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THE ESTIMATION OF THE SAFE DOSE

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Small quantities of toxic substances may be present in food, either because they have been deliberately added to improve the appearance of the food or because they have been used as insecticides or for various other reasons. This can do no harm if the quantities are small enough, and it is sometimes necessary to decide what is the maximum quantity that can be regarded as quite safe. For some of the older poisons evidence on this point may be available from observations on men. The doses of arsenic used by numerous poisoners have been estimated, and from a consideration of the effects produced it is possible to determine the lowest recorded toxic dose; a dose somewhat lower than this can be regarded as safe. With new poisons this method is not immediately applicable, and it is sometimes necessary to get indirect evidence from experiments on animals. Such experiments are difficult to design (Barnes and Denz, 1954b). Preliminary work may show that a given dose has little effect, and experiments are then sometimes undertaken to show that a smaller dose has no effect at all. If 100 rats are fed on a suitable diet for 6 months without obvious ill effect, it may seem reasonable to suppose that the diet is not very toxic, but it is clear that a diet which killed 1% of rats might easily fail to kill any of the particular rats chosen for the test. The probability that any one rat will survive is 0.99 and the probability that 100 rats will all survive is $0{\cdot}99^{100}$. Therefore the probability that this test would fail to detect this toxicity would be 0.99^{100}, or about 0.37, but a diet which killed 1% of the people who ate it would be a very undesirable diet. Some allowance can be made for this weakness in the argument by setting the limit at 1/10 or 1/100 of the quantity shown to be safe, but such factors of safety are a very arbitrary form of extrapolation, and if the variation of the response to the drug is high, they may not be as safe as they seem to be.

Another difficulty about experiments of this type lies in the fact that occasional deaths are liable to occur in a colony of rats even when no poisons are given. The maintenance of 100 control rats without poisons adds considerably to the labour of the experiments and to the error of the result if deaths occur in both groups. Such experiments are costly and dull and the evidence they provide cannot be satisfactory. The present paper discusses alternative types of experiment in which safe doses are determined by extrapolation from the results of experiments with doses which have measurable effects. Extrapolation is not a reliable procedure, but in the present case it is inevitable, and it is thought that the arguments given below are at any rate preferable to the vague form of extrapolation outlined above.

A suitable method of calculation was described by Gray, Trevan, Bainbridge, and Attwood (1931), who measured the relation between the dose of stibamine glucoside and the mortality of mice, and suggested that the maximum tolerated dose should be defined in terms of an arbitrary small mortality, and that it should be calculated by extrapolation on the assumption that the logarithm of the individual lethal dose was normally distributed. Calculations of this type are easily made to-day, and it is known that the basic assumption is generally true (Gaddum, 1933, 1953). Gray *et al.* suggested that the dose which would kill 1 mouse in 742 should be called the maximum tolerated dose. This corresponds to 3 standard deviations from the mean. It is now proposed that the safe dose be calculated from the point corresponding to 6 deviations from the mean. This would be expected to kill only about one animal in 1,000 million. This number is large compared with the population which is likely to be exposed to the drug.

The following mortalities were observed after the intravenous injection of a solution of neoarsphenamine in mice (Perry, 1950):

0.3 ml. 6/20=30% 0.36 ml. 12/20=60%

From these results it may be estimated that

m=log LD50=$\bar{1}.530$

λ=S.D. log tolerance=0.1018

Log safe dose = $m - 6\lambda = \bar{1}.530 - 6 \times 0.1018 = \bar{2}.919$

and the safe dose is therefore estimated as 0.083 ml. The validity of this extrapolation may be questioned, but it is probably more reliable than an arbitrary guess of the factor of safety.

When some animals die in control groups without drugs allowance can be made for this fact by using Abbott's correction (Gaddum, 1953).

The object of most toxicity tests is not only to save life, but also to avoid minor degrees of injury. It may be possible sometimes to decide which animals have suffered minor damage and to observe an increase in the percentage harmed when the dose increases. When this can be done the method outlined above may be used, but it is not easy when the animals do not die. In experiments of this type observations are usually made of the weight of the animals, and various other measurements may be made such as estimates of food and water consumed, blood pressure, blood counts, temperature, etc. When this has been done it is not immediately obvious how the results may be used to calculate a safe dose which will affect only a negligible proportion of the animals.

Measurements of this kind may diminish as the dose increases, but it is convenient to consider the case where the slope of the dose-effect curve is positive. The effect may, for example, be the difference between the weights of treated and control groups. This is likely to increase as the dose increases. Let x be the logarithm of the dose and y the corresponding measurement (the " effect "). Let s be the estimated standard deviation of the measurements. Consider the case where y is linearly related to x, and the measurements are normally distributed, and the standard deviation of y (σ) is independent of the dose (i.e., the measurements are homoscedastic). If these conditions are not fulfilled, it may be possible to replace the original measurements with some suitable metameter which fulfils them.

It should then be possible to express the results as shown in Fig. 1, where the vertical scale represents the effect (y) and the horizontal scale represents log dose (x). The relation between dose and effect is given by the line CD, the slope of which is b_e. The line OA represents no effect at all—that is the measurement when no dose has been given. Suppose that the results are converted into quantal results by means of the convention that effects larger than EF are positive and smaller effects negative. The percentage of positive effects at the dose DF can be estimated from the deviation DF. Let $DF=k\sigma$; then the expected mortality is that corresponding to a normal equivalent deviation (NED) of k. The relation between dose and mortality can be shown by plotting k against log dose. The slope of

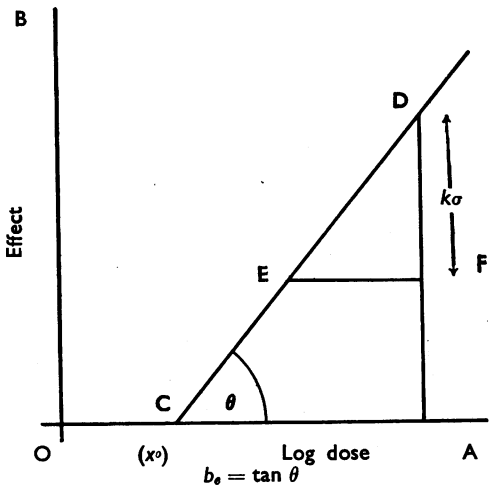

FIG. 1.—Theoretical relation between log dose and effect (see text).

the line relating NED (or probit) to log dose $=b_e=k/EF=1/\lambda$, where λ is the standard deviation of the logarithms of the individual effective doses. This relation between λ and the slope is implicit in the theory of probits. It follows that $EF=k\lambda$. The slope of the line relating effect to log dose $=b_e=DF/EF=k\sigma/k\lambda=\sigma/\lambda$ $\therefore \lambda=\sigma/b_e$. λ can thus be estimated as s/b. This method of calculation has been in use for some time (Gaddum, 1931), but the above argument in support of it has not been given before.

The effect on a single animal is significant when $y=ts$, where t has the appropriate value obtained from tables. For the calculations described here t is taken as equal to 2. This may be called the " threshold effect " and is presumably harmless, since it corresponds to a value of the measurement on which the experiment is based, which is exceeded about once in every 20 of the control animals. The corresponding log dose (x_1) may be called the threshold log dose. It is proposed that the safe dose be calculated from the expression $(x_1-6\lambda)$, which is comparable with the expression $(m-6\lambda)$ used above to calculate the dose which causes a negligible mortality. In both cases, the logarithmic factor of safety is 6λ; in one case it is applied to the LD50 and in the other it is applied to the threshold dose. In practice the safe dose can be estimated as $(x_0-4\lambda)$ where x_0 is the log dose corresponding to no effect and is represented by the point C in Fig. 1.

This argument involves three assumptions which can be tested—that the line is straight and that y is normally distributed and has a constant standard

deviation. It makes no allowance for errors of estimation, but it does provide an estimate, instead of a guess, of the factor of safety.

Example 1: Smith (1950) found that various antimalarials inhibited the growth of young rats and devised a short-term chronic toxicity test in which drugs were mixed with the food for 14 days. The effect of each drug was calculated as the growth suppression (y) which was equal to the difference between the mean weight changes in groups receiving the drug and control groups receiving no drug. When y was plotted against log dose (mg./kg./day) the results could be approximately fitted by straight lines over a certain range of doses in the neighbourhood of the GS50 (the dose causing 50% suppression of growth). Smith gives the standard deviation of the effect for each group and this appears to have been approximately constant for doses less than the GS50. The root mean square of his estimates of the standard deviation of the growth in grams in this range was calculated and taken as an estimate of s. The log dose corresponding to no effect (x_0) was estimated by plotting the actual growth in grams against log dose, fitting a straight line to the results and taking the point on this line corresponding to the growth of the controls. The results did not differ appreciably from the estimates based on the regression line, which were used for the calculations in Table I.

TABLE I
CALCULATION OF SAFE DOSES OF ANTIMALARIALS FROM THE DATA OF SMITH (1950)

	s	b'	λ	Safety Factor	Daily Dose (mg./kg.)		
	S.D.(y) g.	dy/dx g.	s/b'	Anti-log 6λ	GS50	Thres-hold	Safe
Pamaquin hydroiodide	9·87	280	0·035	1·6	30	28	17·3
Quinacrine hydro-chloride	8·85	66·5	0·133	6·3	36	13	2·1
Chloroquine diphosphate	6·0	115	0·052	2·0	43	22	11
Chlorguanide hydro-chloride	8·5	109	0·078	2·9	71	59	20
Amodiaquine (Camoquin)	5·9	88·4	0·067	2·5	112	78	31
Oxychloro-quine di-phosphate	7·0	105	0·067	2·5	123	35	13·9
Quinine hydro-chloride	7·3	106	0·069	2·6	460	250	96
Thiourea	8·0	22·4	0·357	139	575	160	1·2
Sulphadia-zine Sodium	7·6	112	0·068	2·6	1,300	720	290

The value of λ was calculated from Smith's estimates of the slopes of the log-dose-effect lines. His slope function is given as a percentage and was therefore multiplied by the mean weight change of the controls for each drug to give an estimate of the slope (b') in grams. The standard deviation of log tolerance (λ) was then estimated as s/b'. In accordance with the above suggestions the safe dose was estimated from $(x_0-4\lambda)$.

The results of these calculations are shown in Table I. It will be seen that the safety factor was often quite small, but that it was surprisingly high for thiourea because the log-dose-effect curve was very flat ($b'=22.4$ g.).

Example 2: In the experiments by Barnes and Denz (1954a) drugs were mixed with the diet of rats and the effects of sublethal doses were detected by estimates of cholinesterase in the blood or brain. Groups of 6–12 rats were used for each dose and

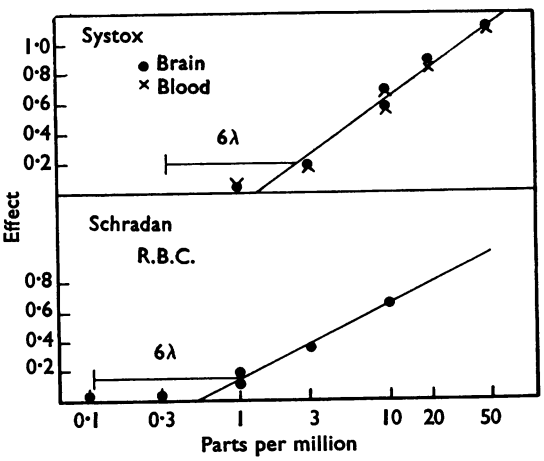

FIG. 2.—Observed relation between the logarithm of the concentration of toxic substances in the diet in parts per million and their effect on cholinesterases, calculated according to the convention described in the text (data of Barnes and Denz, 1954a).

the estimates were made after a period of several weeks on the diet. The cholinesterase was estimated from the rate of production of CO_2 in a Warburg apparatus with acetylcholine as substrate and the results were expressed in μl. CO_2/min./ml. (or g.). The mean result for each group was calculated and in the present calculations its logarithm taken. The difference between this figure and the corresponding figure for control animals in the same experiment was taken as the effect of each dose (y). This was plotted against log dose and the points fitted with straight lines.

The standard deviation of y (s) was estimated from the ranges. This is thought to be justifiable when the number in each group is not more than 12. The maximum and minimum effects in each group of the results, as originally given, were marked and the ratio of one to the other calculated on a

slide rule. The logarithm of this ratio was divided by d_n, which is the expected ratio of the range to the standard deviation.

Table II shows the results with systox (diethyl-*S*-ethylthio-ethanol thiophosphate). The effects on the brain agreed well with the effects on the whole blood and the results could be fitted with a straight line, when y was plotted against log dose.

There was evidence that s tended to increase when the dose was high, and the figures for the highest two doses were therefore omitted in the calculation of the mean values of s. The results for

TABLE II

THE EFFECTS OF DIETARY SYSTOX ON THE CHOLINESTERASES] OF RATS, CALCULATED ACCORDING TO THE CONVENTION DESCRIBED IN THE TEXT

	Dose p.p.m.	Degrees of Freedom	y Effect		s Standard Deviation of y	
			Brain	Blood	Brain	Blood
A	0	6	0	0	0·076	0·080
	1	6	0·033	0·045	0·045	0·065
	3	6	0·181	0·161	0·040	0·063
	10	7	0·697	0·671	0·041	0·077
B	0	11	0	0	0·063	0·103
	10	11	0·580	0·546	0·118	0·193
	20	(10)	0·882	0·832	(0·186)	(0·194)
	50	(11)	1·076	1·080	(3·21)	(4·04)
Total ..		47		Mean ..	0·064	0·097

blood appeared to vary significantly more than those for brain. The higher figure (0.097) was therefore taken as an estimate of s. The threshold effect y_1 (see above)$=2\times0.097=0.194$. This is much smaller than the effect of 20 parts per million (p.p.m.), and this justifies the omission from the calculations of the estimates of s for the highest two doses. The argument is based on the bottom part of the curve only. The following figures were estimated from the regression line:

$$b'=0.66 \qquad x_1=0.414$$

From these figures it may be calculated that

$$\lambda=s/b'=0.097/0.66=0.147$$

The safe log dose$=x_1-6\lambda=0.414-0.832=\bar{1}.532$

The safe dose is thus estimated as about 0.34 p.p.m. It will be seen that the safety factor by which the threshold dose is divided to find the safe dose= antilog $6\lambda=7.6$.

Example 3: Schradan (OMPA, tetrax, octamethylpyrophosphoramide).

When the results were calculated as in Example 2, they could again be fitted by straight lines, but the lines for different tissues did not coincide. The red blood corpuscles appeared to be the most sensitive tissue and then the blood, plasma and brain, in that order. The following calculations are based on the results with the most sensitive tissue, the red blood corpuscles.

It was calculated that $b'=0.458 \quad x_1=0.01$

Therefore $\lambda=s/b'=0.08/0.458=0.175$

Log safe dose$=x_1-6\lambda=0.01-1.05=\bar{2}.96$. Safe dose $=0.09$ p.p.m.

The factor of safety is in this case 11.2.

The results obtained by graphical methods (Fig. 2), in Examples 2 and 3, differed by less than 10% from the results recorded above. Such methods are presumably accurate enough for this type of calculation.

TABLE III

THE EFFECTS OF SCHRADAN IN THE DIET OF RATS ON THE CHOLINESTERASES IN THE RED BLOOD CORPUSCLES

	Dose p.p.m.	Degrees of Freedom	y Effect	s Standard Deviation
A	0	11	0	0·071
	0·1	11	0·03	0·0785
	0·3	11	0·03	0·0693
	1	11	0·19	0·048
B	0	5	0	0·044
	1	5	0·12	0·108
	3	5	0·34	0·150
	10	4	0·63	0·071
Total ..		64	Mean ..	0·080

DISCUSSION

Although it is hoped that the methods described here will help those responsible for chronic toxicity tests, it is clear that many difficulties remain. For example, no allowance has been made for errors in the estimation of m, x_1 and λ.

It was hoped at one time that sublethal doses of drugs would generally inhibit the growth of young animals as they did in the experiments of Smith with antimalarials. This is not, however, a universal rule and the methods of calculation discussed above sometimes cannot be applied unless other methods of measuring sublethal toxic effects are used. The development of new methods of detecting small effects of drugs on the central nervous system may perhaps widen the scope of these methods. These difficulties may be overcome, but a more serious difficulty lies in the fact that results obtained with

animals cannot safely be applied to man. The best that can be done is to test each drug on several different species of animal and to assume that man is no more affected than the most sensitive of the species tested.

Summary

Methods are described for obtaining from the results of a toxicity test a rough estimate of the largest dose which can be considered safe.

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