A method for determining the dermal toxicity of pesticides

DIANA N. NOAKES AND D. M. SANDERSON

Chesterford Park Research Station, Fisons Pest Control Limited, Saffron Walden, Essex

Noakes, D. N., and Sanderson, D. M. (1969). *Brit. J. industr. Med.*, 26, 59-64. **A method for determining the dermal toxicity of pesticides.** A method is described for the measurement of the percutaneous LD₅₀ of a pesticide, using the laboratory rat as a test animal. The results obtained by this method on a selection of pesticide chemicals are tabulated and discussed. The reproducibility of the method was validated by a collaborative study with five other laboratories.

The importance of dermal absorption as the main route of entry in occupational poisoning by agricultural chemicals has been reviewed by Barnes (1959) and Hayes (1960). Their findings have been confirmed by several recent field studies in which the dermal exposure to pesticides during spraying operations was measured and found to be greater in most cases than exposure by other routes (Jegier, 1964a, 1964b; Simpson, 1965; Simpson and Beck, 1965; Durham, 1965). Kay (1964), reviewing occupational poisoning by industrial and agricultural chemicals in California in 1960, found that percutaneous absorption was recorded as the probable route of entry in nearly 80% of cases. Furthermore, Gaines (1960) has demonstrated that the incidence of occupational poisoning by various pesticides is more closely related to their acute dermal toxicities in animals than to their acute oral toxicities.

Despite these observations, there is not yet any generally accepted method for testing the dermal toxicity of pesticides, and variations in technique between different laboratories often result in very different LD₅₀ values being obtained for the same chemical. In animal tests made to compare hazards from different materials it is essential to know the relative acute oral and acute dermal toxicities on the same species, and to use standard techniques for determining these toxicities. This is particularly important for dermal toxicity since so many factors influence the dermal penetration rate.

The relation between the physicochemical properties of a compound and its percutaneous absorption has been reviewed by several authors, including Griesemer (1959), Malkinson and Rothman (1963), Marzulli, Callahan, and Brown (1965), and Blank and Scheuplein (1964).

Biological factors of importance include interspecies variation in dermal penetration rates (Tregear, 1964; McCreesh, 1965) and individual variation within a species (Nabb, Stein, and Hayes, 1966). The condition of the skin and the health of the whole animal, particularly as they affect the integrity of the epidermal 'barrier', also have a considerable influence (Malkinson, 1960; Malkinson and Rothman, 1963; Stoughton, 1965; Vinson, Singer, Koehler, Lehman, and Masurat, 1965).

The environmental conditions under which contact between the skin and chemical occurs also affect absorption. An increase in temperature and humidity usually results in increased penetration (Stoughton, 1965).

The penetration rate also depends on the vehicle, as it affects both partitioning of dissolved chemical at the surface and the integrity of the 'barrier', and on the concentration of active chemical it contains (Stoughton, 1965; Blank and Scheuplein, 1964; Vinson *et al.*, 1965). The concentration factor alone implies a generally far greater hazard from a concentrated formulation than from material diluted for spraying. An occlusive dressing over the contact

site may either increase penetration, by increasing the hydration of the skin and reducing the loss of chemical or vehicle, or reduce it by absorbing the chemical (Stoughton, 1964, 1965; Malkinson, 1965; Vandekar and Komanov, 1963). Another important factor is the contact time, variation of which has been used as a convenient method for assessing the absorption rate. This has obvious safety implications, as different materials are known to penetrate the skin at different rates.

Decontamination should be thorough in order to prevent subsequent absorption or ingestion of any remaining chemical. Also washing with organic solvents should be avoided as they may facilitate absorption.

In this paper we describe the method of testing dermal toxicity adopted in this laboratory, in which the albino rat is used as the test species. The technique involves removal of the hair from the trunk and application of the chemical to the exposed skin, which is covered with an impermeable dressing for the required contact time; after decontamination the animal is observed for toxic effects. We have chosen to measure dermal toxicity under conditions of impermeable covered contact, as this gives maximal absorption of chemical and could be considered analogous to skin contamination under the worst possible conditions, *e.g.*, inside an impervious glove. Some of the variables relating to the experimental conditions have been investigated together with the reproducibility of results in successive tests under similar conditions. The reproducibility of results between different laboratories was assessed by a collaborative exercise carried out with samples of a single chemical preparation tested by a similar technique in five other laboratories.

Experimental

Wistar albino rats (bodyweight 150-200 g.) were housed in wire-mesh cages and were fed and watered *ad libitum* throughout the tests. The hair was removed from the trunk with electric veterinary clippers, usually less than an hour before application of the chemical but in some tests on the previous day. Only animals in which the epidermis then appeared to be intact and healthy were used. All tests were carried out in the same laboratory at a temperature of 18-21°C. and relative humidity 60-80%.

Six commercially available pesticides were tested, some as the technical active ingredient in a laboratory solvent and others as commercial formulations. The active ingredients in the materials referred to by trivial names were as follows: parathion, diethyl 4-nitrophenyl phosphorothionate; dimethoate, O,O-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate; diazinon, diethyl 4-(2-isopropyl-6-methylpyrimidinyl) phosphorothionate; formothion, O,O-dimethyl S-(N-formyl-N-methylcarbamoylmethyl) phosphorodithioate; dinoseb, 2-(1-methyl-n-propyl) 4,6-dinitrophenol. They were

applied as liquid preparations, in solution or aqueous suspension (see Tables). The concentration of active ingredient was kept constant in each test, so that the volume applied and the area of skin contaminated after spreading increased proportionally with the dose level. The chemical was kept in contact with the skin by a 'sleeve' of waterproof plaster which also prevented the rat from bending round to interfere with the contaminated area (Figs. 1 and 2). The sleeve was used alone or

FIG. 1. Method of restraining rat and applying plaster.

FIG. 2. Plaster cover completed.

lined with other materials as required (Fig. 3). For one test three layers of plaster were used to produce a firm sleeve into which an aperture was cut so that the contaminated area of skin was left uncovered (Fig. 4). The contact time was 4 hours or 24 hours, followed by thorough decontamination with a detergent-and-water wash. Rats were caged alone during the contact time to prevent mutual interference.

In some tests (including that using the sleeve with an aperture) dye was added to the chemical to indicate its area of spread, and whether oral contamination occurred. Control rats wrapped in the plaster sleeve showed no signs of distress after 24 hours.

FIG. 3. Application of lining material to plaster.

FIG. 4. Method for 'uncovered' contact.

For each test, three or four groups of four rats were contaminated at dose levels in geometric progression (factor of 2 or $\sqrt{2}$). The LD_{50} and, whenever possible, the 95% confidence limits were calculated from the mortality by the moving average method (Weil, 1952). Some of the tests were repeated up to four times at intervals of one day or several weeks on different animals to check the reproducibility of the method.

The exact procedure for an individual rat was as follows:

1 The rat was held by an assistant (as in Fig. 1) and the hair was removed from the back and flanks with electric clippers; on a 200-g. rat this exposed a skin area of about 6×8 cm. (in some cases the belly hair was also removed to expose a slightly larger area).

2 The calculated volume of chemical was applied to the exposed skin from a syringe fitted with a blunted hypodermic needle. The shaft of the needle was used to spread the chemical evenly over the skin.

3 The trunk of the rat was encircled with a strip of plaster¹ 3 in. wide and 8-9 in. long (with a lining layer of another material or an aperture if required, as in Figs. 3 and 4). The plaster was tightened sufficiently to prevent the rat from wriggling free (Fig. 1) and wrapped round to form a double layer (Fig. 2).

4 The rat was caged alone for the required contact time, after which the plaster was cut through with blunt-tipped scissors and peeled off. The skin and surrounding hair were sponged thoroughly with detergent and warm water, rinsed, and dried with a towel. The animals were then caged in groups for further observation (usually seven days, or until survivors were clinically normal).

Results

Table 1 shows the values obtained for the percutaneous LD_{50} of technical parathion under different conditions of occlusion. The basic figure for comparison is provided by the LD_{50} for penetration through uncovered skin under similar conditions of restraint. In comparison with this result, the toxicity was reduced by occlusion with waterproof plaster alone or plaster backed with thin

¹'Sleek' (Smith & Nephew Limited).

TABLE 1
PERCUTANEOUS TOXICITY OF PARATHION TO THE RAT

Contact time (hrs)	Percutaneous LD_{50} (mg./kg.)				
	Plaster with aperture (= uncovered)	Plaster alone	Plaster + aluminium foil	Plaster + rubber sheet	Plaster + polythene sheet
24	18 [12.5-25]	54 (47-65) 39 (32-46) 50 (33-76) 54 (46-65)	5.2 (3.7- 7.4) 4.4 [3.1- 6.2] 9.5 (6.7-13.0) 8.0 (6.6-12.0)	41 (30-59)	18 [12.5-25]
4	c. 25-30	70 [50-100] 60 (42-84)	8.8 [6.2-12.5]	41 (30-59)	18 [12.5-25]

Animals: female Wistar albino rats.

Chemical: technical parathion, diluted 1/10 with glycerol formal. Acute oral LD_{50} , female rat = 3 mg./kg.

LD_{50} s calculated by the method of Weil (1952) or estimated from the mortalities (indicated by 'c').

() indicates 95% confidence limits calculated by the Weil method.

[] indicates the range from dose causing no mortality to dose causing 100% mortality, in tests where there were no fractional mortalities.

TABLE 2
COMPARATIVE PERCUTANEOUS TOXICITIES OF PESTICIDES IN THE RAT

Chemical	Contact time (hrs)	Sex of rat	Percutaneous LD ₅₀ (mg./kg.)	
			Plaster alone	Plaster + aluminium foil
Parathion 35% w/v ES formulation	24	F	76 (54-107)	16 (11-24)
Undiluted	24	F		8 (5.4-11)
Diluted 1/10 in water				13 (9.5-19)
				11 [8.0-16]
Parathion 25% w/v WP formulation, 1/5 suspension in water	24	F	65 (50-84) 54 (42-71) 46 (39-55) 54 (42-71)	21 (16-27) 35 [25-50]
Dimethoate 32% w/v ES formulation, undiluted	24	M	770 (648-917) 840 (658-1,073) 1,090 (917-1,300) 840 [707-1,000]	700 [500-1,000] 353 [250-500] 460 (385-545)
	4	M	> 1,100	1,000 (670-1,500)
Diazinon 20% w/v ES formulation, undiluted	24	M	> > 1,000	500 (335-745)
	4	M	> > 1,000	c. 1,000-1,200
Formothion 25% w/v ES formulation, undiluted	24	M	600 (420-840)	353 [250-500]
Pentachlorophenol technical 40% w/v solution in glycerol formal	24	F	149 (105-210)	105 (74-148)
Dinoseb amine 18.5% w/v aqueous formulation	24	M	134 (95-190) 113 [80-160]	80 (54-119) 67 (48-95)

LD₅₀s calculated by the method of Weil (1952) or estimated from the mortalities (indicated by 'c').

> indicates toxic effects but no deaths at the highest dose tested.

> > indicates no deaths or toxic effects at the highest dose tested.

() indicates 95% confidence limits calculated by the Weil method.

[] indicates the range from dose causing no mortality to dose causing 100% mortality, in tests where there were no fractional mortalities.

ES = emulsifiable solution; WP = wettable powder.

rubber sheet and was not affected by covering with plaster backed with polythene, but was increased by covering with plaster backed with aluminium foil. Different treatments gave toxicities varying 10-fold.

The results obtained for commercial formulations of parathion and several other pesticides are shown in Table 2. These were tested using the two types of occlusion which gave the highest and lowest LD₅₀ values with technical parathion, *i.e.*, the plaster alone and plaster backed with aluminium foil. Apart from the parathion ES formulation, only diazinon showed a marked increase in toxicity under aluminium foil, although there was a consistent tendency for toxicities to be greater when foil was used.

Data in Tables 1 and 2 show that when a given test was repeated several times under similar conditions, the greatest difference obtained between the highest and lowest LD₅₀ values was two-fold.

In the collaborative exercise (Table 3), the toxicities of the concentrates were also all within a two-fold range and were in close agreement with results obtained in this laboratory. The only results outside this range were from the laboratory which diluted the concentrate.

In the tests using parathion (Table 1) reduction of the contact time from 24 hours to 4 hours did not greatly alter the toxicity for any type of occlusion, indicating that with this material most of the skin absorption occurred within 4 hours, whatever the covering. With diazinon and dimethoate, absorption was apparently slower, since the 4-hour LD₅₀s were higher than the 24-hour LD₅₀s (Table 2).

Discussion

This study has indicated that dermal absorption is maximal under plaster backed with aluminium

TABLE 3
DERMAL LD₅₀S FOR A 35% ES FORMULATION OF PARATHION DETERMINED IN FIVE COLLABORATING LABORATORIES

Laboratory	Material tested	Type of rat	LD ₅₀ (mg./kg.) and 95% confidence limits	
			Female rats	Male rats
1	Concentrate	Porton Wistar	14 (11.7-17)	63.5 (55.6-72.6)
2	Concentrate	Boots Wistar	23.7 (14.6-38.6)	90 (71-114)
3	Aqueous dilutions (to constant volume)	M & B Wistar B.S.L. Wistar	6.3 (5.4-7.4) 10.6 (9.6-11.7)	(no test) (no test)
4	Concentrate	Porton Wistar (S.P.F.)	14.2 (13.1-15.4)	51.4 (47-56.2)
5	Concentrate	Porton Wistar (S.P.F.) C.F.E. (S.P.F.)	20.8 (14.0-30.8) 28.4 (21.5-37.5)	103 (77.8-137) 75 (53.5-105)
This laboratory	Concentrate 1:10 aqueous dilution	S.A.S. Wistar S.A.S. Wistar	16 (11-24) 8 (5.4-11) 13 (9.5-19) 11 [8.0-16]	(no test) (no test)

24-hour covered contact under plaster backed with aluminium foil.

foil, which has proven to be an inert and impermeable covering unlikely to react with the majority of pesticide formulations. The importance of the impermeability of aluminium foil covering is indicated by the abnormally low toxicity values obtained with absorptive coverings such as rubber sheet or plaster alone.

The method described is simple and gives reproducible results as demonstrated by both repeated experiments in this laboratory and collaborative studies involving other laboratories.

For the purposes of occupational safety to man, *i.e.*, the specification of handling precautions and protective clothing, pesticides are usually grouped into fairly broad categories. In the absence of specific data on man himself, the acute toxicity in animals by both oral and percutaneous (and, if appropriate, inhalation) routes is taken into account when assessing hazard. For practical purposes, therefore, very precise measurements of dermal LD₅₀s in laboratory animals are unnecessary, and an indication of the order of toxicity is sufficient.

No method of testing percutaneous toxicity in animals will produce results that are directly applicable to man. An indication of the possible hazard can, however, be obtained and can be checked by field observations of personnel during manufacture and use of the compound, or by comparison with materials of known hazard tested under similar conditions. Percutaneous toxicity should be compared with toxicity by other routes in the same species; and contact conditions should be varied, if necessary, in ways likely to be met in

practice. The aim throughout should be to simulate actual exposure conditions in laboratory tests.

The technique described has three major practical advantages. It has been designed for use with a small species, the rat, which is routinely used for acute and chronic toxicity tests on pesticides by other routes, and which has a comparatively large surface area relative to bodyweight. It can easily be adapted to other species such as the mouse, rabbit or guinea-pig for comparative tests. It is adaptable and thereby permits investigation of the main variable factors which might affect dermal absorption. Such factors include contact time and dilution of the formulation with water.

The recommended method for dermal toxicity testing of pesticides in this laboratory is therefore as follows:

- 1 use young albino rats similar to those in tests by other routes;
- 2 feed and water *ad libitum* throughout the test;
- 3 remove the hair from the dorsal and lateral surfaces of the trunk with electric clippers shortly before the test, using only rats with a healthy, intact epidermis;
- 4 test formulated chemicals undiluted or, if necessary, diluted with water;
- 5 apply chemical to prepared area of skin using a syringe with blunted needle and spread, ensuring constant volume per unit area;
- 6 cover contaminated area, as shown in Figs. 1 and 2, with plaster backed with aluminium foil, as shown in Figure 3;

7 cage animals individually and observe for selected contact time;

8 decontaminate by a thorough soap and water wash;

9 continue to observe animals for seven days.

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