THE EFFECT OF PARTICLE SIZE ON THE TOXICITY OF α-NAPHTHYL THIOUREA (ANTU) TO ALBINO RATS

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INTRODUCTION

It has long been recognized in bio-assay work that particle size can be an important factor in determining the response of the test animals. However, except for arsenious oxide (Schwartze, 1922; Chitty & Southern, 1954), this subject has been little studied in relation to the toxicity of rodenticides.

The only important reference to α-naphthyl thiourea and particle size in the literature is the statement by Richter (1945) that an average diameter of 5–10 μ had proved best for work with poison dusts while a larger size, approximately 100 μ, had given best results in food mixtures. This statement was subsequently repeated by several authors, one of whom (Münchberg, 1951) suggested that particles of 100–200 μ are best for baiting because they are less likely to be tasted. Dr Richter has since informed us (private communication) that the larger particle size was probably chosen for baiting because it was less likely to be blown about during mixing.

When antu was first manufactured in Britain for the control of *Rattus norvegicus* there were no experimental data on which to base a firm specification. Since Chitty & Southern (1954) had found that finely divided arsenious oxide was more toxic than coarse, and this agreed with what might be expected on pharmacological grounds, by analogy it seemed reasonable to recommend that only antu of fairly small particle size should be used. But as this would have involved increased manufacturing costs, it was decided to seek experimental evidence of the effect of differing particle size on toxicity.

The bio-assay of α-naphthyl thiourea presents certain difficulties. Dieke & Richter (1946) showed that in wild *R. norvegicus* resistance to antu varies with age and levels off just after puberty. Suckling rats with an LD_{50} of 58 mg./kg. were found to be seven or eight times as resistant as adults. Gaines & Hayes (1952) reported similar results in simulated field trials. Rogers (1946), in investigating the cause of this increase in susceptibility to antu after puberty, incidentally showed that it occurred in laboratory strains of *R. norvegicus*. Later, Latven, Sloane & Munch (1948) recorded LD_{50}'s in Blaine-Wistar rats varying from 8 to 190 mg./kg. according to the time of the year—but since they used only females weighing 100–125 g. this may reflect a seasonal effect on the time of onset of the change-over after puberty rather than a phenomenon found in adult rats.

However, Dieke & Richter (1946) also found that even among adult domestic rats, different strains vary considerably in resistance to antu. Thus, one of their strains had an LD_{50} of 6·25 mg./kg. and another, an LD_{50} of only 2·5 mg./kg.

https://doi.org/10.1017/S0022172400000814 Published online by Cambridge University Press

Other authors (McClosky & Smith, 1945; Byerrum, 1946; Meyer & Karel, 1948*a*) have noted an effect of certain diet constituents, notably iodine, on the average lethal dose. Meyer & Karel (1948*b*) have further shown that environmental temperature is a factor that can affect toxicity.

To add to the confusion, Rall & North (1952) re-examined the data of Dieke & Richter (1946) on the LD_{50} for wild *R. norvegicus* of different ages, and suggested that making a correction for body weight is fallacious. They hold that a more satisfactory LD_{50} can be expressed in terms of milligrams of antu per rat.

METHODS

To eliminate, as far as possible, any variation in toxicity due to extraneous factors a single, commercial, sample of antu was taken, and this was then separated into fractions of different particle size. The usual methods of doing this include sieving, sedimentation, centrifuge analysis and elutriation. Sieving is a relatively inaccurate method and is not suitable for the preparation of very small particles. For example, 240-mesh B.S. test sieves with a nominal aperture of 66 μ are manufactured to a tolerance of $\pm 6\%$.

In elutriation methods the particles of powder are lifted in an upward-moving fluid stream, separation depending on the different relative velocities of the coarse and finer particles. The fluid is usually air or water. Although the technique is otherwise satisfactory, complicated apparatus is required, and in the present instance sedimentation was preferred.

Sedimentation methods are based on Stokes's Law (Stokes, 1846), which states that a spherical particle falling through a fluid medium reaches a limiting velocity. For the preparation of the chosen particle size ranges a 4 l. beaker was fitted with a mechanical stirrer and a draw-off tube which could be fixed at any chosen depth. The draw-off tube was bent in the form of a U with the orifice pointing upwards to prevent material in the lower part of the beaker from being drawn into it. A 1% dispersion of antu, with a wetting agent (Teepol), was prepared and mechanically stirred until no evidence of agglomeration could be detected by microscopic examination of a drop taken from the stirred-up liquid. The stirrer was then stopped and the time noted. After sufficient interval had elapsed for particles of the required size (and greater) to fall from the surface of the suspension to a chosen level in the liquid, the part of the suspension above that level was removed by the application of vacuum to the draw-off tube. Constant repetition of this process yielded fractions of the desired particle size range, the final suspension simply being filtered and dried.

This procedure offers the objection that any impurities may become concentrated in certain fractions thus reducing their purity. The three particle size ranges used here were analysed by determination of their nitrogen contents (Elmore, 1948) with the following results: 5 μ or smaller, 99·06 % purity; 50 to 55 μ, 98·93 %; 100–110 μ, 99·96% purity.

In the assays all animals came from a single colony of Wistar rats and were reared at a fairly constant temperature on a cubed diet with a weekly supplement

of greens. Since the work was done over 2 years, as opportunity offered, there was time, presumably, for a change in susceptibility to antu to occur.

In all but the last assay, in order to obtain sufficient numbers of rats, animals of both sexes were used and these varied considerably in weight. No significant difference in susceptibility of adult male and female rats to antu appears to have been recorded, though Dieke & Richter (1946) looked for it in wild *R. norvegicus*. However, care was taken to distribute the same proportion of the two sexes, and animals of similar weight, to each particle size and each dosage level, on each day of a particular test. By this means it was thought that whatever variations in susceptibility may have occurred in the test animals from one assay to the next, any relative toxicity effects due to particle size would be preserved.

This expectation was supported by the experience of Latven *et al.* (1948), who found that the potency of their standard antu with respect to commercial samples remained constant however much the actual LD_{50}'s altered.

The experimental procedure (which was also carried out at constant temperature) was to weigh the animals a few days before the assay for assignment to dosage groups. The day before a particular batch of rats was due to be poisoned each animal was weighed and allowed only three or four cubes of diet overnight. In the following afternoon it was offered a plain flour and water pill which, if eaten satisfactorily, was followed by a similar pill containing antu. From then onwards diet cubes were supplied *ad lib*. Water was available throughout. All rats died within 3 days or recovered. Rats refusing to eat the whole of the poison pill were rejected whether they survived or not. They were very few in number.

In order to weigh accurately, in the time available, the many small quantities of antu which were required, dosages were accepted if they were within 5 % of the target dosages. The dosages quoted below are thus averages of the individual dosages at that particular level. In most instances weighings were well within ± 5 % and no standard deviations have been calculated.

The data obtained were analysed by the probit method following the procedure recommended by Finney (1947). There was no evidence that calculations based on weight of antu per rat would have yielded more information.

RESULTS AND DISCUSSION

In all, four assays were carried out. In the first, in October 1951, two samples of antu consisting of particles of $< 5\ \mu$ and of 50–55 μ were administered to batches of twelve rats at the dosages shown in Table 1. Each batch originally included six males and six females. The total weight ranges of the animals were 267–420 g. (males) and 179–280 g. (females), but this considerable variation caused no apparent heterogeneity in the results of the assay.

The LD_{50} of the coarse particles was estimated as 4·93 mg./kg. of body weight compared with 7·98 mg./kg. for the fine antu. There was thus a constant log dosage difference of 0·210 with a standard error of $\pm 0{\cdot}058$, which corresponds to a relative toxicity of 1·62. Fiducial limits (5 %) were calculated as 1·236 and 2·123.

In a second assay in July 1952, the $< 5\ \mu$ sample used previously was compared

with a fraction consisting of particles of 100 μ or more. Again, six male and six female rats were used at each dosage, with weights ranging from 291 to 408 g. (♂'s) and from 185 to 248 g. (♀'s). As a result of a preliminary siting test, the dosage levels chosen were somewhat lower than in the first assay. Table 2 shows that in spite of this, for the < 5 μ antu, all four dosage levels were found to lie above the LD_{50} point. This was now estimated at only 5·39 mg./kg. However, the coarser particles with an LD_{50} of 3·98 mg./kg. were apparently again more toxic than the fine. The constant log dosage difference was 0·132 with a standard error of $\pm$ 0·068, giving a relative toxicity of 1·354.

Table 1. *Relative toxicity of* 5 *and* 50–55 μ *antu*

50–55 μ			< 5 μ			50–55/< 5 μ	
Mean dosage (mg./kg.)	Mortality	Log LD_{50} and LD_{50}	Mean dosage (mg./kg.)	Mortality	Log LD_{50} and LD_{50}	Constant log dosage difference	Relative toxicity
4·008	3/12	0·692 ± 0·048 = 4·93 mg./kg.	6·012	4/11	0·902 ± 0·041 = 7·98 mg./kg.	0·210 ± 0·058	1·620
6·015	8/11		7·816	5/12			
9·068	11/12		10·993	9/12			
11·985	12/12		14·875	11/12			

Table 2. *Relative toxicity of* < 5 *and* 100–110 μ *antu*

100–110 μ			< 5 μ			100</5 μ	
Mean dosage (mg./kg.)	Mortality	Log LD_{50} and LD_{50}	Mean dosage (mg./kg.)	Mortality	Log LD_{50} and LD_{50}	Constant log dosage difference	Relative toxicity
3·507	2/12	0·600 ± 0·055 = 3·98 mg./kg.	5·524	7/11	0·732 ± 0·077 = 5·39 mg./kg.	0·132 ± 0·068	1·354
4·421	7/10		7·535	10/12			
6·074	11/12		9·401	9/12			
7·958	11/12		12·862	11/12			

In the following month this assay was repeated. Again, owing to shortage of rats equal numbers of males and females had to be used. The males varied between 377 and 514 g. and the females between 230 and 328 g. They were thus rather heavier than on the previous occasion.

Once more the susceptibility of the animals to antu seemed to have increased (Table 3). Although the < 5 μ dosages selected were lower than before, only the lowest contributed much information to the assay.

However, the data available again support the view that the coarser particles are the more toxic. The LD_{50}'s work out at 3·23 mg./kg. for the 100 μ sample and 3·79 mg./kg. for the < 5 μ antu. This represents a constant log dosage difference of 0·070 ± 0·049 and a relative toxicity of 1·174. The value arrived at for the standard error of the log dosage difference, though large, does not contain any correction for possible heterogeneity.

https://doi.org/10.1017/S0022172400000814 Published online by Cambridge University Press

The fourth assay, in August 1954, was a repetition of the second and third. Only male rats were used, their weights varying from 305 to 397 g. This greater uniformity in the experimental animals did not prevent somewhat anomalous results from the coarser particle size antu, but analysis indicated no obvious heterogeneity. The data are given in Table 4.

Table 3. *Relative toxicity of* < 5 *and* 100–110 μ *antu*

100/110 μ			$< 5\mu$			$100 < / < 5\mu$	
Mean dosage (mg./kg.)	Mortality	Log LD_{50} and LD_{50}	Mean dosage (mg./kg.)	Mortality	Log LD_{50} and LD_{50}	Constant log dosage difference	Relative toxicity
3·509	9/12	0·509 and 3·23 mg./kg.	4·014	5/12	0·579 and 3·79 mg./kg.	0·070 ± 0·049	1·174
3·998	8/11		5·264	12/12			
4·966	10/12		7·5	12/12			

Table 4. *Relative toxicity of* < 5 *and* 100–110 μ *antu*

100–110 μ			$< 5\mu$			$100 < / < 5\mu$	
Mean dosage (mg./kg.)	Mortality	Log LD_{50} and LD_{50}	Mean dosage (mg./kg.)	Mortality	Log LD_{50} and LD_{50}	Constant log dosage difference	Relative toxicity
2·973	0/12	0·641 ± 0·033 = 4·37 mg./kg.	2·984	0/12	0·803 ± 0·033 = 6·36 mg./kg.	0·162 ± 0·045	1·450
4·477	9/12		4·441	2/12			
5·905	9/12		5·920	6/12			
			7·483	7/12			

For the fourth time the coarser particles were more toxic, and on this occasion the difference was significant. The LD_{50}'s were 4·37 and 6·36 mg./kg., with a constant log dosage difference of $0{\cdot}162 \pm 0{\cdot}045$ equivalent to a relative toxicity of 1·45. The 5% fiducial limits for the relative toxicity were estimated as 1·18 and 1·845. Once more the susceptibility of the experimental animals seemed to have changed—this time in the direction of greater resistance to antu.

Using a method due to Cochran, and described by Finney (1947), it is possible to combine the results of the three assays in which the fine-particle antu was compared with particles measuring 100–110 μ. When this is done a mean constant log dosage difference of $0{\cdot}122 \pm 0{\cdot}030$ is obtained, which is significant at well beyond the 0·05 probability level and corresponds to a relative toxicity of 1·32 in favour of the coarse particles.

Since there is no apparent statistical objection, the results of the 100–110 μ tests may be further combined with a data from the first assay in which particles of 50–55 μ were used. This gives an overall mean log dosage difference of $0{\cdot}141 \pm 0{\cdot}0267$ which is even more significant. The equivalent relative potency is 1·38. In other words, the larger particle size samples of antu under test were probably about one and a third times as toxic to domestic rats as particles of size 5 μ or less.

https://doi.org/10.1017/S0022172400000814 Published online by Cambridge University Press

The concentration of antu in baits which is recommended at present is such that with optimum conditions, including prebaiting, poisoned rats ingest several times the average lethal dose. Bearing this in mind, and also the fact that in the present assays white rats were used, there is perhaps insufficient justification for recommending that in future control work only large particle antu should be used. It is reasonable to conclude, however, that there is now no case for specifying particles of less than 100 μ.

It would be interesting to know why the coarser particles are more toxic. No convincing explanation can be offered here.

SUMMARY

1. Particles of antu of 5 μ diameter were significantly less toxic to domestic rats than particles of 50–55 μ.

2. In each of three bio-assays they were less toxic than particles of antu of 100–110 μ.

3. It is concluded that the specification for antu for use in poison baits against *R. norvegicus* should not require it to be in the form of fine particles.

This work was initiated at the suggestion of Dr E. E. Turtle of this Division, and his advice during its progress is gratefully acknowledged. Thanks are also due to Mr L. E. Hammond for much help in the preparation of the test doses, and to Dr H. Egan and Mr E. I. Johnson of the Government Chemist's staff who carried out a number of the many micro-weighings involved.

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(*MS. received for publication* 19. IV. 55)