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Reentry Research: Establishment of Safe Pesticide Levels on Foliage

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Field reentry studies (KAHN 1979) are often required by the California Department of Food and Agriculture to establish reentry intervals for pesticide-treated orchards or vineyards. These studies are difficult to conduct and the reentry intervals cannot be readily enforced without application records. KNAAK (1978c) proposed that safe pesticide levels on foliage, in $\mu g/cm^2$ of leaf surface, be established. The establishment of safe levels would allow growers and governmental agencies to determine by test when orchards or vineyards are safe to reenter.

This study reports a procedure for the establishment of safe pesticide levels on foliage using dermal dose red cell cholinesterase response curves and field reentry data.

METHODS

Male albino rats (Simmonsen, Gilroy, CA) weighing 220 to 240 g were used throughout the study. The animals were individually housed in galvanized steel cages (9"W x 11"D x 9"H) prior to and during the three day study.

Preparation of animals for treatment. One day prior to dosing, the animals were individually weighed and hair was clipped from their backs using an electric clipper (Sears and Roebuck, Chicago, Ill). A Queen Anne collar made of polyethylene sheeting (.040 in. in thickness) was placed around the neck of each animal (Fig. 1) and fastened using three staples (Bostitch B8 stapler, East Greenwich, RI). A template (.040 in. in thickness) made from neoprene rubber sheeting (Fig. 1) was then glued to the clipped back of each animal using Eastman 910 Adhesive, Eastman Chemical Products Inc., Kingsport, TN. This was accomplished by using a strip of adhesive tape to transfer the template to the back of the rat and to hold it in place while the glue dried. Animals were then returned to their cages to await treatment on the following day.

Treatment of Animals. Table 1 indicates the pesticides applied, the dose rates and the number of rats treated per dose. Doses were applied to the clipped backs (25 cm^2) in 1.0 mL of acetone using a digital microliter pipette (Pipetman, Model P-1000D, West Coast Scientific, Oakland, CA) and a glass stirring rod to insure uniform application. In practice, increments of 0.5 mL were applied

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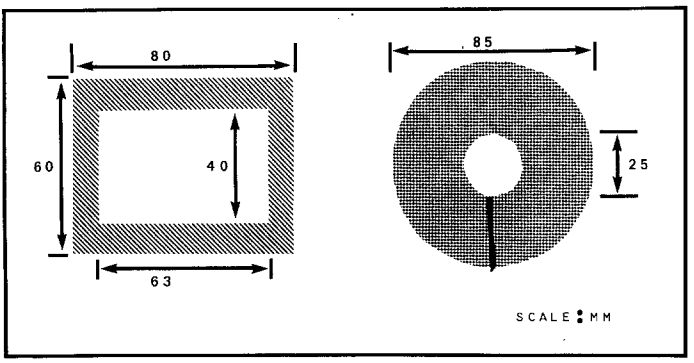

Figure 1. Diagram of Queen Anne Collar and Rubber Template used in the Study.

to skin inside the rubber template and allowed to dry prior to application of a second increment. The animals were returned to their cages and allowed free access to food (Rat Chow 5012, Ralston Purina Co., St. Louis, Mo.) and water for 72 h. During this time the animals were observed once a day for symptoms of poisoning, loose collars and chewed rubber templates. Twenty-five control animals were used in the study.

Collection of Blood Samples and Determination of Blood Cholinesterase Activity. All rats were sacrificed 72 h after dosing by decapitation. Blood was collected in a 100 mL polypropylene beaker containing 0.1 mL of sodium heparin (1,000 USP units/mL, Lipo-Hepin, Riker Labs., Northridge, CA) to prevent coagulation. The collected blood was placed in heparinized vacutainers and refrigerated to await analysis.

Cholinesterase activity in blood was determined using a Technicon Autoanalyzer II system and the colorimetric procedure of KNAAK et al. (1978a).

The cholinesterase activity of the red cells was obtained by subtracting the activity of the plasma from the activity of whole blood using the sample's hematocrit as follows:

$$RBC = \frac{\text{Whole Blood Activity} - \left(\frac{[1-\text{Hematocrit}]}{100}\ [\text{Plasma Activity}]\right)}{\frac{\text{Hematocrit}}{100}}$$

The activity is reported in terms of micromoles of -SH released per min per mL of sample. A plasma sample from Sigma Chemical Co., St. Louis, MO, was used as the enzyme standard and reduced glutathione was used for instrument standardization.

Whole blood was diluted 1:1 with pH 7.7 buffer for analysis while plasma was used without dilution.

TABLE 1

Treatment Schedule

Pesticides	Dose (ug/cm^2)[a/] and Number of Animals Treated											
	4000	2000	800	600	400	200	80	40	20	6.0	4.0	2.0
Paraoxon										3	3	3
Parathion							3	3	3		3	
Dialifor				3	3	3	3	3			3	
Phosalone	3		3		3			3				
Azinphosmethyl	3				3	3		3				
Methidathion		3			3	3	3	3				
Dimethoate	3		3		3	3		3				

a/ Dose applied to the clipped back (25 cm^2) of 220-240 g male Sprague-Dawley rats. Analytical grade chemicals were used.

Paraoxon, diethyl 4-nitrophenyl phosphate, was obtained from W. Winterlin, University of California, Davis, Ca.
Parathion, O,O-diethyl O-(4-nitrophenyl) phosphorothioate, was obtained from Monsanto Company, Agricultural Products, St. Louis, MO.
Dialifor, O,O- diethyl S-[2-chloro-1-(1,3-dihydro-1,3-dioxo-2H-isoindol-2yl) ethyl] phosphorodithioate, was obtained from Hercules Inc., Agricultural Chemicals, Wilmington, DEL.
Phosalone, O,O-diethyl S-[(6-chloro-2-oxo-3H)-benzoxazolyl-methyl] phosphorodithioate, was obtained from Rhone-Poulenc Inc., Agricultural Chemicals, Monmouth Junction, N.J.
Azinphosmethyl, O,O-dimethyl S-[(4-oxo-1,2,3-benzotriazin-3(4H)-yl) methyl] phosphorodithioate, was obtained from Mobay Chemical Corp., Agricultural Chemicals Division, Kansas City, MO.
Methidathion, O,O-dimethyl S-[(5-methoxy-2-oxo-1,3,4-thiadiazol-3(2H)-yl) methyl] phosphorodithioate, was obtained from Ciba-Geigy Corp., Agricultural Division, Greensboro, N.C.
Dimethoate, O,O-dimethyl S-[2-(methylamino)-2-oxoethyl]phosphorodithioate, was obtained from American Cyanamid, Agricultural Research Division, Princeton, N.J.

Statistical Analysis of Cholinesterase Data. The cholinesterase data was statistically analyzed according to the Log-Probit Analysis procedure of FINNEY (1972) for determining LD_{50} and ED_{50} values. The basic computer program written by O'NEIL et al. (1979) was modified to compute % inhibition and ED_{50} for red cell and plasma cholinesterase using the Burroughs 6700 computer, Computer Center, University of California, Davis, CA. The procedure of LITCHFIELD and WILCOXON (1949) was used to test for parallelism.

RESULTS

Dermal Dose-ChE Response. Figures 2 and 3 show the dermal dose ChE response curves, slopes, ED_{50} and $(Chi)^2$ values for the effects of parathion, methidathion, dialifor, azinphosmethyl, phosalone, dimethoate and paraoxon on red cell and plasma cholinesterase activity. The dermal dose producing 50% cholinesterase inhibition (ED_{50}) is given in ug/cm^2 of skin. Approximately 25cm^2 or 7.7% of the total body surface was treated. Paraoxon was the most toxic organophosphate tested while dimethoate was the least toxic.

The $(Chi)^2$ values for the dermal dose-ChE response curves were not significant at the .05 level. The $(Chi)^2$ test is a measure of the heterogeneity of the data. The control means, for red cell and plasma cholinesterase activity, were respectively, 3.5 and 1.4 micromoles of -SH released per min per mL of sample.

The slopes of the red cell regression lines varied between 0.7 and 2.9, while the slopes for plasma varied between 0.37 and 1.4. A dermal dose-plasma ChE response curve was not obtained with dimethoate. Dialifor was the only pesticide with similar slopes for both plasma and red cell cholinesterase.

Exposure to pesticides, in general, resulted in larger slopes for red cell cholinesterase and smaller slopes for plasma. The smaller plasma slopes make this enzyme a poorer index of exposure than red cell ChE. The smallest plasma slope was obtained using parathion, while methidathion and dialifor gave the largest response.

Table 2 gives the ED_{50} values in terms of ug/cm^2 of total body surface, mg/kg of body weight and the safety index (dermal LD_{50}/ED_{50}). An LD_{50} value was not available for dialifor in the rat, however, a dermal LD_{50} was determined in the rabbit, 67.7 mg/kg, by GALLO (1977).

Safe Levels on Foliage. Table 3 gives a procedure for determining safe levels on tree foliage in ug/cm^2 using the ED_{50} values in ug/cm^2 of body surface, slopes of the dermal dose-inhibition curves and safety data from field reentry studies. In this procedure, the pesticides are grouped according to their slopes as indicated in Table 3. At least one pesticide with safety information must be included in each group. These are designated as pesticide standards. The standards are underlined in Table 3. The relative dermal toxicities were determined by dividing the

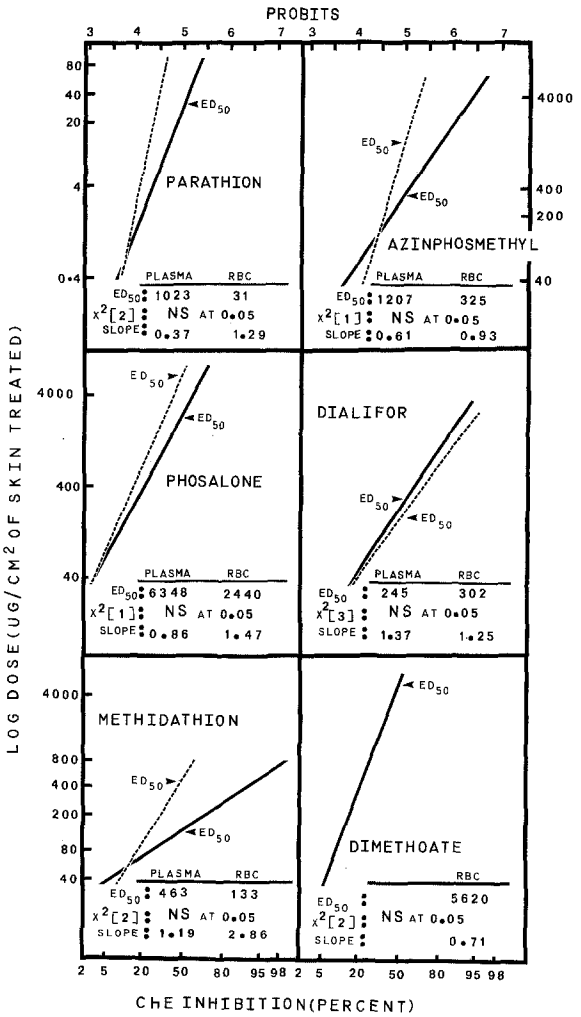

Figure 2. Dermal Dose-ChE Response curves for six organophosphate pesticides. Male Sprague-Dawley rats weighing 220-240 g were used. A 25 cm^2 area of skin was treated.

--------- plasma ChE
_____ RBC ChE

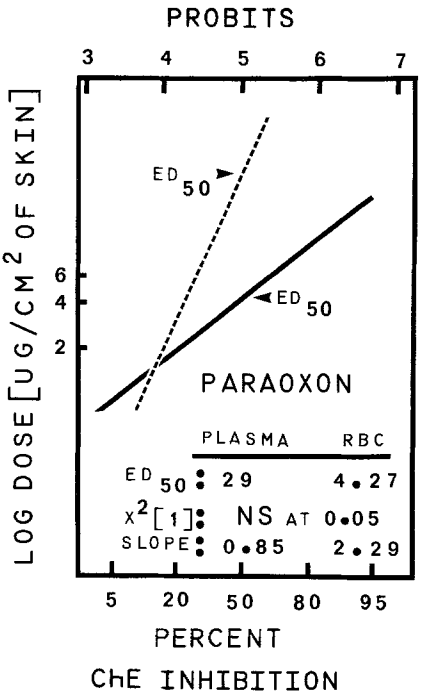

Figure 3. Dermal Dose-ChE Response curves for Paraoxon. Male Sprague-Dawley rats weighing 200-240 g were used. A 25 cm^2 area of skin was treated.

-------- plasma ChE
_____ RBC ChE

ED_{50} of the pesticide under investigation by the ED_{50} of the pesticide standard. The relative toxicity was then multiplied by the safe level determined for the standard to obtain a safe level for the pesticide under investigation.

The conditions of exposure for the standard and the pesticide under investigation must be similar. In Table 3, a safe level for dialifor was estimated using a safe level for phosalone on peaches. Dialifor is not registered for use on peaches.

DISCUSSION

Ethical questions, inadequate funding, and a limited number of scientists available for conducting reentry studies suggested a new approach in setting reentry intervals; the development of safe levels using dermal dose-response data in conjunction with field reentry data. This procedure, extends the knowledge gained from a limited number of field studies to other field situations.

TABLE 2

Dermal Dose-ChE Reponse expressed in terms of total body surface, body weight and safety index

Pesticides	ED_{50} ug/cm^2 of body Surface[a/]	ED_{50} mg/kg[b/]	Dermal LD_{50} mg/kg	Safety Index LD_{50}/ED_{50} mg/kg
Paraoxon	0.33 ± 0.2	0.5	2.0 c/	4.0
Parathion	2.4 ± 0.3	3.4	21.0 d/	6.2
Methidathion	10.0 ± 0.3	15.0	150 e/	10.0
Dialifor	23.0 ± 0.3	33.0	-	-
Azinphosmethyl	25.0 ± 0.5	35.0	220 d/	6.3
Phosalone	188.0 ± 0.4	265	1450 f/	5.5
Dimethoate	432 ± 2	611	1420 g/	2.3

a/ Pesticides were individually applied in 1.0 mL of acetone to the clipped backs (25 cm^2) of 220-240 g male rats. Blood was taken 72 h after application for ChE determination. Response expressed in terms of total body surface (325 cm^2) from dermal dose-ChE response curves in Figures 2 and 3. Values given with 95% confidence limits.

b/ Values determined from dermal dose-ChE response curves.

c/ Estimated.

d/ GAINES (1960).

e/ CIBA-GEIGY Toxicology Data Bulletin.

f/ MAZURET (1971).

g/ GAINES (1969).

GAINES in 1960 and 1969 successfully determined the dermal LD_{50} of a series of pesticides in the rat. The pesticides were individually applied to the clipped back of the rat in xylene. The rat, however, has not been used extensively for dermal toxicity studies. The rabbit has been the animal of choice because it is easier to dose and restrain for longer periods of time.

Rats may be held in restraining devices for periods up to four hours without serious problems. In this study, exposure periods up to 72 h were desired. This necessitated using Queen Anne collars to prevent the rat from reaching the treated area with his mouth. The animals were able to reach the treated area with a front paw and place the paw in the mouth. The ChE data did not indicate the animals received an oral dose.

Rubber templates were glued to the backs of each animal to mark the area to be treated. After treatment, a light weight fiber glass screen was to be glued to the template to prevent the rat from contacting the treated area with his paws. Gluing a screen to the surface of a template proved to be a difficult

TABLE 3

Establishment of Safe Levels on Tree Foliage (in ug/cm^2) using the results of Dermal Dose-ChE Response Studies in Male Rats and Field Reentry Studies

Pesticides[a]	Slopes	ED_{50} ug/cm^2 of body Surface	Relative Toxicity[b]	Safe Level on foliage in ug/cm^2[c]
Paraoxon	2.3	0.33	1.0	0.02[d]
Methidathion	2.9	10	30	0.6
Dialifor	1.3	23	0.12	0.8[e]
Parathion	1.3	2.4	0.013	0.09
Phosalone	1.5	188	1.0	7.0[f]
Azinphosmethyl	0.9	25	1.0	3.1[g]
Dimethoate	0.7	432	17	53

a/ Pesticide standard is underlined.
b/ ED_{50} of pesticide under investigation divided by ED_{50} of pesticide standard.
c/ Relative toxicity multiplied by safe level of standard. Safe levels for standards determined by reentry studies: d/ SPEAR et al.(1977); f/ POPENDORF et al. (1979); g/ RICHARDS et al. (1978).
e/ Safe level on grape foliage estimated to be 0.09 ug/cm^2 by KNAAK et al. (1978b).

task. This procedure, therefore, was not utilized to prevent oral ingestion. The animals tolerated the collars reasonably well for periods up to 72 h. Templates remained intact on the back of the animals indicating that the rats were not reaching them with their teeth.

The automated colorimetric procedure of KNAAK et al. (1978a) was sensitive enough for this study and provided a rapid and uniform procedure for determining cholinesterase activity. Values between 10 and 90% inhibition were used to construct the dermal dose-ChE inhibition curves.

Dermal dose-plasma and red cell ChE inhibition curves were obtained on all pesticides with the exception of dimethoate. A dose-plasma ChE response relathionship was not obtained with dimethoate.

Pesticides and pesticide standards with parallel slopes (LITCHFIELD and WILCOXON 1949) were paired in order to compare their relative toxicities (BLISS 1935). This was necessary because the relative toxicity of the pesticide and standard must be the same at doses below their ED_{50} if they are to be useful.

Conditions of exposure (harvest operations, dustiness, temperature, etc.) involving the pesticide of interest and the pesticide standard must be similar if the procedure based on relative toxicity is to work. If different conditions of exposure are anticipated, a field reentry study may be necessary.

Safe levels should be established for all dislodgeable, dermally toxic pesticides and their oxons.

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