the rat by the inhalation route than by the oral route (Table 2). With chlorpyrifos formulated in xylene, the inhalation toxicity is about twice the oral toxicity value for the rat, although it is recognized that the toxicity of xylene may be a factor contributing to this difference. Mice displayed less difference between oral and inhalation toxicity values of naled than did rats. This fact may be explained by the observation (BERTEAU and BIERMANN, 1977) that that portion of a soya-bean oil aerosol deposited in the lung was higher in rats than in mice.

It is recognized that these inhalation LD50 values include some input from absorption through the skin and stomach because whole-body exposure was used. However, toxic effects of such additional absorption would be minimally significant due to the more rapid effect of absorption from the lung alveoli.

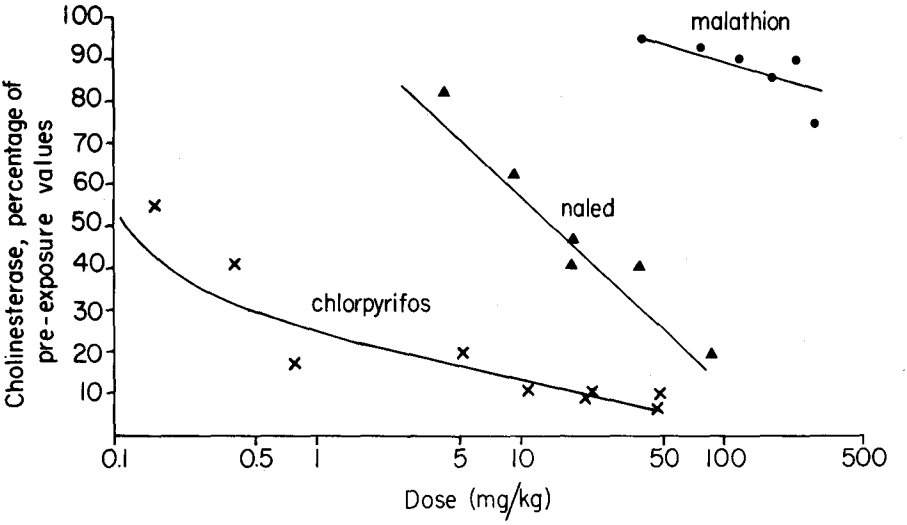

Fig. 1. Relationship between plasma cholinesterase depression and inhalation dose of three insecticides to female mice.

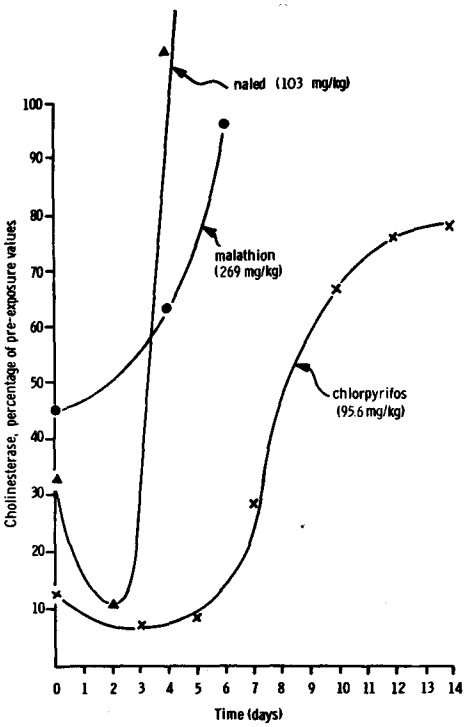

Fig. 2. Plasma cholinesterase recovery when mice received given inhalation doses of three insecticides.

The ip LD50 value for naled to rats of 35 mg/kg (Table 2) is still higher than the inhalation LD50 of 7.7 mg/kg. Thus, even if 100% of the amount inhaled was initially deposited in the animal, the toxicity would be higher when naled is administered by the inhalation route.

HOBEN, et al. (1976) report a similar increase in toxicity when pentachlorophenol is administered by the inhalation route as contrasted to the oral or intraperitoneal routes.

It is recognized that the respiratory minute volumes reported by GUYTON (1947) refer to animals in the resting state. These rates may vary under the conditions of exposure. However, oxygen intake must at least be basal, i.e., approximately half the resting values. With naled, for the calculated inhalation LD50 value to approach the ip LD50, the breathing rate would have had to increased 5 - 6 times. Such change in ventilation was not observed.

The high inhalation toxicity of naled may be caused in part by its corrosive property, or naled may be metabolized to an even more toxic material in the lung. We are currently studying the metabolism of naled when the pesticide is administered by the inhalation route.

Inhalation doses of chlorpyrifos below those needed to induce mortality produced significant lowering of the plasma cholinesterases. However, with naled, the dose needed to kill some animals more closely parallels that needed to produce a significant lowering of cholinesterase. Pre-exposure plasma cholinesterase levels are rapidly achieved in animals that survived exposure to naled, but are reached more slowly in those exposed to chlorpyrifos. One reason may be that chlorpyrifos is retained to a limited extent in body-fat (SMITH, et al., 1967), whereby small but toxic amounts may be released into the bloodstream for a few days following exposure.

The effect of malathion on cholinesterase depression was variable; in one five-hour exposure, depression was barely significant but in another the depression was 45% of the pre-exposure value (Fig. 2). This variation may be related to the level of circulating aliesterases, which are known to detoxify malathion by hydrolyzing the ethoxycarbonyl group of the succinic acid moiety (COOK, et al., 1958; DUBOIS, et al., 1968).

The MMD of ULV aerosols has been reported to be around 10 μm. However, such aerosols are heterodisperse and a significant number of particles in the 1-3 μm

range will be encountered. Naled is considered to be a relatively non-toxic pesticide based upon its oral LD50 value to rats; we found 160 mg/kg to female rats and 222 mg/kg to female mice. Based upon whole body deposition after inhalation of aerosols, we found inhalation LD50 values of 156 mg/kg for mice but 7.7 mg/kg for rats. Thus, in extrapolating these animal data to assess the hazards to humans, we believe naled should be considered to be one of the more toxic pesticides when exposure is by the inhalation route.

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