On the Estimation of Worker Entry Intervals into Pesticide Treated Fields with and without the Exposure of Human Subjects[1]

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Determination of a time interval between the application of pesticides to agricultural fields and safe entrance of agricultural workers back into those fields has, over the past few years, been a subject of great concern to agricultural, industrial, health and political communities. Articles have appeared which describe efforts to study the effect of specific pesticides on the well being of those who subsequently enter the fields for such operations as thinning, harvesting or insect counting (WARE, et al 1973, 1974a, 1974b; FOSTER and YONAN 1974; SPEAR, et al 1974; SERAT 1973). These studies in general give rise to suggested entry intervals based on the particular experience from an individual study while one describes a general calculation of entry intervals from the kinetics of a change both in pesticide residues and in their physiological effect (SERAT 1973).

Because of the inherent difficulties in fielding and maintaining crews of subjects to work the pesticide-treated areas and because of the potential danger involved it would greatly facilitate the establishment of entry intervals for all concerned if they could be estimated without the need for human exposure in field studies. Considering the number of pesticides in use and crops involved, as well as varying ambient conditions, it is obviously most important that methods alternative to those now used be developed for reentry studies so that directed human exposure might be eliminated. Information currently available suggests that such an alternative will be possible.

KINETIC FACTORS INVOLVED IN CALCULATING SPECIFIC ENTRY INTERVALS FOR A GIVEN DEGREE OF CHOLINESTERASE INHIBITION

Many studies have been conducted to determine the loss of pesticide residues from the surface of foliage. Pesticide disappearance is generally first order or pseudo first order, though a rapid erosive or mechanical loss may preceed the onset of that which is kinetically describable.

For a first order loss:

$$\frac{dP}{dt} = -kP \text{ and upon integration}$$

$$P = P_0 10^{-kt/2.303} \quad (1)$$

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where k is the specific rate constant, P_o is the deposit level of pesticide on the day of application (t = o) and P is the level at time t. Appropriate expressions could be derived should other than first order kinetics prevail.

A first order change in blood cholinesterase activity with total pesticide exposure has also been observed (GROB and HARVEY 1949, SERAT 1973). Therefore,

$$\frac{dE}{dP} = k' E \text{ and thus}$$

$$(2) \qquad P_t = \frac{2.303}{k'} \log \frac{E_o}{E} ,$$

where k' is the specific rate constant and P_t is the total level of pesticide which has produced a fractional enzyme activity loss, $1 - E/E_o$. The enzyme activity after exposure is E while the baseline value prior to exposure is E_o. Since exposure to pesticide in the field usually occurs over several consecutive working days it was shown (SERAT 1973) that a value of P_t which produces some fractional loss in cholinesterase, $1 - E/E_o$, may be expressed for a working period of one week (5 days) in the same field, by:

$$(3) \qquad P_t = P_E \left[1 + 10^{-k/2.303} + 10^{-2k/2.303} + 10^{-3k/2.303} + 10^{-4k/2.303}\right] ,$$

where P_E is the pesticide level on the day of entry. The level of pesticide each day during exposure in the field is, from equation (1), $10^{-k/2.303}$ times that of the previous day.

From equation (2) it is noted that in order to attain no loss in cholinesterase activity the value of P_t must be zero. Theoretically, an infinite time period must pass before residues on foliage reach a zero level. However, since there is a biological threshold of exposure to pesticides which must be exceeded before measurable enzyme losses appear and since present analytical techniques can not discern very small changes in cholinesterase activities, values of P_t calculated for several percent change in enzyme activities would perhaps represent pesticide levels for which no significant loss in activity occurs.

The value of P_E can, from equation (1), be related to the time (entry interval) required for its appearance on foliage, as $P_o 10^{-kt/2.303}$; and thus, from equations (1-3), a time interval can be calculated which allows pesticide to decay to a level which produces an arbitrary loss in enzyme activity, $1 - E/E_o$. If in addition, some parameter can be established which relates a function of toxicity to pesticide level, then for those pesticides whose toxicity is dependent upon producing a common physiological effect and for which the relative toxicity values as determined on

a test system parallel approximately those found in man, the entry intervals can be estimated without the need to expose human subjects.

In the assignment of relative toxicity values for each of a mixture of pesticide residues on foliage, a factor, the toxicologic potential

$$T = \frac{P}{LD_{50}}$$, has been proposed (SERAT and BAILEY 1974).

Since the value of P from equation (1) is time dependent, the toxicologic potential is time-dependent also. If P is chosen as P_t, the level of pesticide residue which produces an arbitrary change in enzyme activity, by equation (3) and substituting for P_E,

$$(4) \quad T = P_o 10^{-kt/2.303}\left(1 + 10^{-k/2.303} + 10^{-2k/2.303} + 10^{-3k/2.303} + 10^{-4k/2.303}\right)\frac{1}{LD_{50}}.$$

With the qualifications given above and for specified losses in cholinesterase activity, which in turn determine the entry intervals, t, the values of T for different pesticides of similar physiological function are thus predicted to be similar. It is this fact that suggests that entry intervals could be calculated from data not requiring human exposure for their procurement.

There exists a great paucity of field data of a quality adequate to allow substantial verification of the concept of estimating entry intervals without human exposure, but estimations have been made from the results of three field studies involving organic phosphate materials. Basic data from two of these studies are considered proprietary by those who supplied them; accordingly, the respective pesticides will not be identified. Since the thrust of the development of this procedure is to fit existing data to theory in order to test a concept, it detracts nothing when the names of the materials are omitted.

ESTIMATING ENTRY INTERVALS WITH AND WITHOUT HUMAN EXPOSURE

Three organic phosphate pesticides applied to crops have been examined as to their effect on workers harvesting fruit or otherwise laboring in contact with the foliage. Details and results regarding one of these materials (A), applied to orange groves, have been described (SERAT 1973). Pertinent data and kinetic constants were determined as $k = -7.16 \times 10^{-2}$ days^{-1}, $k' = -4.75 \times 10^{-3}$ ppm^{-1}, $P_o = 92$ ppm and the entry interval for a loss of 30 percent in plasma cholinesterase activity (a value chosen for illustrative purposes only) was calculated (equations 1-3) as 23.4 days. An LD_{50} of 62 mg/kg has been reported for this compound following dermal application of a xylene solution of technical grade material to female Sherman strain rats (GAINES 1969).

The second pesticide (B) was also applied to orange groves while the third (C) was used to treat cotton (WARE, et al 1974b). For pesticide (B), k was estimated as -4.0×10^{-2} days $^{-1}$,k' was -1.94×10^{-3} ppm $^{-1}$, P_o was 100 ppm and the entry interval calculated as 23.0 days for an illustrative 30 percent loss in enzyme activity. For this material the LD_{50} was estimated as 220 mg/kg under the same conditions used in evaluating the toxicity of (A) (GAINES, 1969).

For pesticide (C) the data reported were used to calculate $k = -3.62 \times 10^{-1}$ days^{-1} and P_o, by extrapolation of a plot representing a well defined first order loss of pesticide from foliage, was 12.9 mg/m^2 or 90.3 ppm (calculated from the units given on the basis that 7 ppm are equivalent to one mg/m^2). Since pesticide loss was rapid, a calculation was made for the total exposure to reflect the average change in residues over a working day of 7 hours, whereas the actual exposure had been 5 hours. Similarly, the loss in cholinesterase activity reported for 5 hours exposure was used to estimate maximal loss for 7 hours exposure. Since both pesticide concentration and enzyme activity were changing, only an approximate value of the activity could be determined by direct proportion. The data again suggest a distinct first order loss of enzyme activity with total exposure. The value of k' was calculated to be -3.43×10^{-2} m^2/mg, and the dermal LD_{50}, measured under the same conditions used with pesticides (A) and (B), is reported as 112 mg/kg (GAINES, 1969). For an illustrative 30 percent loss in plasma cholinesterase activities, an entry interval of 3.4 days was obtained considering as before a working week in the same field of 5 days at 7 hours per day.

By use of equation (4) the respective toxicologic potentials, T, calculated for pesticide levels in units of ppm, for various arbitrarily chosen levels of cholinesterase activity loss were estimated for the three pesticides. Values are given in Table 1. Considering the confidence limits in measuring LD_{50} values and with the implicit assumption that the relative values as measured on female Sherman strain rats approximate relative toxicologic effects in man, these values calculated for T at any one level of enzyme activity loss are in reasonable agreement. As they suggest a common average value for a common loss in enzyme activity, those values were used (equation 4) to calculate entry intervals (Table 1) without the use of data relevant to human exposure to the pesticides. These calculated intervals c ompare favorably with those obtained by use of data resulting from human exposure.

DISCUSSION

Organic phosphate pesticides have as their principal mode of physiological activity the inhibition of cholinesteratic enzymes with the concomitant impairment of the central nervous system. A priori then, relative measurements of toxicity might be expected for different mammalian systems. Such an assumption, however, is fraught with hazard since the capacities of organic phosphates to function as cholinesterase inhibitors in no way predicts that factors involved in absorption or metabolism will be comparable in

those different systems. Thus, to assume that for measurable physiological effects in man, comparable relative values will exist for a determined toxicologic quantity (dermal LD_{50}) in test animals is speculation whose validity must be borne out by comparing the results obtained from tests involving human subjects and those produced by calculation from contrived parameters of toxicity (toxicologic potential). It would hardly be fortuitous if for the greater number of organic phosphate pesticides such correlations proved the validity of the assumptions.

Three organic phosphate pesticides for which field data on human exposure exist have been shown, within reason, and considering the precision of measurements, to give approximate relative correlations in their toxic effects on an experimental animal and on man. From such information entry intervals expected to produce a given degree of cholinesterase inhibition in farm workers can be calculated either with or without the involvement of human subjects.

The development of the expressions above follows from a first order loss both for residues on crops, and for plasma cholinesterase activities as a function of the total potential pesticide exposure. Presumably other corresponding expressions could be developed for other kinetic orders depicting the changes. It is of particular interest that for the three pesticides, the best defined first order losses of residues from foliage, or of enzyme activity, were noted with (C), a pesticide whose structure, as used, is the phosphate. Less certain kinetic constants were obtained for pesticides (A) and (B). These materials are applied as phosphorodithioates which must be converted to the phosphates or oxygen analogs to exhibit much toxicity and perhaps to initiate a sequence of reactions leading to loss of the phosphorodithioates from foliage. Thus, first order changes which were observed with these latter materials may represent a series of other reactions or processes which are not of sufficient time duration to be rate limiting if they are other than first order. With each of the pesticides, the first order loss of enzyme activity with total potential exposure signifies a dose response rather than a dose-rate response. This implication has been discussed previously (SERAT 1973).

Calculations above were based on pesticide levels and physiological effects attributable to the parent pesticidal materials, the only substances measured. Should perhaps even minor amounts of the notably more toxic phosphate analogs of pesticides (A) or (B) been present, the correlations obtained between entry intervals calculated with and without data on humans (table 1)would probably have been far less significant. Thus,it is probable that only low, if detectable, levels of the respective phosphates existed on foliage. With the appropriate data, however, calculations of entry intervals can be made for phosphate derivatives or combinations of these with parent pesticides.

Since on foliage the phosphate forms of many organic phosphates are produced <u>in situ</u>, studies devoted to the loss in residues of parent compounds should also include measurements of the development

TABLE 1. Worker Entry Intervals Calculated from Exposure Data for Various Losses in Plasma Cholinesterase Activity and from Common Values for the Toxicological Potential

Pesticide	$\frac{E}{E_o}$	T Calculated	Entry Interval (Days)				
			Calculated From Exposure Data	Calculated for a Common Value			
				T = 0.126	T = 0.266	T = 0.562	T = 0.900
A	0.95	0.17	50.5	55.0	-	-	-
	0.90	0.36	40.4	-	44.6	-	-
	0.80	0.76	29.9	-	-	34.1	-
	0.70	1.21	23.4	-	-	-	27.5
B	0.95	0.12	71.5	70.4	-	-	-
	0.90	0.25	53.5	-	51.7	-	-
	0.80	0.52	34.8	-	-	33.0	-
	0.70	0.84	23.0	-	-	-	21.2
C	0.95	0.09	8.8	7.9	-	-	-
	0.90	0.19	6.8	-	5.9	-	-
	0.80	0.41	4.7	-	-	3.8	-
	0.70	0.65	3.4	-	-	-	2.5

and loss of the more toxic phosphate analogs. Adequate data are obtainable only if foliage samples are taken often enough and from several representative areas of the field(s). In addition, the present determination of LD_{50} values is often made on various formulations and on an insufficient number of test animals to minimize the limits of variation of the values. For application to entry interval calculations, LD_{50} values should have limits of $\pm$ 10 percent and should be highly standardized to represent the toxicity of residues and degredation products as they appear on foliage. This may include the addition of oxygen analogs to parent compounds for test purposes.

The value of 30 percent, chosen arbatrarily as an illustrative loss in plasma cholinesterase activity, refers to an average loss experienced by a crew of field workers. In so choosing, it is recognized that whatever maximum loss may eventually be allowable for an individual in a field study, it is quite different from the value which would be considered a statistically significant loss for the crew. The latter figure,if very high,would include individual cholinesterase losses which would be totally unacceptable.

Data obtained from the studies with the three pesticides mentioned above relate to areas of the county where ambient weather conditions were hot and dry. For cooler or more humid locations and for situations involving a high level of dust deposit on foliage, the kinetic constants obtained may well be different. Upon sufficient testing under these latter conditions, rates and energies of activation may become available so that kinetic information can be calculated for use in setting worker entry intervals for the differing locations and conditions.

As future reports appear in which data involving humans are presented for estimation of entry intervals it will be of considerable interest to see if indeed with other pesticides common values exist for the toxicologic potentials. If so, other worker entry intervals can then be estimated without exposure of human subjects.

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