

Age and Species Variation in the Acute Toxicity of Alpha-Naphthyl Thiourea.*

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The general properties of α -naphthyl thiourea (ANTU) have been described in a previous paper,¹ in which it was shown that this substance, besides being an effective poison for the field control of Norway rats, has interesting possibilities as a tool for research because of its actions leading to pulmonary edema, increased lymph flow² and other physiological and anatomical effects.^{3,4}

The present work was performed to elucidate the practical questions of whether ANTU is toxic to other animals than Norway rats and would therefore present hazards in use, and also whether, as was indicated in field trials, young Norway rats are less susceptible than adults. Differences between males and females were sought at the same time.

Although our primary objectives were practical ones, bearing on the use of ANTU as a rodenticide, the results obtained are presented here because of the many applications of ANTU and other thiourea derivatives to

various fields of research.

Age and Sex Variation in Rats—Wild Norway rats were chosen for test animals, because of the large strain differences in response to thiourea poisoning which had been found between wild and laboratory rats,⁵ and which it was felt might exist for ANTU as well.

The rats were all wild brown Norways, trapped in the alleys and back yards of Baltimore, Maryland, in special wooden box traps which have been described elsewhere.⁶ They were predominantly from residential districts. Care was taken to use only rats trapped in areas where no systematic field poisoning with ANTU had been done. All rats (except some classed as suckling) were held in the laboratory a minimum of 4 days after trapping to allow them to recover from that ordeal and to make sure they were healthy. During this period they were fed Purina fox chow cubes and had access to an ample water supply.

The ANTU was suspended in olive oil and administered by intraperitoneal injection, the dose being adjusted to body weight by giving a volume corresponding to 0.1 cc per 10 g body weight. The rats were not starved; they were, however, injected in the late afternoon, just before the normal feeding time of wild rats.

To restrain the rats for weighing and injecting use was made of the "sock" developed by Emlen.⁷ This device made it possible to

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¹ Richter, C. P., *J. Am. Med. Assn.*, 1945, **129**, 927.

² Drinker, C. K., *Pulmonary Edema and Inflammation*, Harvard University Press, 1945, 39-43.

³ Du Bois, K. P., *Fed. Proc.*, 1946, **5**, 174.

⁴ McClosky, W. T., and Smith, M. I., *Public Health Rep.*, 1945, **60**, 1101.

⁵ Dieke, S. H., and Richter, C. P., *J. Pharm. and Exp. Therap.*, 1945, **83**, 195.

⁶ Richter, C. P., and Emlen, J. T., Jr., *Public Health Rep.*, 1945, **60**, 1303.

⁷ Emlen, J. T., Jr., *J. Wildlife Management*, 1944, **8**, 264.

TABLE I.
Age and Sex Variation in Susceptibility of Wild Norway Rats to ANTU Poisoning.

Age						
Wt. range (g)		No. rats used			Average body wt. (g)	LD ₅₀ ± S.E. (mg/kg body wt.)
		Male	Female	Total		
0- 50	suckling	17	7	24	39.3	58±4.4
51-100	weanling	23	17	40	80.7	43±5.7
101-125	young	11	24	35	112.5	22±3.2
126-150	"	15	7	22	140.4	18±4.3
151-200	"	15	14	29	170.1	16±2.7
201-300	young adult	10	26	36	263.1	8.1±0.9
301-400	adult	45	37	82	348.3	7.7±1.0
401-546	"	39	17	56	447.6	6.2±0.6
Total				324		
Sex						
Above 301 (adult males)		84	—	—		7.0±0.7
" 301 (" females)		—	54	—		7.4±0.9

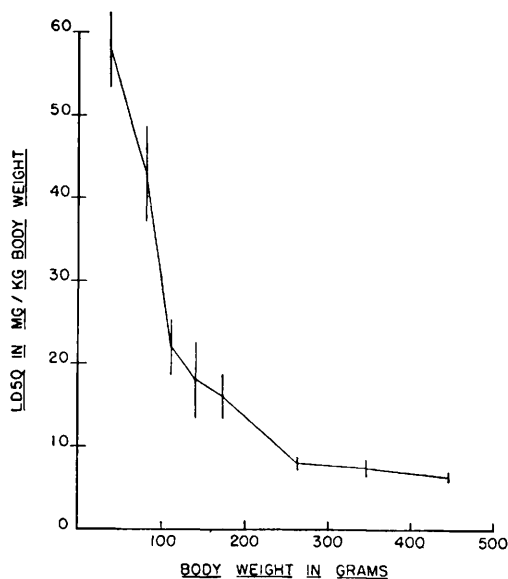


Fig. 1.

Change in acute LD₅₀ of ANTU with body weight of wild Norway rats. The LD₅₀ for each weight range is plotted against an abscissa representing the average body weight of rats in that range. The standard error of each LD₅₀ is indicated by a vertical line of appropriate length.

handle wild rats without anaesthetics in much the same way as laboratory rats.

The assays on rats weighing more than 200 g were carried out over the course of a year and a half (January 1944 to June 1945) with no seasonal variation being noted. The assays on rats below 200 g were performed during April, May and June 1945.

The results obtained with a series of 324 rats are summarized in Table I. The median lethal doses (LD₅₀'s) given in the last column were estimated, together with their standard errors, by the method of Litchfield and Fertig,⁸ using logarithmic-probit graph paper.

A comparison of the LD₅₀ values given in Table I, and presented graphically in Fig. 1, shows that the resistance to acute ANTU poisoning was greatest in the youngest rats, and then decreased fairly rapidly to level off between 8 and 6 mg/kg for rats weighing 200 g or more. Suckling rats (weighing less than 50 g) and weanling rats (up to 100 g) were respectively about 7 and 5 times as resistant as old rats. Between 100 and 200 g the resistance was still more than twice as great as it was above 200 g. It may be mentioned in this connection that a corresponding (but larger) difference in response to acute poisoning with the parent compound thiourea has been observed between young and adult laboratory rats.^{9,5}

The correlation of weight with age, which is indicated by the classifications into suckling (birth to approximately 30 days of age), weanling (30 to 60 days) and adult (over 90 days), is only an estimate, based on observations such as whether the rats were trapped in a group with their mother, were

⁸ Litchfield, J. T., Jr., and Fertig, J. W., *Bull. Johns Hopkins Hosp.*, 1941, **69**, 276.

⁹ MacKenzie, J. B., and MacKenzie, C. G., *Proc. Soc. Exp. Biol. and Med.*, 1943, **54**, 34.

TABLE II.
Estimated Acute LD₅₀ Values for Various Animals (Adult).

	Administered*	No. used	LD ₅₀ [†] mg/kg body wt.
Norway rat (<i>Rattus norvegicus</i>)			
domestic, Strain I	i.p.	51	2.5±0.5
" Strain II	"	26	6.25±0.7
wild	"	(See Table I)	
"	s.t.	50	6.9±0.5
Dog (<i>Canis familiaris</i>)	i.p.	9	below 16
"	s.t.	7	38 (20-50)‡
Mouse (<i>Mus musculus</i>) albino	i.p.	19	56±4
Alexandrine rat (<i>R. rattus</i> subsp.)	"	14	250 (75-450)
Guinea pig (<i>Cavia cobaya</i>)	"	11	350 (300-400)
Rabbit (<i>Oryctolagus cuniculus</i>)	" s.t.	6	above 400
Cat (<i>Felis libyca domestica</i>)	s.t.	10	500 (75-1000)
Chicken (<i>Gallus bankiva</i>)			
Barred Plymouth Rock pullets	i.p.	4	2500 (?-5000)
"	s.t.	7	4250 (2500-5000)
Monkey (<i>Macaca mulatta</i>)	i.p.	6	175 (150-200)
"	s.t.	12	4250 (3500-5000)

* ANTU was administered either "i.p." (by intraperitoneal injection, usually in olive oil suspension) or "s.t." (by stomach tube, usually suspended in 10% acacia solution).

† Whenever material could not be treated statistically an estimate of the LD₅₀ is followed by the range between the highest dose observed to kill none and the lowest dose killing all.

‡ Three puppies given ANTU by stomach tube survived doses up to 75 mg/kg.

small but trapped alone and able to fend for themselves, or afforded obvious external indications of maturity such as an open vagina or large descended testes. It is moderately certain that no rat weighing less than 100 g was sexually mature, while all those weighing over 200 g were indubitably adults. The age of puberty thus fell in the weight range of 100 to 200 g; more precisely, in the range 100-150 g for females and 150-200 g for males. This last statement is based upon observations made on 11 female and 5 male wild Norway rats that were raised from birth in the laboratory, which indicated that around puberty the males of a given age are heavier than the females: between 60 and 90 days old these females weighed 100-150 g and the males 150-200 g, with considerable individual variation being found within these limits, particularly between rats of different litters. (In laboratory Norways from our colony the sex difference in weight is even larger, as 60-day-old males often weigh more than 200 g while females of that age rarely weigh more than 140 g.)

In the series of rats receiving ANTU the vagina was open in about half the females with weights between 100 and 125 g, and in all the females weighing more than 150 g,

which agrees well with the above statement. No definite difference in resistance to ANTU poisoning was found between individual females with open vaginas and others in the same weight range with closed vaginas, but this external sign is admittedly not the best criterion of sexual maturity. It is therefore not yet clear whether the levelling off of the LD₅₀ which occurred after puberty is directly related to sexual maturity or is rather an associated phenomenon dependent on general growth factors.

Separating the 138 rats weighing more than 300 g into males and females led to respective LD₅₀ values of 7.0 and 7.4 mg/kg (Table I). Thus adult females were little if any more resistant than males, indicating the absence of a marked sex variation in response to acute ANTU poisoning.

Species Variation. The differences in susceptibility to acute poisoning with ANTU which we have found between animals of various species are shown in Table II. Norway rats are seen to be the most susceptible animals of those tested, and the only ones killed by amounts less than 10 mg/kg. The dog and the mouse were the only others killed below 100 mg/kg. An intermediate group comprising the Alexandrine rat, the

guinea pig, the cat, and probably the rabbit[‡] is followed by the chicken and the Rhesus monkey, both of which were extremely resistant, at least to ANTU given by stomach tube.

It is interesting to note that in contrast to the large variations in the toxicity of thiourea to Norway rats from different sources,⁵ little difference was found in the response of the same rats to ANTU. Rats from Dr. Astwood's colony in Harvard (designated Strain II) were only twice as resistant as rats from our colony (Strain I), and hardly differed at all from the wild Norways.

The mode of administering the ANTU did not influence the toxicity in rats, but in other species less ANTU was required to kill by intraperitoneal injection than when given by stomach tube. For instance monkeys died following 200 mg/kg of ANTU given intraperitoneally but withstood 17 times that amount by stomach tube, and dogs likewise showed a several-fold difference. In part this may be ascribed to emesis, which occurred fairly often in dogs, cats, and monkeys, but elimination in this manner before absorption could take place was minimized by starving the animals overnight before they received doses by stomach tube, and withholding food until 6 to 8 hours later.

The effects characteristic of ANTU poisoning in Norway rats, namely pulmonary edema and pleural effusion, were not found in some species even after the administration of fatal doses. Examination of sections from the lungs of Alexandrine rats, guinea pigs, rabbits and monkeys has not revealed edema

in anything like the amounts found in the lungs of Norway rats, dogs, mice and cats.¹⁰ It would therefore seem likely that the mechanism of action of ANTU is different in these 2 groups of animals.

Both males and females were used in most species tested (the rabbits and chickens were all females) without any striking sex difference in susceptibility becoming evident. Sex differences cannot be ruled out, however, on the basis of these limited data. An indication that an age difference exists in dogs, comparable to that observed in rats, is found in the fact that 3 puppies survived doses which would have been fatal to adult dogs.

Summary. Marked age variation in the susceptibility of wild Norway rats to acute poisoning with α -naphthyl thiourea has been found, with suckling rats about 7 times as resistant as adults. This resistance was found to decrease with increasing body weight, levelling off for adults at an LD₅₀ between 6 and 8 mg/kg body weight. The difference found between adult males and females was within the limits of error and is therefore not considered significant.

Adult animals of other species also varied markedly in susceptibility to acute ANTU poisoning. Norway rats, dogs and mice were killed by amounts less than a hundred milligrams per kilogram. Alexandrine rats, guinea pigs, and cats required several hundred milligrams, while chickens and monkeys survived doses of several grams per kilogram body weight. Large amounts of pulmonary edema were found only in Norway rats, dogs, mice and cats.

[‡] McClosky and Smith⁴ found an LD₅₀ for rabbits of about 1000 mg/kg.

¹⁰ Latta, H., unpublished results.

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Bactericidal Action of Streptomycin.

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Streptomycin,¹ which inhibits the growth of a variety of gram positive, gram negative and acid fast microorganisms¹⁻⁷ has been found

by Waksman and Reilly⁸ to be bactericidal for *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*. According to Wallace