Aust. Wildl. Res., 1981, **8**, 369–83

The Sensitivity of Australian Animals to 1080 Poison I. Intraspecific Variation and Factors affecting Acute Toxicity

J. C. McIlroy

Division of Wildlife Research, CSIRO, P.O. Box 84, Lyneham, A.C.T. 2602.

Abstract

The calculated sensitivity (LD_{50}) of a species to 1080 poison (sodium fluoroacetate), used for control of vertebrate pests, is affected by the experimental procedures employed. Variation can be minimized if the most obvious sources are avoided, as described in this paper. Very young mammals and female waterfowl in breeding condition may be more sensitive to 1080 than other members of their populations. No other substantial differences in sensitivity were found between males and females, immatures and adults, or within and between different populations of six species of birds and mammals in eastern Australia.

Introduction

Sodium fluoroacetate (compound 1080) is a tasteless and odourless water-soluble poison used in a number of countries throughout the world against rodents and other vertebrate pests. It was initially introduced into Australia and New Zealand over 25 years ago to help control rabbits, *Oryctolagus cuniculus*, but has since been used against a number of other pests, particularly brush-tailed possums *Trichosurus vulpecula*, wallabies *Macropus rufogriseus*, pademelons *Thylogale billardierii*, dingoes *Canis familiaris dingo*, and feral pigs *Sus scrofa*.

Since 1972 the Division of Wildlife Research, CSIRO, has been investigating whether these poisoning programs affect non-target fauna. The aim is to provide data which will be of use to vertebrate pest control authorities and to fauna conservation authorities for designing the best ways in which to use the poison. An essential part of the research is obtaining data on the sensitivity to 1080 of target and non-target species.

In toxicological work the sensitivity of different animals to a poison is usually expressed as the LD_{50} or median lethal dose, a statistical estimate of the dose, in milligrams of poison per kilogram body weight, that will kill 50% of a large population. [For lucid explanations of why LD_{50}s are used in preference to other LD values, see Griffith (1964), Hodge (1965) and Tucker and Crabtree (1970).]

The LD_{50} of a poison is not a physical constant like its density or solubility, nor strictly a value for the species as a whole. Instead, it and its 95% confidence limits are only an indication of the values that might be expected from repeated trials on the same strain of animals under the same experimental conditions. Because of this, many procedures for LD_{50} trials on laboratory animals have been specified in detail in recent years, largely as the result of legislative acts and regulations (Weil *et al.* 1966).

The necessity for such a standardized procedure has been questioned by Griffith (1964), Weil *et al.* (1966) and Weil and Wright (1967). Although they found statistically significant differences in LD_{50} values (up to 3·2-fold) within and between laboratories, related to differences in experimental procedure, they maintained that these were not great enough to change the interpretation of relative hazards of the test chemicals involved. However, because I was concerned with a controversial poison and its toxicity to a variety of wild animals, I felt it was important to assess the effects that differences in experimental procedure might have on LD_{50} values of 1080 and, if necessary, design a procedure to minimize such sources of variation. This paper reports the results of an assessment of six major experimental factors, outlines the experimental procedure followed in all subsequent LD_{50} trials, and describes the variation in acute toxicity to 1080 that can exist within and between different populations.

Methods

The six experimental factors which initially appeared the most important were the age, sex and nutritional status of experimental animals, the numbers used per dose level, the populations from which they were obtained, and the route of administration of the poison. Trials to evaluate their importance were carried out on rabbits, brush-tailed possums, tammar wallabies *Macropus eugenii*, brown antechinus *Antechinus stuartii*, galahs *Cacatua roseicapilla*, and black ducks *Anas superciliosa*. Every effort was made to treat the animals as humanely as possible under conditions similar to those recommended in a code of practice for the care and use of animals in research in Australia (National Health and Medical Research Council and CSIRO 1979).

Briefly, graded doses of 1080 were given to groups of three or five animals and the resultant mortality observed over the next 7 days. All trials were carried out with AR sodium fluoroacetate (*c.* 100% purity), dissolved in distilled water. The same concentration was maintained for each successive dose and the volume of solution always kept below 5% of the body weight. Dosing was restricted to only those individuals falling within a ±25% range of the mean weight of the specimens obtained. All animals were acclimatized to captivity for at least 2 weeks before the trials began. Animals being dosed were held either in the hands or in cotton or jute bags. Wire-loop incisor holders or wooden mouth gags were used to facilitate oral dosing. The same persons handled and dosed animals throughout each trial. Each group was dosed at approximately the same time of day, and one extra animal per group received a control dose of water. Ambient temperatures during the trials ranged between 10 and 15°C (tammar wallabies), 16 and 19°C (black ducks) and 19 and 27°C (remainder of species). Pilot doses (see 'Discussion') were given to some individuals to establish which dose level to use to begin some trials. A ratio of 1·26 was then maintained between successive dose levels. LD_{50}s and 95% confidence limits were calculated according to Thompson (1947) and Weil (1952).

Details of the variations in the age, sex, nutritional status and source of specimens, the numbers used per dose level and the route of administration of the poison are shown in Table 1. Because of constraints on the availability of specimens over half of the trials were carried out with two experimental variables simultaneously present. Student's *t* test was used for statistical comparisons of LD_{50}s, as used by Stoner (1969).

Results

Age of Experimental Animals

The LD_{50} trials on animals of different ages demonstrated that there are no significant differences in sensitivity to 1080 between immatures and adults of rabbits, marsupial mice (including adults of different age), and galahs, respectively (Table 1). Immature possums superficially appear *more tolerant* to 1080 ($P = <0·05$) than adult females either with or without pouch young (Table 1). However, because there are no significant differences in sensitivity between them and adult males (Table 1) and adult males and females (see next section) I suspect

Table 1. Variation in LD_{50} values of 1080 poison according to age, sex, other intraspecific factors or variations in trial procedures

Regions: C, Canberra; Y, Yaouk, N.S.W.; B, Bombala, N.S.W.; KI, Kangaroo I., S.A.; Br, Brindabella Range, N.S.W.; H, Hay, N.S.W.; G, Griffith, N.S.W. Nutrition: F, fasted; NF, not fasted. *Significant difference, *t*-test ($P<0.01$)

Trial No.	Age	Sex	No. per dose level	Method of administration	Region	Nutrition	LD_{50} and 95% CL (mg kg^{-1})
				Rabbits			
1	Ad.	M	5	Oral	C	F	0·35 (0·30–0·41)
2	Ad.	M	5	Oral	Y	NF	0·40 (0·32–0·50)
3	Ad.	F	5	Oral	Y	NF	0·40 (0·29–0·54)
4	Ad.	F	3	Oral	Y	NF	0·34 (0·30–0·39)
5	Imm.	F	5	Oral	Y	NF	0·36 (0·30–0·43)
6	Imm.	M	3	Oral	Y	NF	0·34 (0·30–0·39)
				Brush-tailed possums			
1	Ad.	M	5	Oral	C	F	0·68 (0·59–0·78)
2	Ad.	M	5	Oral	B	NF	0·67 (0·49–0·92)
3	Ad.	M	5	I.p.	B	NF	0·57 (0·51–0·64)
4	Ad.	F + p.y.	5	Oral	B	NF	0·62 (0·55–0·71)
5	Ad.	F + p.y.	3	Oral	B	NF	0·58 (0·40–0·84)
6	Ad.	F	3	Oral	B	NF	0·58 (0·40–0·84)
7	Imm.	M & F	5	Oral	B	NF	0·86 (0·67–1·09)
				Tammar wallabies			
1	Ad.	M	5	Oral	KI	NF	0·27* (0·23–0·31)
2	P.y.	M	3	Oral	KI	NF	0·15* (0·12–0·20)
				Antechinus stuartii			
1	Imm.	M	5	Oral	Br	NF	1·42 (1·24–1·61)
2	Ad.	M	5	Oral	Br	NF	1·37 (1·15–2·16)
3	Ad.	M	5	Oral	Br	NF	1·42 (1·07–1·88)
4	Ad.	M	5	Oral	Br	F	1·85 (1·43–2·40)
				Galahs[A]			
1	Ad.	M	5	S.c.	C	NF	5·69 (5·08–6·37)
2	Ad.	M	5	I.p.	H	NF	4·67 (3·10–7·04)
3	Ad.	M	3	S.c.	C	NF	5·77 (3·75–10·46)
4	Ad.	F	5	S.c.	C	NF	5·34 (3·97–7·20)
5	Ad.	F	5	S.c.	G	NF	5·72 (4·57–7·16)
6	Imm.	M & F	5	S.c.	G	NF	5·18 (4·48–6·00)
				Black ducks[B]			
1	Ad.	M	5	S.c.	C	NF	18·91* (16·33–21·89)
2	Ad.	F	5	S.c.	C	NF	10·01* (7·43–13·48)
3	Ad.	F	5	S.c.	C	NF	23·80 (15·30–37·03)

[A]Trial 4, non-breeding females; trial 5, breeding females.
[B]Trial 2, breeding females; trial 3, non-breeding females.

that this difference is only an artefact of the data. The pouch young of tammar wallabies are significantly *more susceptible* to 1080 than adults ($P = <0 \cdot 01$) (Table 1). The pouch young of brush-tailed possums and northern native cats, *Dasyurus hallucatus*, similarly appear to be more sensitive than adults. More pouch young possums than adults died at each dose level, although only their mothers were dosed with 1080; presumably the young ingested lethal amounts of 1080 in the milk. The eight pouch young of one northern native cat also all died within 24 h after their mother received a non-lethal dose (84% of a LD_{50}) but the five pouch young of a tiger cat, *Dasyurus maculatus*, survived in similar circumstances (74% of a LD_{50}). Williams (1948) similarly reports young rats being killed by milk from their poisoned mothers.

Sex of Experimental Animals

The LD_{50} trials also demonstrated that males and females of rabbits and brush-tailed possums (with or without pouch young), respectively, are equally sensitive to 1080 (Table 1). There is, similarly, no significant difference in galahs between breeding and non-breeding adult females and males, nor in black ducks between males and non-breeding adult females (Table 1). Female black ducks in breeding condition (i.e. containing rapidly developing ova), however, were significantly *more* sensitive to 1080 than males ($P = <0 \cdot 01$) or females in non-breeding condition ($P = <0 \cdot 05$).

Nutritional State of Experimental Animals

There were no significant differences in sensitivity to 1080 between fasted and non-fasted rabbits, brush-tailed possums and brown antechinus, respectively (Table 1).

Source of Experimental Animals

There were no significant differences in sensitivity to 1080 between samples of rabbits, brush-tailed possums and galahs, respectively, from different regional populations in eastern Australia (Table 1).

Number of Animals used per Dose Level

Trials on rabbits, brush-tailed possums and galahs indicated that there is no significant difference in LD_{50}s of 1080 when either three or five animals are used per dose level (Table 1). The 95% confidence interval, though, was narrower with five animals per dose level for brush-tailed possums and galahs, compared with that with three animals per dose level. This did not occur with the rabbits.

Route of Administration of Poison

Results from trials on brush-tailed possums and galahs revealed that, although intraperitoneal injections produce lower LD_{50} values than oral dosing (possums) or subcutaneous injections (galahs), the differences are not significant (Table 1).

Discussion

Many of the experimental factors which may affect the determination of LD_{50} values have been discussed by Hurst (1958), Hagan (1959), Keplinger *et al.* (1959),

Ferguson (1962), Griffith (1964), Dearborn (1967) and Weil and Wright (1967). I considered most of these factors, together with the results from my LD_{50} trials, in designing a reasonably standardized experimental procedure for obtaining subsequent LD_{50}s for a variety of species under different environmental conditions. The factors involved and procedure adopted were as follows.

Age of Experimental Animals

With increasing age, considerable differences can occur in the absorption, distribution, metabolism and excretion of toxic compounds in animals. This can result in considerable variations in sensitivity between newborn animals, young actively growing animals, mature animals and senescent animals (Bender 1964, 1965; Yeary *et al.* 1966; Dearborn 1967; Goldenthal 1971; Matsumura 1975).

Very little information exists in relation to differences in sensitivity to 1080 with age. Hereford calves, *Bos taurus*, are more sensitive than cows (0·22 *v.* 0·49 mg kg^{-1}) (Robison 1970) and 7-day-old ducklings of the mallard, *Anas platyrhynchos*, are more sensitive than 3-month-old ducklings (5·97 *v.* 9·11 mg kg^{-1}) (Tucker and Crabtree 1970). In contrast, 24-h-old dogs, *Canis familiaris*, are reportedly less sensitive to fluoroacetate than adults (Chenoweth 1949). Because of this and the variations discovered in acute toxicity of 1080 amongst the test animals of different age, I do not include very young or senescent animals in LD_{50} trials (see also Tucker and Crabtree 1970).

Sex of Experimental Animals

There are often sex-related differences in susceptibility to pesticides or poisons among mammals (Pallota *et al.* 1960; Tucker and Crabtree 1970; Matsumura 1975). Females, for example, are often more sensitive to poisons than males or more likely to produce variable LD_{50}s because of variations in their hormonal state or reproductive condition (Weil *et al.* 1953; Hurst 1958; Weil and Wright 1967). Pronounced sex-related differences in acute toxicity are apparently uncommon amongst birds (Tucker and Crabtree 1970).

Evidence of sex-related differences in sensitivity to 1080 is equivocal. Although Kalmbach (1945), Dieke and Richter (1946), Rosell (1965) and Stoner (1969) found no significant variations related to sex in LD_{50}s for wild Norway rats, *Rattus norvegicus*, 'ricefield' rats and 'laboratory' rats, Williams (1948) noted that pregnant and lactating *Rattus rattus alexandrinus* require about 50% more 1080 to kill them than non-breeding females or males.

A similar equivocal situation appears to exist amongst birds. Males of two species of wild ducks have been reported to be more sensitive to 1080 than females but males of a third species were less sensitive (Ward and Spencer 1947). No such differences have been noted in other species (Chenoweth 1949), particularly young non-breeding birds (Tucker and Crabtree 1970), but it may not hold for mature birds during the breeding season (Tucker and Haegele 1971). Laying hens, *Gallus gallus*, for example, are more sensitive to 1080 than non-layers (Cottral *et al.* 1947).

Because of this equivocal information and the results obtained from the trials on rabbits, brush-tailed possums, galahs and black ducks, LD_{50} trials, wherever possible, are restricted to adult males. In certain circumstances, such as when there

are only a limited number of specimens available or, as with certain birds, there is no simple, rapid method for distinguishing between sexes, non-breeding females are included.

Nutritional State of Experimental Animals

Although generally animals are fasted before dosing in LD_{50} trials, supposedly to avoid the effects of variable stomach contents on absorption and to bring each individual closer to a more comparable metabolic state (Tucker and Crabtree 1970), I eventually rejected the procedure. Griffith (1964), and my results reported earlier, demonstrated that various fasting regimes have no effect on LD_{50} values. I also felt that dosing animals just before their regular feeding time in captivity (e.g. carnivores) would better reflect the absorption likely to take place under field conditions. All captive animals, therefore, have continual access to water and are fed regularly or *ad libitum* with either natural foods or a laboratory diet.

Source or Strain of Experimental Animals

The scientific literature is replete with reports of apparent differences in the response of various strains of animals to drugs and other chemicals (Griffith 1964; Dearborn 1967; Weil and Wright 1967). Weil *et al.* (1966), for example, found that faster growing strains of rats are associated with consistently higher LD_{50} values for various chemicals compared with slower growing strains. More specifically, Chenoweth and Gilman (1946) recorded that the 'small short-eared breed' of rabbits is less sensitive to fluoroacetate than other breeds. Oliver *et al.* (1977) have similarly discovered large differences in tolerance to 1080 between eastern and western Australian populations of a number of native mammals. They attributed this difference to the greater capacity of animals in the western populations to detoxify fluoroacetate, which occurs naturally in their plant diets.

Wheeler and Hart (1979), in contrast, found no sigificant differences in the sensitivity to 1080 of rabbits from three well separated sites in Western Australia. The rabbits had never been exposed to 1080 at one of the sites, but at the other two fluoroacetate occurs naturally in certain plants and is also used in baits for rabbit control. Although they initially observed significant differences in LD_{50} values between my samples from eastern populations and theirs from western populations, they concluded that these differences were apparently due to differences in experimental technique, because when they closely duplicated my trial procedure they obtained a similar LD_{50} to mine (S. H. Wheeler, personal communication 1978).

Although the results of my LD_{50} trials on rabbits, brush-tailed possums and galahs revealed no significant differences in respective sensitivity to 1080 between the regional populations sampled, I still advocate caution in carrying out trials on animals from different populations. Regional variations in toxic susceptibilities of brush-tailed possum populations, for example, have recently been reported in New Zealand (Anon. 1978).

Weight Range of Experimental Animals

Weil *et al.* (1966) concluded that differences in body weight of laboratory rats significantly affected their LD_{50}s for a range of chemicals. Howard *et al.* (1973),

also working with rats, found in contrast that their LD_{50} for 1080 was not affected by differences in body weight. As a compromise to standardization I try to restrict dosing of each species to only those animals falling within $\pm 25\%$ range of the mean weight. The only exception is with species that are difficult to obtain and which vary considerably in weight; under such circumstances I select individuals on the basis of the smallest range in weight possible. I also assign individuals to each dose group according to the lowest range in weight possible, but make a random selection when deciding which particular dose each group receives.

Condition of Experimental Animals

Generally, animals are kept in captivity for at least 2 weeks before dosing. On five occasions during field trials I, for practical reasons, had to restrict the period of acclimatization to only 1 week. In each case the species concerned (e.g. feral pigs; little crow *Corvus bennetti*), appeared to rapidly adjust to captivity. In contrast, a number of larger macropods and common wombats, *Vombatus ursinus*, kept in captivity for much longer periods (i.e. 1–2 months), proved to be particularly susceptible to disease or stress associated with captivity, such as pneumonia and capture myopathy. I believe that LD_{50} data which are directly relevant to a field situation will be obtained for such species only by dosing free-ranging specimens and subsequently radio-tracking their movements and fate.

Environmental Factors

The effects of variations in environmental factors on LD_{50} values are not well known (Dearborn 1967). Extremes of temperature are known to influence the action of toxic chemicals in homeotherms, probably by altering the rate of absorption, distribution or metabolism of the compound and influencing the rate of reaction. Strychnine, for example, is more toxic to laboratory rats at environmental temperatures of 8 and 36°C than at the more normal temperature of 26°C (Keplinger *et al.* 1959). Mice, *Mus musculus*, are similarly appreciably more sensitive to fluoroacetate at elevated environmental temperatures (Chenoweth 1949). This has also recently been demonstrated by A. J. Oliver (personal communication, 1979). He found that different ambient temperatures can cause almost fivefold differences in the susceptibility of mice to 1080 and just over twofold differences amongst guinea pigs, *Cavia porcellus*. Both species were most susceptible at low (e.g. <13°C) and high (e.g. >30°C) temperatures. Thus it is imperative to carry out comparative LD_{50} trials with 1080 on different species at similar temperatures. If this is not possible, the range of ambient temperatures encountered during the trial, particularly during the first 24 h, should be reported, to allow a critical examination of the LD_{50} values obtained.

Dieke and Richter (1946) found that carrying out LD_{50} trials in different seasons did not significantly affect the toxicity of 1080, whereas Chenoweth (1949) noted that frogs were more sensitive to fluoroacetate in summer than in winter. This emphasizes the need to measure rectal temperature of cold-blooded animals before dosing, to confirm that they are active. My results also suggest that the seasonal condition of animals (particularly in regard to breeding) should be taken into account in determining the acute toxicity of 1080 to any species.

Number of Animals per Dose Level

Weil *et al.* (1953) have shown that, for practical purposes, there is little difference between LD_{50}s for a range of chemicals when either three or ten animals are used per dose level with a ratio of 1·26 or 2·0 between dose levels. This supports my findings from the trials on rabbits, brush-tailed possums and galahs that there is no significant difference in LD_{50}s for 1080 on three or five animals per dose level. Both the number of animals per dose level and the ratio between successive doses, however, can affect the fiducial range or 95% confidence limits. Generally, as seen with the brush-tailed possums and galahs, the greater the number of animals per dose level and smaller the ratio between doses, the narrower the 95% confidence interval.

My policy, therefore, is to use five animals per dose level with a ratio of 1·26 between doses for common species, but only three animals per dose level and the same ratio for less common species or those difficult to obtain.

Route of Administration of Poison

The effect on resultant LD_{50} values of administering 1080 by different routes is not very clear. Although Ward (1946), Quin and Clark (1947), Chenoweth (1949) and Spector (1956) maintained that fluoroacetate is equally toxic to most animals no matter how administered, Ward and Spencer (1947) have pointed out that the lack of difference is only wholly true for methyl fluoroacetate and not entirely true for sodium fluoroacetate (i.e. 1080). Chenoweth and Gilman (1946) also found slightly increased toxicities after oral administration into some animals, but they labelled these increases as 'probably not significant'. Jarrett and Packham (1956), in contrast, found that intraperitoneal and intravenous injections of 1080 are more toxic to sheep, *Ovis aries*, than oral doses and subcutaneous injections.

Because the trials on brush-tailed possums and galahs revealed no significant differences in LD_{50} values resulting from differences in the route of administration of 1080, I continue to use the oral route of administration with all mammals because it is the route by which the poison is ingested in the field. Actual dosing is accomplished with an oesophageal catheter (stomach tube) for large mammals or ball-pointed needle for smaller species, both with the appropriate hypodermic syringe. With birds, however, I find it easier, because of the presence of the crop in some species and not in others, to inject the 1080 subcutaneously in the hind-neck region.

It is well known that vomiting is a characteristic and early symptom of 1080 poisoning in certain species (Ward and Spencer 1947; Atzert 1971) even, as I found, when the 1080 is administered by intraperitoneal injection. Because of this, oral LD_{50}s probably more closely reflect the sensitivity of animals under actual field poisoning conditions. LD_{50}s obtained through injections, on the other hand, represent the susceptibility of the species when its first line of defence against being poisoned (i.e. vomiting) has been bypassed, and are therefore less relevant to field situations.

Purity and State of Poison

All my LD_{50} trials are carried out with AR sodium fluoroacetate (*c.* 100% purity). The purity of the 1080 used (e.g. commercial 1080 is of *c.* 90% purity) should

always be stated, to allow a correct comparison of LD_{50} values, and any impurities present should be non-toxic. Chenoweth (1949) points out that most commercially produced 1080 contains fluoride impurities and this can be a source of error in interpreting the results of studies with fluoroacetate.

I administer the prescribed amount of 1080 in a solution of distilled water. Atzert (1971) maintains that 1080 has substantially the same oral toxicity regardless of whether the carrier is water, meat, grain, oil, gum acacia suspension or gelatin capsule. Although some earlier scientists claimed that aqueous solutions of 1080 decrease in toxicity, even when refrigerated (Chenoweth 1949; Harrison *et al.* 1951), recent studies demonstrate that they are very stable. According to C. G. Rammell (personal communication, 1978) standard solutions of 1080 prepared in distilled water and stored at room temperature for 10 y showed no significant breakdown, while solutions of 1080 prepared in stale algae-infested water were just as toxic after 12 months as they were initially.

Volume of Poison Administered

The volume of 1080 solution administered is rarely stated in reports of LD_{50} trials. Ferguson (1962) found significant differences in LD_{50} values between doses of various compounds given at 5% and 1% of the body weight; the general trend was that the greater the volume of the solution, the greater was the death rate (see also Treon 1962; Griffith 1964). In contrast, Weil *et al.* (1966) found that the LD_{50} of 26 chemicals was relatively unaffected by their dilution. Matsumura (1975) advises, for physiological reasons, that the volume be kept at a minimum, certainly below 5% of the body weight. I follow such a course, maintaining the same concentration for each successive dose and administering volumes of solution ranging between 0·01 and 3·76% of the body weight, depending on the size of the species involved and the accuracy required, particularly in administering very small volumes to small species. Equivalently calculated doses of distilled water are also administered to one control animal per dose level.

Time of Dosing

The ability of some animals to metabolize drugs varies according to a circadian rhythm (Radzialowski and Bousquet 1967). Although I did not know if and what rhythms might be involved in the metabolism of 1080, for the sake of standardization I dose each group of species at approximately the same time of the day.

The Handling of Experimental Animals

I use a variety of methods to restrain each species during dosing, including holding them in the hands, in cotton or jute bags or in wire cones and weldmesh 'crushes', with wire-loop incisor holders or wooden and metal mouth gags to facilitate oral dosing. Although Stoner (1969) found that the LD_{50} of 1080 for laboratory rats is not significantly affected by traumatic but non-fatal injuries, it still seems common sense to prevent the animals from struggling violently and injuring themselves, and to provide uniform handling conditions during each trial.

Changes in Personnel

Weil *et al.* (1966) demonstrated that changes in personnel performing LD_{50} trials

did not significantly affect LD_{50} values. Despite this I ensure the same persons handle and dose animals throughout a particular trial.

Pilot LD_{50} Values

Tucker and Haegele (1971) maintained that the widespread use of 'representative species' for LD_{50} trials is undesirable, and recommended that extrapolation of toxicity data from one species to another should be avoided. Their studies showed that the range of LD_{50}s for 1080 amongst six species of birds was sixfold, and therefore that predicting the LD_{50} for one species on the basis of that for another could lead to considerable errors. Despite this, I basically prefer this 'similar species' approach for range-finding purposes with 1080 to that of Weil *et al.* (1953), who used a ratio of 2·0 rather than 1·26 between dose levels and based their first two doses on information on the LD_{50} of a similar chemical. They reasoned that if these first two doses were far enough apart, at least one of them would be included in the actual calculation of the LD_{50}.

My method is to give one animal a pilot dose estimated, from LD_{50}s for similar species (similar either taxonomically or in terms of their food and feeding behaviour or metabolic rate), to be close to the expected LD_{50}. If this animal shows pronounced symptoms of poisoning I begin the main trial at or close to that dose level and administer successive lower, higher, or both lower and higher doses (ratio of 1·26) to groups of from three to five animals according to the resultant values for mortality. If the initial pilot animal exhibits no symptoms or dies, I administer higher or lower doses to successive individuals until one either shows symptoms of poisoning, dies or survives. The dose at which this happens is then used as the basis for selection of the initial dose level in the main trial. This approach has proved to be completely satisfactory and usually involves only one, two or three animals, far less than that required by Weil *et al.* (1953).

Post-dosing Observation Period

I generally observe animals for only the first 7 days after dosing. This may not be long enough but has often been the most practical, especially under field conditions. Many other workers extended their observations over 14 days or even longer if the animals were still showing evidence of intoxication (Dieke and Richter 1946; Tucker and Haegele 1971).

Chenoweth (1949) maintains that in most species individuals surviving a dose of 1080 completely recover within 24 h. Rats require 48–72 h. Emlen and Strecker (1951) similarly found that wild house mice either die within a few hours of ingesting 1080 or suffer a period of debility and convalescence for 4–5 days. My results confirm variability. Forester kangaroos *Macropus giganteus*, for example, took up to 3 days to die, the reaction of dingoes was similar to that of the mice, and wedge-tailed eagles *Aquila audax* and feral pigs sometimes were still recovering after 7 days.

Emlen and Strecker (1951) maintain that the results of laboratory tests on wild mice are reasonably indicative of the lethality of 1080 in actual field suppression programs. Their studies demonstrated that the survival of individuals incapacitated by sublethal doses of 1080 is not reduced by competition with normal unpoisoned members of the population nor benefited by the artificial protection of laboratory conditions.

During the 7-day post-dosing observation period any symptoms, changes in behaviour and mortalities are recorded. This usually involves semi-continuous observations during the first day and repeated inspections or observations on subsequent days. Survivors are then released (preferably at their capture site), used in other scientific studies, or in some cases (see next section) rested and redosed later. Gross autopsies are performed on all dead animals and tissue or organ samples taken for subsequent measurement of 1080 levels.

Re-use of Animals

Some species can acquire a tolerance to fluoroacetate after repeated sublethal doses (Quin and Clark 1947; Chenoweth 1949) and others can accumulate sublethal amounts until they finally reach a lethal quantity (Quin and Clark 1947; Foss 1948; Rowley 1963). Although both phenomena are only temporary and can be avoided provided a sufficient interval is left between doses, the extent of this interval can be critical.

Chenoweth (1949) showed that a sublethal dose provides rats with some protective effect for 24–36 h against further doses, and that the portion of a dose that is exerting a toxic action can be eliminated within 24 h. Foss (1948) discovered that some dogs will not accumulate lethal amounts of fluoroacetate provided there is an interval of 2–3 days between sublethal dosings, but Annison *et al.* (1960) noted in sheep that a fatal cumulative effect can still occur in animals receiving a second, similar normally sublethal dose 1 month after they had received the first. Continued sublethal doses for 1080 may also cause a subtle loss or temporary impairment of cell function or organ damage which could render the animals more susceptible to further challenging doses of 1080 or diseases. Such doses, for instance, are known to cause regressive changes in the germinal epithelium of the seminiferous tubules (Mazzanti *et al.* 1965; see also Howard *et al.* 1973). These changes, though, are reversible.

Strictly speaking, if all possible sources of procedural variation in LD_{50} trials are to be eliminated, each animal should be used only once. Initially, I did not appreciate the problems of tolerance or long-term cumulative effect (which are attributed largely to the slowness with which fluoroacetate is excreted) and because of a shortage of specimens re-dosed some individuals at a higher level after an interval of only 8–74 days. Although in these instances no animals died after the initial dose and only 14% showed any symptoms, mainly slightly increased sensitivity to stimuli or vomiting, for the sake of standardization they should not have been re-used. Survivors of doses which kill some individuals should certainly not be used, because they have undergone a degree of selection and do not strictly conform to the requirements of an LD_{50} test.

Method of Calculating LD_{50}

I use the moving-average method and tables first proposed by Thompson (1947) and Weil (1952) to calculate LD_{50}s. This allows the LD_{50} (with 95% confidence limits) to be calculated by interpolation, by use of four consecutive dosage levels without any assumption that the data fit or must be transformed to any type of curve. The LD_{50} and its confidence limits obtained by this method agree very closely with those obtained by more complex and time-consuming methods such as probit

analysis (Bliss 1938). The method used is supported by Armitage and Allen (1950), following their practical comparison of several methods.

In summary, although Griffith (1964) maintains that there is no single 'correct' way to determine LD_{50}s, there is still an obvious need for a certain amount of standardization of experimental procedure. The studies reported here suggest that such a procedure need not be highly inflexible provided obvious sources of variation such as those described are avoided and details of the specimens and procedure published (see, e.g., Tucker and Haegele 1971). Too often in the past, because no details have been included about the experimental procedure or because of inadequate numbers or arbitrary changes in the numbers of animals per dose level and ratio between doses, it has been impossible to calculate a precise LD_{50}. Instead *approximate* LD_{50}s are given, e.g. 'while not *accurately* established the LD_{50} [of methyl fluoroacetate for mixed-breed dogs] lies in the *vicinity* of 0·06 mg/kg' [my italics] (see Chenoweth and Gilman 1946). Such values then have a tendency to be requoted or accepted as *the* LD_{50}s applicable for the species in all situations, even, in this case, erroneously for sodium fluoroacetate, by scientists and vertebrate pest controllers (e.g. Ward and Spencer 1947; McGirr and Papworth 1955; Spector 1956; Tomlinson and Gooding 1971). Alternatively, LD values other than the LD_{50} are given, such as the LD_{47}, LD_{92} or LD_{100} (e.g. Ward and Spencer 1947). Although a LD_{100} may be a more desirable figure in relation to a pest species, such a value, for statistical reasons, is less precise than the LD_{50} and does not facilitate the comparison of the sensitivity of different species to 1080 poison.

Conclusions

The LD_{50} is only a guide to or the most rapid and convenient indicator of the potential hazard to a species from a poison. Much additional information is required before the species' actual vulnerability in the field can be assessed, such as what proportion of the population discovers and eats poisoned baits or carcasses and how much poison they then ingest. However, from a practical view, my results indicate:

(1) Very young animals, such as the pouch young and possibly young-at-heel of marsupials, are more sensitive to 1080 poison than older animals. This could mean that a greater proportion of them might be killed during a poisoning campaign than adults, either directly through eating baits or indirectly by ingesting 1080 in their mother's milk. This might then show up as a lack of appearance of many immatures in the next year's cohort.

(2) Individuals experiencing seasonally increased metabolic demands, such as female waterfowl during the breeding or moulting periods, may also be more sensitive to 1080 than other members of the population. Again, depending on the date of poisoning, this could affect the numbers of the next generation.

(3) There appear to be no other significant differences in sensitivity within and between different populations of six species of birds and mammals in eastern Australia.

(4) The LD_{50} or measurement of the sensitivity of a species to 1080 is very much a function of experimental procedure, particularly environmental temperatures. For this reason caution is necessary in extrapolating laboratory-derived LD_{50}s to field situations. Traditional laboratory trials are difficult

with some species, such as certain macropods and wombats, because of problems with diseases and stress in captivity, and the only practical alternative in these cases appears to be dosing and then radio-tracking free-ranging animals.

Acknowledgments

I gratefully acknowledge the assistance given by Messrs M. Jones, V. Kowalski and, in particular, R. Cooper and E. Gifford in obtaining the animals and carrying out the LD_{50} trials. All animals were obtained under permits provided by the Conservation and Agriculture Branch, Department of the Capital Territory, Canberra, and the National Parks and Wildlife Services of New South Wales and South Australia.

References

Annison, E. F., Hill, K. J., Lindsay, D. B., and **Peters, R. A.** (1960). Fluoroacetate poisoning in sheep. *J. Comp. Pathol.* **70,** 145–55.

Anon. (1978). Bait development and toxicology. N.Z. For. Serv. For. Res. Inst. Annu. Rep. 1978, pp. 68–70.

Armitage, P., and **Allen, I.** (1950). Methods of estimating the LD_{50} in quantal response data. *J. Hyg.* **48,** 298–322.

Atzert, S. P. (1971). A review of sodium monofluoroacetate (compound 1080) – its properties, toxicology and use in predator and rodent control. U.S. Fish Wildl. Serv. Spec. Sci. Rep. Wildl. No. 146.

Bender, A. D. (1964). Pharmacologic aspects of aging: a survey of the effect of increasing age on drug activity in adults. *J. Am. Geriatr. Soc.* **12,** 114–34.

Bender, A. D. (1965). A pharmacodynamic basis for changes in drug activity associated with aging in the adult. *Exp. Gerontol.* **1,** 237–47.

Bliss, C. I. (1938). The determination of the dosage-mortality curve from small numbers. *Q. J. Pharm. Pharmacol.* **11,** 192–216.

Chenoweth, M. B. (1949). Monofluoroacetic acid and related compounds. *J. Pharmacol. Exp. Ther.* **97,** 383–424.

Chenoweth, M. B., and **Gilman, A.** (1946). Studies on the pharmacology of fluoroacetate. I. Species response to fluoroacetate. *J. Pharmacol. Exp. Ther.* **87,** 90–103.

Cottral, G. E., Dibble, G. D., and **Winter, B.** (1947). The effect of sodium fluoroacetate ("1080" rodenticide) on white leghorn chickens. *Poult. Sci.* **26,** 610–13.

Dearborn, E. H. (1967). Comparative toxicity of drugs. *Fed. Proc.* **26,** 1075–7.

Dieke, S. H., and **Richter, C. P.** (1946). Comparative assays of rodenticides on wild Norway rats. I. Toxicity. U.S. Public Health Rep. No. 61, pp. 672–9.

Emlen, J. T., Jr., and **Strecker, R. L.** (1951). The effect of laboratory confinement on survival of poisoned house mice. *Ecology* **32,** 331–2.

Ferguson, H. C. (1962). Dilution of dose and acute oral toxicity. *Toxicol Appl. Pharmacol.* **4,** 759–62.

Foss, G. L. (1948). The toxicology and pharmacology of methyl fluoroacetate (MFA) in animals, with some notes on experimental therapy. *Br. J. Pharmacol.* **3,** 118–27.

Goldenthal, E. I. (1971). A compilation of LD_{50} values in newborn and adult animals. *Toxicol. Appl. Pharmacol.* **18,** 185–207.

Griffith, J. F. (1964). Interlaboratory variations in the determination of acute oral LD_{50}. *Toxicol. Appl. Pharmacol.* **6,** 726–30.

Hagan, E. C. (1959). Acute toxicity. In 'Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics'. pp. 17–25. (U.S. Food and Drug Administration: Texas.)

Harrison, B. L., Bransford, A. V., and **McNamara, B. P.** (1951). Deterioration of sodium monofluoroacetate in water and saline solutions. *Fed. Proc.* **10,** 306–7.

Hodge, H. C. (1965). The LD_{50} and its value. *Am Perfumer Cosmet.* **80,** 57–60.

Howard, W. E., Marsh, R. E., and **Palmateer, S. D.** (1973). Selective breeding of rats for resistance to sodium monofluoroacetate. *J. Appl. Ecol.* **10,** 731–6.

Hurst, E. W. (1958). Sexual differences in the toxicity and therapeutic action of chemical substances. In 'A Symposium on the Evaluation of Drug Toxicity'. (Eds A. L. Walpole and A. Spinks.) pp. 12–25. (J. & A. Churchill Ltd: London.)

Jarrett, I. G., and **Packham, A.** (1956). Response of the sheep to sublethal doses of fluoroacetate. *Nature (Lond.)* **171,** 580–1.

Kalmbach, E. R. (1945). "Ten-eighty", a war-produced rodenticide. *Science (Wash. D.C.)* **102,** 232–3.

Keplinger, M. L., Lanier, G. E., and **Deichmann, W. B.** (1959). Effects of environmental temperature on the acute toxicity of a number of compounds in rats. *Toxicol Appl. Pharmacol.* **1,** 156–61.

McGirr, J. L., and **Papworth, D. S.** (1955). The toxicity of rodenticides. I. Sodium fluoroacetate, Antu and Warfarin. *Vet. Rec.* (12 Feb. 1955), 124–31.

Matsumura, F. (1975). 'Toxicology of Insecticides.' (Plenum Press: New York.)

Mazzanti, L., Lopez, M., and **Berti, M. G.** (1965). Atrophy of the testes produced by sodium monofluoroacetate in albino rats. *Experientia (Basel)* **21,** 446–447. [Not seen; cited by Atzert (1971).]

National Health and **Medical Research Council and CSIRO.** (1979). Code of practice for the care and use of animals in research in Australia. (Australian Government Publishing Service: Canberra.)

Oliver, A. J., King, D. R., and **Mead, R. J.** (1977). The evolution of resistance to fluoroacetate intoxication in mammals. *Search (Syd.)* **8,** 130–2.

Pallota, A. J., Kelly, M. G., Rall, D. P., and **Ward, J. W.** (1960). Influence of sex and body weight on the toxicity of E-73 acetate (acetoxyecleheximide). *Pharmacologist* **2,** 84.

Quin, J. I., and **Clark, R.** (1947). Studies on the action of potassium monofluoroacetate (CH_2 FCOOK) [*Dichapetalum cymosum* (Hook) Engl.] toxin on animals. *Onderstepoort J. Vet. Res.* **22,** 77–90.

Radzialowski, M., and **Bousquet, W. F.** (1967). Circadian rhythm in hepatic drug-metabolizing activity in the rat. *Life Sci.* **6,** 2545–8.

Robison, W. H. (1970). Acute toxicity of sodium monofluoroacetate to cattle. *J. Wildl. Manage.* **34,** 647–8.

Rosell, N. C. (1965). The durability of toxicity of 1080 impregnated corn grain. *Philipp J. Plant Ind.* **27,** 169–77.

Rowley, I. (1963). The effect on rabbits of repeated sublethal doses of sodium fluoroacetate. *CSIRO Wildl. Res.* **8,** 52–5.

Spector, W. S. (1956). 'Handbook of Toxicology. Vol. 1. Acute Toxicities.' (W. B. Saunders Co.: London.)

Stoner, H. B. (1969). Studies on the mechanism of shock. The effect of trauma on the toxicity of 3,5,dinitro-*o*-cresol and sodium fluoroacetate. *Br. J. Exp. Pathol.* **50,** 277–84.

Thompson, W. R. (1947). Use of moving averages and interpolation to estimate median-effective doses. I. Fundamental formulas, estimation of error, and relation to other methods. *Bacteriol. Rev.* **11,** 115–45.

Tomlinson, A. R., and **Gooding, C. D.** (1971). Wildlife hazards from 1080 baiting. *J. Agric. West. Aust.* **12,** 268–70.

Treon, J. F. (1962). Toxicological aspects of cosmetic formulation – a synopsis. *Am. Perfumer* **77,** 35–41.

Tucker, R. K., and **Crabtree, D. G.** (1970). Handbook of toxicity of pesticides to wildlife. U.S. Fish Wildl. Serv. Res. Publ. No. 84.

Tucker, R. K., and **Haegele, M. A.** (1971). Comparative acute oral toxicity of pesticides to 6 species of birds. *Toxicol. Appl. Pharmacol.* **20,** 57–65.

Ward, J. C. (1946). Rodent control with 1080, Antu, and other war-developed toxic agents. *Am. J. Public Health* **36,** 1427–31.

Ward, J. C., and **Spencer, D. A.** (1947). Notes on the pharmacology of sodium fluoroacetate – compound 1080. *J. Am. Pharm. Assoc.* **36,** 59–62.

Weil, C. S. (1952). Tables for convenient calculation of median-effective dose (LD_{50} or ED_{50}) and instructions in their use. *Biometrics* **8,** 249–63.

Weil, C. S., Carpenter, C. P., and **Smyth, H. F.** (1953). Specifications for calculating the median effective dose. *Am. Ind. Hyg. Q.* **14,** 200–6.

Weil, C. S., Carpenter, C. P., West, J. S., and **Smyth, H. F.** (1966). Reproductivity of single oral dose toxicity testing. *Am. Ind. Hyg. Assoc. J.* **27,** 483–7.

Weil, C. S., and **Wright, G. J.** (1967). Intra- and interlaboratory comparative evaluation of single oral test. *Toxicol. Appl. Pharmacol.* **11,** 378–88.
Williams, A. T. (1948). Sodium fluoroacetate poisoning. *Hosp. Corps Q.* **21,** 16–18.
Wheeler, S. H., and **Hart, D. S.** (1979). The toxicity of sodium monofluoroacetate to wild rabbits (*Oryctolagus cuniculus* L.) from three sites in Western Australia. *Aust. Wildl. Res.* **6,** 57–62.
Yeary, R. A., Benish, R. A., and **Finkelstein, M.** (1966). Acute toxicity of drugs in newborn animals. *J. Pediatr.* **69,** 663–7.

Manuscript received 3 March 1980; accepted 6 October 1980