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THE TOXICITY OF 2-HYDROXYIMINOMETHYL-*N*-METHYLPYRIDINIUM METHANESULPHONATE (P2S)

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The toxicity of 2-hydroxyiminomethyl-*N*-methylpyridinium methanesulphonate (P2S) has been determined in a number of species by various routes. It is approximately equally toxic in the rat, mouse, and guinea-pig. It is much more toxic in the dog. Atropine influences the toxicity of P2S differently in different species. From the results obtained attempts have been made to assess the maximum safe dose which can be given intramuscularly to man.

The value of oximes administered with atropine in the treatment of organophosphate poisoning has been demonstrated by Kewitz and Wilson (1956), Kewitz, Wilson and Nachmansohn (1956), Hobbiger (1957), Wills, Kunkel, Brown and Groblewski (1957), Askew, Davies, and Green (1957), and Askew (1957). The toxicity of the more effective oximes is therefore a matter of some importance, particularly if they are to be used in man.

No systematic studies upon the toxicity of oximes have been described, although Askew (personal communication) screened some of them in rats with a maximum dose of 150 mg./kg. i.p. as a preliminary to examining their efficacy in organophosphate poisoning. Most of the oximes she examined were non-toxic at 150 mg./kg., but di*iso*nitrosoacetone and *p*-methoxy*iso*nitrosoacetone were lethal at 30 mg./kg., and glyoxime, *iso*nitrosoacetylacetone, mono*iso*nitrosoacetone and phenylglyoxime at 100 mg./kg. Askew also found that signs of oxime poisoning were similar for all the compounds studied and consisted of marked lethargy, followed by prostration, muscular tremors and loss of reflexes. Signs of poisoning usually developed within 15 min. of injection. When animals survived, recovery began within an hour. Glyoxime was exceptional in that poisoning was delayed for several hours.

Dultz, Epstein, Freeman, Grey, and Weil (1957) determined the LD50 values for some oximes given intraperitoneally to mice; they varied from 20 mg./kg. with di*iso*nitrosoacetone to about 4,000 mg./kg. with acetone oxime. Di*iso*nitrosoacetone, *iso*nitrosoacetylacetone, *iso*nitrosoacetophenone, *iso*nitrosoacetanilide and 1-diethylamino-2:3-butanedione 2-oxime methiodide were all toxic at less than 150 mg./kg.

Three oximes have proved particularly promising as antidotes to organophosphate poisoning. These were mono*iso*nitrosoacetone (MINA), diacetyl monoxime (DAM) and pyridine-2-aldoxime methiodide (2-hydroxyiminomethyl-*N*-methylpyridinium iodide; P2AM), and their toxicities have been studied more fully, but still no systematic studies of the effect of species and route of administration upon their toxicity have been reported. MINA has been examined in greatest detail. The intraperitoneal LD50 value for mice is 150 mg./kg. (Dultz *et al.*, 1957), but the corresponding values for male and female rats are 50 mg./kg. and 75 mg./kg. respectively (unpublished observations). MINA is toxic because it is converted in the body into cyanide, a reaction which is probably specific to 2-oxo-aldoximes (Askew, Davies, Green, and Holmes, 1956).

DAM is relatively non-toxic, and its toxicity by the intraperitoneal route to mice is 900 mg./kg. (Dultz *et al.*, 1957). No other results are available. Although P2AM is the most promising of the three compounds therapeutically, information concerning its toxicity is very limited. Thus Kewitz and Wilson (1956) give the LD50 for mice as 136 ± 6 mg./kg. (i.p.) and add that with doses of this order death occurs in about 20 min. Askew (unpublished observation) showed that 100 mg./kg. (i.p.) of P2AM was non-lethal to rats and indeed did not seem to cause any obvious effects.

From the above it is clear that toxicity estimates on oximes are scanty and incoordinated.

P2AM is relatively insoluble, so that its use in large mammals might well be limited. The sub-

stitution of a methanesulphonate for the iodide of P2AM gives a much more soluble substance, 2-hydroxyiminomethyl-*N*-methylpyridinium methanesulphonate (P2S) without loss of therapeutic efficacy.

Materials and Methods

Three oximes were used in this investigation. Pyridine-2-aldoxime (P2A), 2-hydroxyiminomethyl-*N*-methylpyridinium iodide (pyridine - 2 - aldoxime methiodide; P2AM) and 2-hydroxyiminomethyl-*N*-methylpyridinium methanesulphonate (P2S).

Toxicity was determined in female rats (180 to 200 g.), mice (18 to 22 g.), rabbits (2 to 3 kg.), and guinea-pigs (350 to 400 g.). Both sexes were used in determinations on monkeys (3 to 4 kg.).

All solutions were made up in water and the *p*H adjusted to 7.4 by the addition of NaOH. In any one determination of the LD50 the volume of the injection/kg. body weight was constant. Owing to differences in the weight of different species this volume varied from species to species and also with the route of administration. The volume used is recorded in the appropriate section of the text.

LD50 values were calculated by the method of moving averages (Thompson, 1947) using the Tables for convenient calculation of the median effective dose (Weil, 1952), except where indicated in the text. A four-point assay was used and six or four animals were used at each point.

Results

The Signs of Poisoning by P2S.—Sub-lethal intramuscular injections of P2S produce lethargy and increased respiration. Lethal doses cause muscular tremors, convulsions, dyspnoea, cyanosis, and death. Doses of 2 to 3 × LD50 produce death in 3 to 4 min. when given intravenously, and in 20 to 30 min. intramuscularly.

The minimum doses at which onset of signs of poisoning were seen in various species are given in Table I together with the maximum doses at which no signs were observed.

Table I

THE APPEARANCE OF SIGNS IN VARIOUS SPECIES FOLLOWING INTRAMUSCULAR INJECTION OF P2S

Species	Maximum Dose at which NO Signs were Observed (mg./kg.) (1)	Minimum Dose at which Signs were Noted (mg./kg.) (2)	Severity of Signs at Minimum Dose (see Col. 2) (3)
Mouse ..	100	120	Very slight
Rat ..	126	159	,, ,,
Guinea-pig	159	200	,, ,,
Rabbit ..	126	159	,, ,,
Dog ..	30	60	Marked
Monkey ..	—	200	,,
Sheep ..	60	c. 80	Slight

In the mouse, rat, guinea-pig and rabbit, doses of 100 mg./kg. were given without any obvious toxic effects, although slight signs, such as lethargy and increased rate of respiration, were obvious when the dose was increased by about a quarter over that shown in column 1.

The minimum dose given to monkeys was 200 mg./kg. Although there were no deaths, it produced restlessness, lethargy and tremors in each animal. Two of the four animals collapsed for from 10 to 20 min.

The first indications of toxic effects in sheep were seen with approximately 80 mg./kg., but these effects were slight.

The dog was the most sensitive mammal studied. No signs of poisoning occurred at 30 mg./kg., but at 60 mg./kg. they were marked, consisting of gasping respiration, tremors, vomiting, inability to hold up its head, sometimes followed by collapse. Recovery was complete in 1 hr.

In rats killed with P2S, moderate to severe cyanosis was seen in the lungs and abdominal organs. Histologically the lungs showed generalized congestion with areas of collapse and some haemorrhages; the kidneys and the liver were also congested.

Rats given six intramuscular injections of 80 mg./kg. at approximately 1 hr. intervals showed no obvious ill effects other than slight lethargy. Microscopic examination of the tissues on the next day showed slight congestion of the lungs, liver, spleen and kidney, in one animal. There was, however, some damage to muscles at the site of injection, but there was no visible damage to the muscles in animals killed a fortnight later.

The Toxicity of P2S in Various Species by Various Routes. — The intravenous and intramuscular LD50 values of P2S in the mouse, rat, rabbit and guinea-pig, together with the LD50 value by the intramuscular route in the monkey, are recorded in Table II.

Table II

INTRAVENOUS AND INTRAMUSCULAR TOXICITIES OF P2S TO VARIOUS SPECIES

24 animals were used in each determination of LD50 except in the case of monkeys, where only 16 animals were employed.

Species	Intravenous			Intramuscular		
	Vol. of Soln. Injected ml./kg.	mg./kg. LD50	mg./kg. Limits (95%)	Vol. of Soln. Injected ml./kg.	mg./kg. LD50	mg./kg. Limits (95%)
Mice ..	10·0	122	111–135	1·0	231	215–248
Rats ..	10·0	109	96–123	1·0	218	208–227
Rabbits ..	1·0	147	126–169	1·0	233	203–269
Guinea-pigs				1·0	305	268–342
Monkeys ..				1·0	356	303–419

P2S, intravenously, is equally toxic in the mouse, rat and the rabbit, the LD50 values lying between 110 and 150 mg./kg.

The intramuscular LD50 values for mice, rats and rabbits are about twice the intravenous value (approximately 230 mg./kg.) but are less than the intramuscular LD50 for monkeys which is 356 mg./kg.

These differences in the intramuscular and intravenous LD50 values prompted a more detailed examination of the effect of route of administration upon the toxicity of P2S. Table III gives the LD50 estimates obtained by administering the oxime to rats and mice in various ways.

TABLE III

VARIATION OF TOXICITY OF P2S IN RATS AND MICE WITH ROUTE OF ADMINISTRATION

24 animals were used in each determination of LD50.

Route of Admin.	Rats			Mice		
	Vol. of Injection (ml./kg.)	mg./kg.		Vol. of Injection (ml./kg.)	mg./kg.	
		LD50	Limits		LD50	Limits
i.v. ..	10·0	109	96–123	10·0	122	111–135
i.m. ..	1·0	218	208–227	1·0	231	215–248
s.c. Flank	4·0	527	438–635	10·0	191	159–230
,, Scruff	4·0	332	282–392	10·0	165	145–188
i.p. ..	10·0	262	237–288	10·0	216	188–249
Oral ..	2·5	>800		5·0	>2000	

Excluding oral administration, LD50 values varied in the rat from 109 mg./kg. intravenously to 527 mg./kg. when the oxime was given subcutaneously into the flank. When the oxime was injected subcutaneously into the scruff of the neck the toxicity was significantly less (332 mg./kg.).

In mice such variations were less marked. In fact, differences in the LD50 values by the parenteral routes were not significant.

TABLE IV

THE RELATIVE TOXICITY OF P2S BY A GIVEN ROUTE, EXPRESSED IN TERMS OF THE INTRAVENOUS TOXICITY

Route of Administration	Relative Toxicity	
	Rats	Mice
i.v.	1=109	1=122
i.m./i.v.	2·0	1·9
s.c. Flank/i.v.	4·9	1·6
,, Scruff/i.v.	3·0	1·4
i.p./i.v.	2·4	1·5

The effect of route of administration in these two species is best seen by a comparison of the toxicity by a given route, expressed in terms of the intravenous toxicity (see Table IV). In rats this varies from 2.0 to 4.9, whereas in mice it is between 1.4 and 1.9.

The Influence of Sex upon the Toxicity of P2S.—In view of the sex difference found in the toxicity of MINA to rats, intraperitoneal LD50 values were determined in both sexes in rats and mice. These are given in Table V.

No significant difference was demonstrated by this route.

TABLE V

INTRAPERITONEAL TOXICITY OF P2S TO MALE AND FEMALE RATS AND MICE

The volume of P2S injection was 10·0 ml./kg. in both species. 24 animals were used in each LD50 determination.

	Male		Female	
	mg./kg.		mg./kg.	
	LD50	Limits	LD50	Limits
Rats ..	317	260–387	262	237–288
Mice ..	147	130–167	178	146–217

A Comparison of the Toxicity of Some Derivatives of Pyridine-2-Aldoxime (P2A).—In the course of the general programme, the toxicities of three closely related compounds, P2A, P2AM and P2S, have been compared. Intraperitoneal LD50 values are given in Table VI for rats and mice. All three compounds were equally lethal to rats, but both P2AM and P2S were significantly more toxic than P2A to mice. Again P2AM was less toxic to rats than to mice.

TABLE VI

A COMPARISON OF THE INTRAPERITONEAL TOXICITY OF DERIVATIVES OF PYRIDINE-2-ALDOXIME (P2A)

The volume of P2S used was 10·0 ml./kg. and of P2A and P2AM was 20·0 ml./kg. 24 animals were used in each LD50 determination.

Compound	Rats		Mice	
	mg./kg.		mg./kg.	
	LD50	Limits	LD50	Limits.
P2A ..	299	237–377	305	268–347
P2AM ..	305	277–336	233	216–252
P2S ..	262	237–288	216	188–249

Although the LD50 values expressed in mg./kg. for each of these compounds does not differ markedly, a major difference in toxicity becomes apparent when the mg./kg. values are converted to moles/kg. The molecular weight of P2A is only half that of P2AM or P2S. Expressed in this way, P2A is only half the toxicity of the others. This difference in toxicity is also shown by a comparison of their speed of action (Table VII).

In mice a "sure lethal" dose of approximately 2 × LD50 given intravenously of either P2AM or P2S resulted in immediate convulsions with death from 1 to 6 min. With P2A, the shortest time to

death observed in a series of 35 animals was 120 min. and the longest 280 (Table VII).

TABLE VII

THE TIME TO DEATH IN MICE POISONED WITH ABOUT 2 × LD50 OF P2A, P2AM, AND P2S

Compound	No. of Animals	Mean Time to Death (min.)	Range (min.)	Development of Symptoms
P2A	35	200	120–280	Immediate collapse: rapid recovery in 1–10 min. Death in about 3 hr.
P2AM	15	2	1–6	Immediate convulsion with rapid onset of death
P2S	10	1	1–3	Immediate convulsion with rapid onset of death

The Effect of Atropine upon the Toxicity of P2S.—As an antidote in alkyl phosphate (anticholinesterase) poisoning, oximes are only markedly effective in conjunction with atropine. The toxicity of P2S has therefore been determined in mice, rats and guinea-pigs in the presence of atropine. A standard dose of 17.4 mg./kg. atropine sulphate was given intramuscularly, together with various doses of P2S, and regression lines constructed. The constants of such curves are shown in Table VIII.

TABLE VIII

CONSTANTS OF PROBIT-KILLED/LOG$_{10}$ DOSE REGRESSION LINES FOR P2S IN DIFFERENT SPECIES AND IN THE PRESENCE AND ABSENCE OF ATROPINE

P2S plus atropine sulphate (17·4 mg./kg.) given together i.m. 80 animals were used for the construction of each probit line.

Species	Agent	Regression Coefficient ± Standard Error	LD50	95% Fiducial Limits LD50	Probability Level of χ^2 for Parallelism
Rat ..	P2S	14·8±2·26	218	208–227	70%
	P2S+Atrop.		319	304–338	
Guinea-pig	P2S	11·0±2·10	305	268–342	70%
	P2S+Atrop.		238	208–260	
Mouse	P2S	12·7±1·31	231	215–248	40%
	P2S+Atrop.		218	204–233	

Three interesting points are apparent from Table VIII. First, the slopes of the regressions are steep. This is important, for, as will be shown later, it indicates that a relatively small increase below the LD50 results in doses whose lethal effects are considerably reduced. Secondly, regressions are essentially parallel for mice, rats and guinea-pigs. Atropine does not alter this parallelism. Thirdly, the effect of atropine upon the toxicity of P2S varies with the species. Thus, in rats, atropine raises the LD50 value by not more than about 20%, whilst in mice it has no effect.

DISCUSSION

The object of this work was to obtain information about the toxicity of P2S so that the likely maximum safe dose for man might be assessed.

Death has been used as the criterion of toxicity. Probit/log. dose regression lines have been constructed for rats, guinea-pigs and mice and, by assuming linearity, extrapolations to the calculated lines have been made. The results of these calculations are shown in Table IX where some theoretical dose/response relationships are given for the extreme end of the lines quoted in this Table.

TABLE IX

THEORETICAL DOSE/RESPONSE RELATIONSHIP OF I.M. INJECTED P2S AT THE EXTREME ENDS OF THE LINES. QUOTED IN TABLE VIII

Lethal Expectation	Rat (mg./kg.)		Guinea-pig (mg./kg.)		Mouse (mg./kg.)	
	LD	Limits	LD	Limits	LD	Limits
1/1 = LD50	218	208–227	305	268–342	231	215–248
1/100 ..	152	108–152	187	120–226	151	139–166
1/1,000 ..	135	94–151	160	91–201	132	118–147
1/10,000	122	83–132	140	71–183	118	103–134

In Table I the minimum observed dose is given at which signs were recorded and these values are by no means inconsistent with those in Table IX. In each species there is only a one in hundred chance of death at doses which cause the first signs of poisoning. For example, in rats given 150 mg./kg. the expectation of death is 1/100; very slight toxic effects were seen with 159 mg./kg. In the guinea-pig the 1/100 lethal probability occurs at 187 mg./kg. and again slight signs of poisoning were noted at 200 mg./kg. In mice the corresponding figures were 151 and 120 mg./kg. respectively. Although lethal probabilities are quoted at the 1/10,000 level, such estimates must be quite theoretical, since, at this level, death from other causes may well occur and cannot easily be distinguished from those attributable to P2S.

The fact that oximes will almost certainly be used with atropine also complicates the issue. However, since the probit/log. dose regression lines are essentially parallel not only for a given species in the presence and absence of atropine, but also for different species, provided that it is known in which direction atropine influences the toxicity in a given species, an appropriate adjustment in such estimates of "safe" dose can be made.

Although estimates of the intramuscular LD50 of P2S have been made for both the monkey and the dog, the number of animals upon which such estimates have been based are too few to per-

mit the construction of adequate dose/response curves. However, the fact that such lines are parallel for the rat, guinea-pig and mouse suggests that some approximate estimates can be made if it is permissible to assume that for monkeys and dogs the lines are also parallel.

Intramuscular LD50 values of P2S for the dog have only been assessed as a result of few observations and a provisional value of 70 to 80 mg./kg. has been given. On this basis, the dog is markedly more sensitive than any of the other species. The 1/100 lethal probability level is approximately two-thirds of the LD50 and, as has been shown, is the dose level at which symptoms first tend to appear. Two-thirds the LD50 value in the dog is about 50 mg./kg. At 60 mg./kg. poisoning was marked.

Approximately half the LD50 is the dose at which the chances of death are only 1/10,000 and at this dose no signs of poisoning would be expected. Half the LD50 is about 37 mg./kg. and at 30 mg./kg. there were no signs in any of the dogs so treated. These observations were made in the absence of atropine.

The LD50 for the monkey is greater than in any of the other species studied so that it does not seem illogical to assume that the safe dose in this species should not be less than either of the three in which more detailed studies have been carried out.

The value of estimates of the "safe" dose such as have been made above must lie in the use to which they are to be applied. Obviously they should not be treated as estimates of high precision, but they are a guide to dose levels above which P2S may only be given with the possibility of unpleasant side-effects and even death. However, in practice the choice of dose must depend upon whether it is to be given prophylactically or therapeutically. If the former it must be free from any lethal or incapacitating effects: in the latter, since the urgent requirement is saving the life of a seriously poisoned person, temporary inconvenience and even some risk of death from the oxime may be acceptable.

In general, then, it may be stated that doses of 100 mg./kg. intramuscularly of P2S may be given to the rat, mouse, guinea-pig or monkey with a lethal probability of less than 1/1,000. The dog appears to be much more sensitive, but the results are very much less reliable.

However, other factors tend to limit the size of a single dose. Thus therapeutically it is shown elsewhere that in the fully atropinized animal there is an optimum dose of P2S, approximately 30 mg./kg. intramuscularly beyond which there is no proportional increase in effectiveness. For convenient use in the field the total volume of injection must be limited. Again 30 mg./kg. appears to be indicated as a probable upper limit. Thus all the available results at the moment suggest that an intramuscular dose of 30 mg./kg. of P2S may be given to man without any serious side-effects and with a negligible risk of death.

These assessments have been arrived at without considering the effect of atropine upon the toxicity of P2S and this may, as shown, materially alter the picture.

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