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COMMERCIAL-IN-CONFIDENCE

PTA/15/123

THE TOXICITY OF HYDROGEN CYANIDE BY INHALATION TO FERRETS AND BADGERS

Chemical Defence Establishment,
Porton Down, Salisbury, Wilts.

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INTRODUCTION

In order to give some idea of the toxicity of hydrogen cyanide to badgers and the 'humaneness' of this method of killing when badgers are gassed in the tuberculosis eradication campaign, CDE has been asked to carry out some experiments on these animals. Because of the scarcity of badgers, it was suggested by Lord Zuckerman that ferrets be used as a model for the quantitative toxicity testing. What follows is a report which in Part 1 defines the LCt_{50}, LCt_{90} and minimum concentrations to kill 80% and 90% of ferrets exposed, and in Part 2 describes the short series of badgers exposed to HCN and the extrapolation of the ferret results to badgers.

March 1982

PART 1 EXPOSURE OF FERRETS TO HCN

Animals

177 female ferrets were obtained from a commercial supplier. Although the animals were especially bred for the contract it was apparent from visual inspection that there were two different groups of animals involved.

This impression was borne out by an examination of all the body weights, Appendix 1, Table 1, from which the histogram, Appendix 2, Fig 1 is derived. It can be seen that there is a significant number of large animals which are outliers, from the main population. The numerical results of a simple analysis were as follows:

From all the animals used:-

Mean body weight	711.2 g
Standard deviation	174.3 g

From the same figures, less the lightest animal and the twenty-seven heaviest ones a new set of statistics was derived. Comparison of the two sets showed that the suspicion of these being a significantly bi-model population was well founded. In particular the values for skewness were:

For all 177 animals	0.754
For the 149 animals	0.005

This abnormality in the experimental animals may account for some of the scatter in the results.

METHODS

Hydrogen cyanide was generated by reacting 3.0 mol ℓ^{-1} sodium cyanide in 0.1 mol ℓ^{-1} sodium hydroxide with 3.1 mol ℓ^{-1} hydrochloric acid. All reagents used were of analytical reagent grade. See Fig 1.

As may be seen from the diagram the two solutions were supplied at a consta rate by a common syringe drive. The resultant mix of HCN, sodium chloride and water was sprayed into the exposure air flow through a heated tube into the exposure chamber of approximately 125 litres capacity. Because of the particle size of the salt spray the droplets evaporated and precipitated within the heated portion of the tube. The air flow through the chamber was monitored using the pressure drop across a calibrated orifice plate and was set at 200 ℓ min^{-1}.

Sampling of the atmosphere was carried out by drawing gas concentrations from the chamber, for the period of complete exposures, through bubblers containing 0.1 mol ℓ^{-1} sodium hydroxide at a nominal rate of 1.0 ℓ min^{-1}. The individual sampling flow rates were measured and used in calculating the concentrations. Estimation of the cyanide in the bubbler was performed using a CN^- specific ion electrode, referring the results to a calibration curve prepared freshly each day, Fig 2. Recoveries of HCN varied between 90-95% of theory. Animals were exposed in individual cages, using groups of three animals for each exposure. Twenty groups of three animals were used for the one and five minute exposures, nineteen groups for the twenty-five minute ones.

Statistical analyses of the results were carried out using methods described generally in 'Probit Analysis' by D J Finney, 3rd edition, 1971 and more particularly in Sections 4.6, 4.7 and 10.2.

RESULTS

The results are listed as Tables 1, 2 and 3, referring to one, five and twenty-five minute runs respectively.

The entry headings are to be read as, body weight in grammes, Ct in concentration time, that is mg min m^{-3} - this may be divided by the 'time' in minutes to give the equivalent concentration in mg m^{-3} or µg ℓ^{-1}. The 24 h mortality is deaths from the three animals exposed in each group.

TABLE 1

RESULTS FROM ONE MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
1	817	1158	3
	576		
	660		
2	794	919	3
	981		
	1040		
3	1010	618	1
	1009		
	758		
4	804	618	1
	601		
	752		
5	715	786	3
	965		
	868		
6	696	707	1
	548		
	636		
7	741	751	2
	639		
	644		
8	884	833	1
	615		
	784		
9	685	833	3
	776		
	743		
10	615	813	2
	305		
	961		
11	530	760	3
	424		
	612		
12	697	786	2
	843		
	679		
13	561	898	3
	541		
	664		
14	505	723	3
	585		
	574		
15	695	778	2
	450		
	817		
16	651	794	1
	539		
	685		
17	490	798	3
	524		
	695		
18	673	848	2
	564		
	575		
19	648	767	2
	500		
	622		
20	754	763	3
	829		
	592		

TABLE 2

RESULTS FROM FIVE MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
1	575, 1050, 776	3806	3
2	808, 604, 702	3282	3
3	558, 1027, 720	2396	3
4	600, 778, 858	2034	1
5	987, 747, 933	1541	2
6	897, 973, 853	1129	0
7	770, 756, 799	1510	2
8	975, 588, 984	1575	1
9	637, 625, 1058	1313	1
10	963, 823, 1006	1968	2
11	536, 972, 669	2000	3
12	940, 952, 1001	2034	1
13	1259, 1266, 1103	2199	3
14	787, 724, 645	2199	3
15	710, 722, 654	2131	1
16	678, 977, 666	2100	3
17	685, 858, 722	2131	3
18	758, 1266, 901	2131	2
19	724, 1056, 446	2100	3
20	787, 786, 966	2165	2

TABLE 3

RESULTS FROM 25 MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
1	812 633 594	2014	1
2	505 721 605	2535	2
3	553 620 563	2980	3
4	488 625 543	2951	2
5	695 764 650	2988	3
6	685 438 825	2320	3
7	672 438 759	2427	3
8	565 658 455	2320	3
9	436 571 503	2298	3
10	603 645 713	2500	3

Run No	Body Wts in g	Ct	24 morta
11	437 503 506	2427	3
12	640 656 573	2157	3
13	639 645 508	2253	2
14	631 807 612	2342	[illegible]
15	548 582 587	2838	[illegible]
16	737 567 754	2689	[illegible]
17	660 699 737	2155	[illegible]
18	649 598 660	2120	[illegible]
19	769 597 648	2095	

The respective mean and standard deviation for the animal body weights were:

	Mean wt g	SD g
1 minute	685	155
5 minutes	743	186
25 minutes	620	99

The results for the LCt_{50} and LCt_{90} with their 95% fiducial limits were:

	LCt_{50}	Limits	LCt_{90}	Limits
1 minute	670	360 → 730	910	820 → 1910
5 minutes	1600	1210 → 1800	2390	2090 → 3450
25 minutes	1740	1440 → 2109	2600	2142 → 3156

Cts to be read as milligram minutes per cubic metre.

The equations for the regression lines for the dose-response curves are:

1 minute $Y - 5.579 = 9.666 (x - 2.885)$

5 minutes $Y - 5.512 = 7.382 (x - 3.273)$

25 minutes $Y - 5.967 = 7.315 (x - 3.372)$

where Y is the probit of the desired % response and x is the log of Ct.

More importantly, the limits on the percentage kill have been estimated for any particular dose, whereas the classical probit analysis results give the limits for the dose for any percentage kill. These limits allow one to postulate the dose required to 'at least' kill any percentage of the population with a probability in this case of P = 0.975.

Exposure Time	80% ← minimum kill		→ 90%
1 minute	1025)	mg min m^{-3}	(1450
5 minutes	2575)		(3450
25 minutes	2775)		(3625

Full plots of the values of the minimum probable kill versus Ct are in Fig 3.

PART 2 EXPOSURE OF BADGERS TO HCN

Animals

Four badgers were available for experiment from the Ministry of Agricultur station at Tangley Place. These were captive wild animals obtained for the programme.

METHODS

Two variants from the ferret equipment obtained. A larger chamber, of approximately 0.3 m^3 volume, was used with an air flow of 1,000 $\ell\ min^{-1}$ for the lowest concentrations, down to 250 $\ell\ min^{-1}$ for the two highest ones.

An addition, also, was the use of video-recording equipment to obtain a permanent film of the exposures.

Animals were exposed singly.

Exposure No 1. Badger weight 10.7 kg.

This exposure lasted 30 mins, giving a Ct of 2560 mg min m^{-3}, or a concentration of 85 $\mu g\ \ell^{-1}$.

The sequence of events during the exposure was, zero plus;

1 minute	The respiration appeared affected.
3 minutes	Animal was restless.
6 minutes	Showed major signs of intoxication, gasping, vomiting, swaying about.
23 minutes	Collapsed.

On withdrawal from the chamber at approximately zero plus one hour the animal was unconscious. It regained consciousness at about plus 3 hours and was returned to Tangley Place four days later in good health.

Exposure No 2. Badger weight 9.8 kg.

Exposure lasted 30 minutes, giving a Ct of 5575 mg min m^{-3} or a concent of 186 $\mu g\ \ell^{-1}$.

There were no obvious signs until 27 minutes after the beginning of the exposure, then vomiting, gasping, and staggering occurred.

On withdrawal at approximately zero plus one hour after the end of the exposure the animal was semi-conscious, but was returned with badger No 1 i normal condition.

Exposure No 3. Badger weight 8.8 kg.

Exposure lasted 21 minutes. At about 17 minutes from the start of the exposure the badger had ceased breathing and was dead upon withdrawal from the chamber.

Ct = 6560 mg min m^{-3}
Conc. = 312 µg ℓ^{-1}

Exposure No 4. Badger weight 8.8 kg.

Ct = 4020 mg min m^{-3}
Conc. = 335 µg ℓ^{-1}

At 12 minutes from the beginning of the exposure the animal ceased breathing and the gas flow was stopped. Respiration started spontaneously within one to two minutes. The animal was taken back to Tangley Place unwell but recovering.

Video-records of all four exposures and the three immediate post exposure periods are available.

DISCUSSION

The sole reason for using ferrets, which are in good supply, was as an exposure model for badgers, which are not easily available. It is therefore necessary to be able to extrapolate from ferrets to badgers.

The apparent toxicity of hydrogen cyanide by inhalation decreases as the time of exposure lengthens and this difficulty tends to confound the problem. However, it is possible to make some guess as to what should happen when badgers are exposed, and to test whether this is at odds with the short series of experiments.

Starting with the proposition that for any dose rate expressed as mass of hydrogen cyanide/mass of tissue/unit time, the absolute toxicity of cyanide is the same for the two species, then;

1. The dose/kg for any one exposure time will be governed by the volume inhaled/unit time, ie the respiratory minute volume.

2. The apparent difference in toxicity will be due to any differences in respiratory minute volume in the two species.

The relationship between body weight and minute volume is not unity, but has the form:

minute volume ∝ (body weight)$^{0\cdot 7}$

(Rubner, M. 1883.
Über den Einfluss der Körpergrösse auf Stoff - und Kraftwechs
Z. Biol., 19, 535 - 62.)

The mean weight of the badgers was 10 kilogrammes whilst that of
was 700 grammes, a ratio of 14:1, this predicates a minute volume rat
approximately 6.5:1. The badger will, therefore, need to inspire a cc
twice as high as a ferret to maintain the same inhaled dose/kilogramme

Because of the non-linear relationship between Ct and time of exp
is not possible to say that a badger would survive for twice the time
inhaling a similar concentration to a ferret.

Taking that the probable 90% kill for 5 minutes in ferrets is 345
and for 25 minutes is 3625, the equivalent figures for badgers would b
7500.

Similarly the LCt_{50} and LCt_{90} may also be multiplied.

	Ferrets			Badgers	
Time	LCt_{50}	LCt_{90}		LCt_{50}	LCt_{90}
1 min	670	910		(1300	2000
25 min	1600	2390	approximately	(3000	5000
25 min	1740	2600		(3500	5500

In fact for two 30 minutes exposures at 2560 and 5575 Ct units re
both badgers survived.

At a 17 minute exposure with a Ct of 6560 one badger died, and on
survived a Ct of 4000 over 10 minutes. These results support the very
figures in the right hand column.

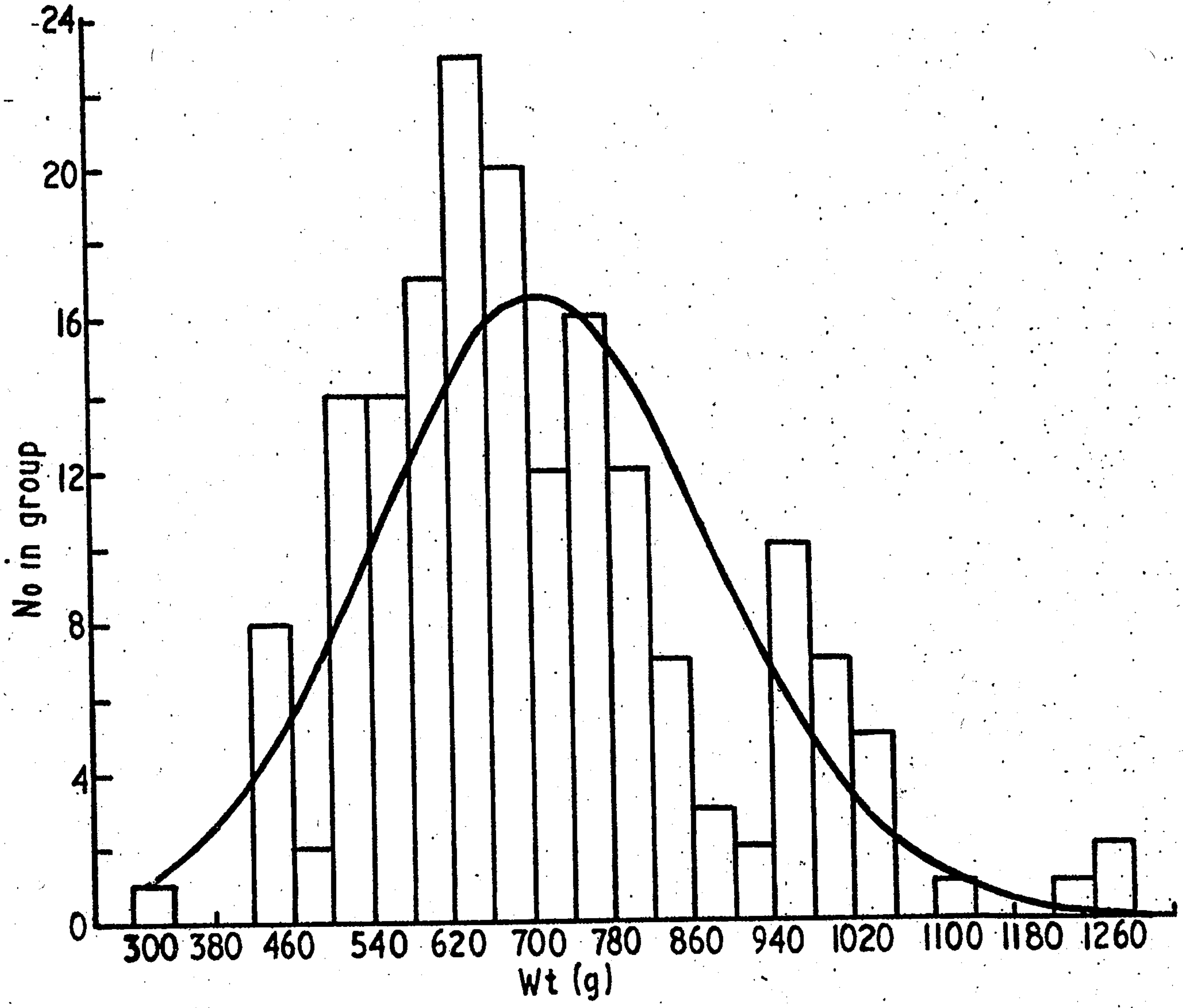
CONCLUSIONS

Using the figures found for the various Cts for ferrets exposed t
cyanide, it appears both from extrapolation and from real exposure of
that a Ct to obtain 90% kill with reasonable certainty would need to b
7000 mg min m^{-3} for a 30 minute exposure, or a concentration of approx
230 $\mu g\ \ell^{-1}$.

Appendix 1. Table 1.

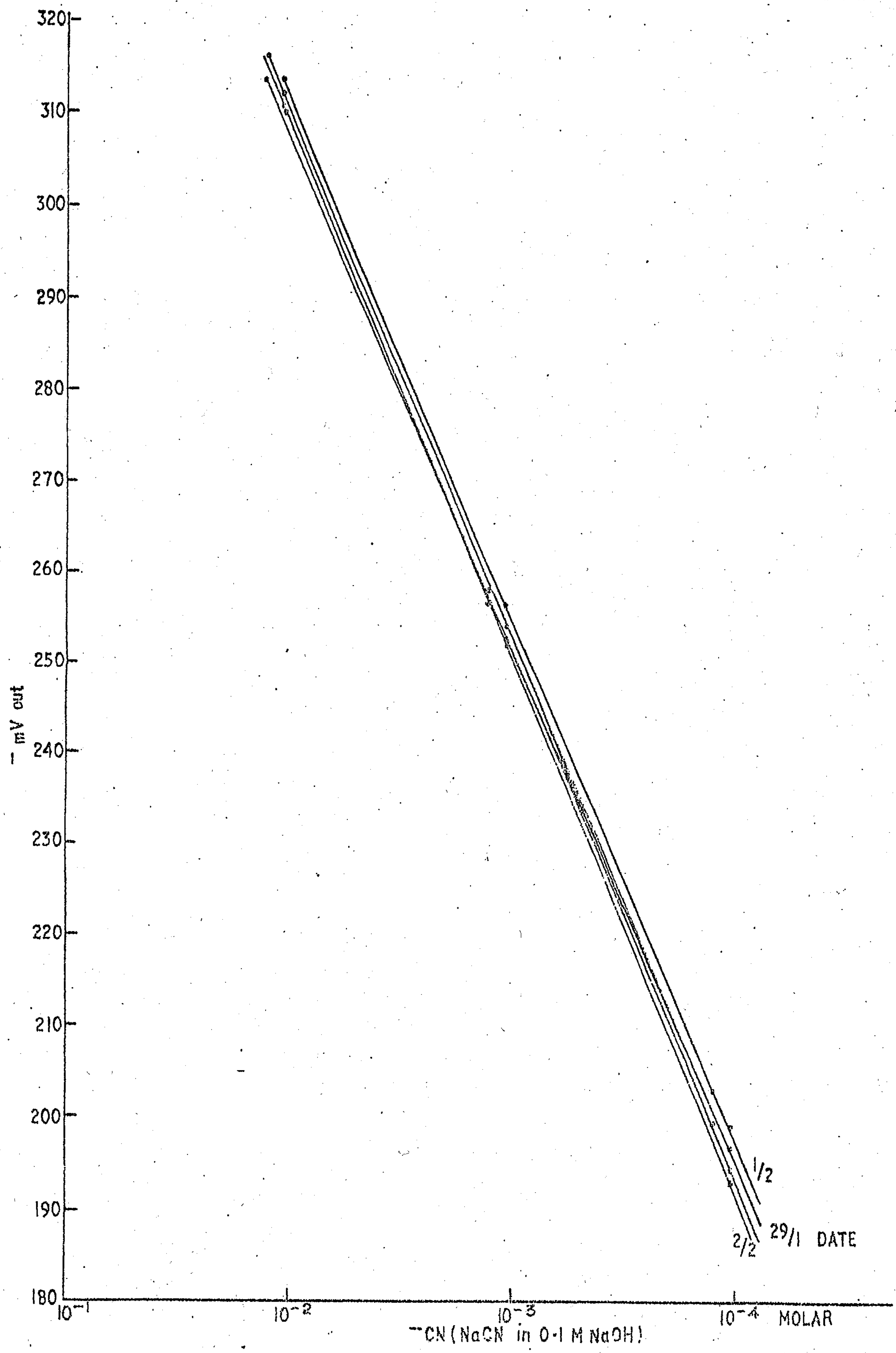
LIST OF FERRET BODY WT (g)

305	541	604	651
424	543	605	654
436	548	612	656
437	548	612	658
438	553	615	660
438	558	615	660
446	561	620	660
458	563	622	664
455	564	625	666
488	565	625	669
490	567	631	672
500	571	633	673
503	575	636	678
503	576	637	679
505	582	639	685
505	585	639	685
506	587	640	685
508	588	644	685
513	592	645	695
514	594	645	695
515	597	645	695
524	598	648	696
530	600	648	697
536	601	649	699
539	603	650	702
710	776	933	1266
713	778	940	1266
715	784	952	
720	786	961	
721	787	963	
722	787	965	
722	794	966	
724	799	972	
724	804	973	
737	807	975	
737	808	977	
741	812	981	
743	817	984	
747	817	987	
752	823	1001	
754	825	1006	
754	829	1009	
756	843	1010	
758	853	1027	
758	855	1040	
759	858	1050	
764	868	1056	
769	884	1058	
770	897	1103	
776	901	1259	



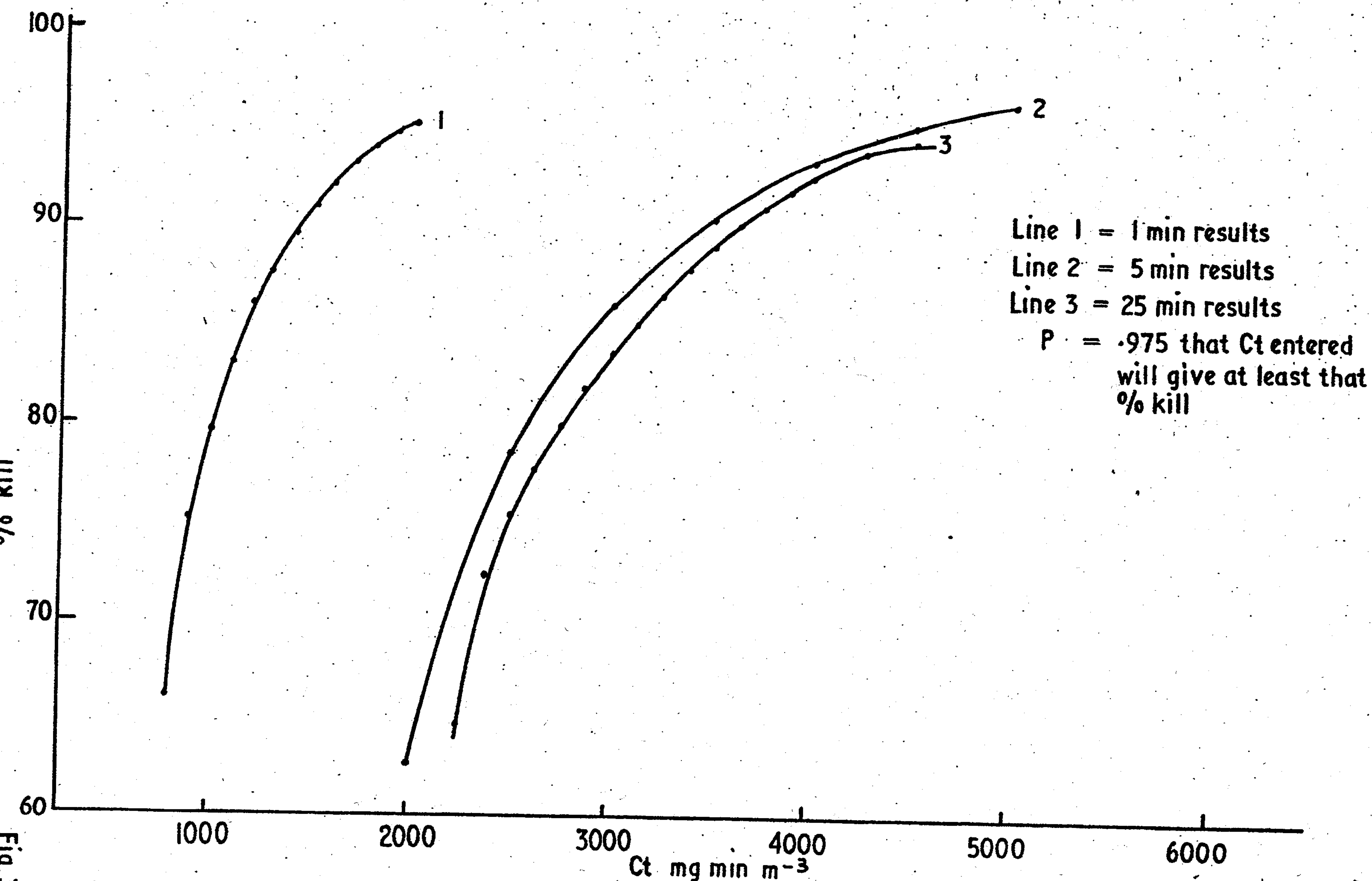
ANIMAL BODY WEIGHTS

Appendix I. Fig. 1



CALIBRATION CURVES FOR CYANIDE ESTIMATION

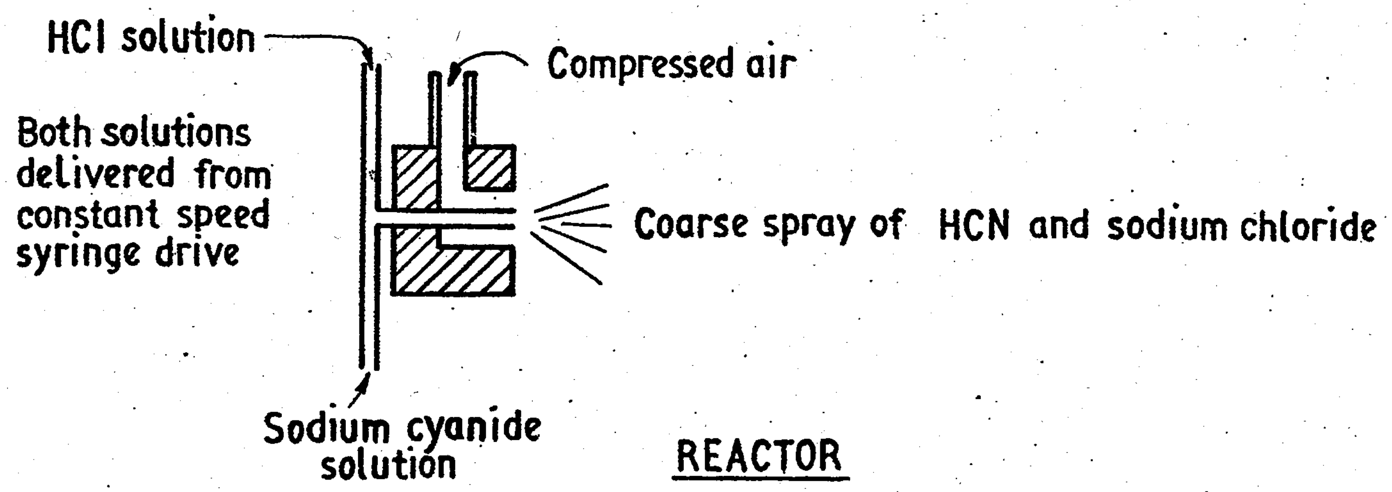
Fig.2.



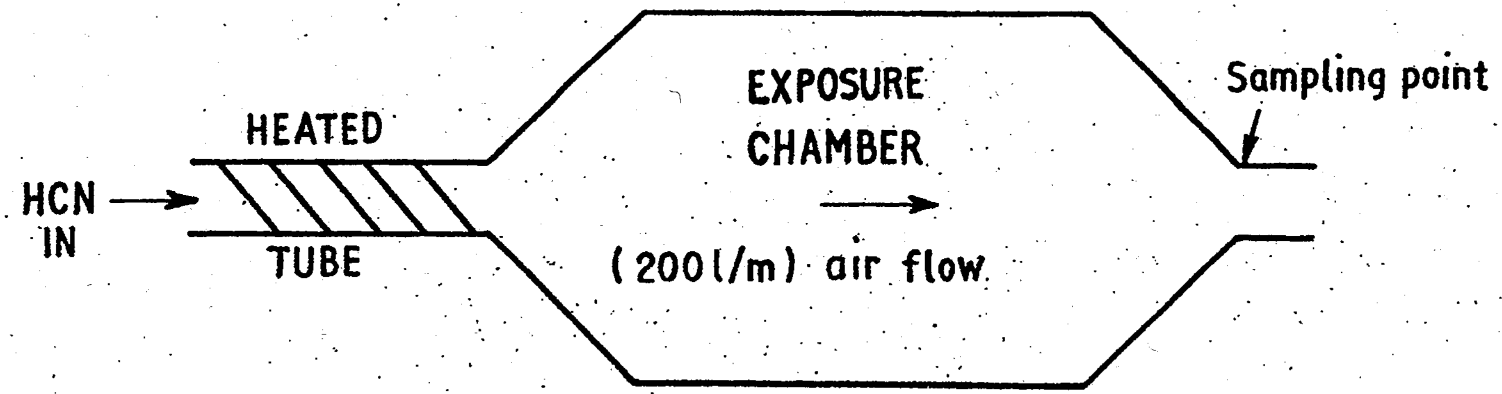
MINIMUM PROBABLE KILL FOR VARYING Cts

Fig.3.

Commercial in Confidence



REACTOR



CHAMBER

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March 1982

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Standard deviation	174.3 g

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As may be seen from the diagram the two solutions were supplied at a consta rate by a common syringe drive. The resultant mix of HCN, sodium chloride and water was sprayed into the exposure air flow through a heated tube into the exposure chamber of approximately 125 litres capacity. Because of the particle size of the salt spray the droplets evaporated and precipitated within the heated portion of the tube. The air flow through the chamber was monitored using the pressure drop across a calibrated orifice plate and was set at 200 ℓ min^{-1}.

Sampling of the atmosphere was carried out by drawing gas concentrations from the chamber, for the period of complete exposures, through bubblers containing 0.1 mol ℓ^{-1} sodium hydroxide at a nominal rate of 1.0 ℓ min^{-1}. The individual sampling flow rates were measured and used in calculating the concentrations. Estimation of the cyanide in the bubbler was performed using a CN^- specific ion electrode, referring the results to a calibration curve prepared freshly each day, Fig 2. Recoveries of HCN varied between 90-95% of theory. Animals were exposed in individual cages, using groups of three animals for each exposure. Twenty groups of three animals were used for the one and five minute exposures, nineteen groups for the twenty-five minute ones.

Statistical analyses of the results were carried out using methods described generally in 'Probit Analysis' by D J Finney, 3rd edition, 1971 and more particularly in Sections 4.6, 4.7 and 10.2.

RESULTS

The results are listed as Tables 1, 2 and 3, referring to one, five and twenty-five minute runs respectively.

The entry headings are to be read as, body weight in grammes, Ct in concentration time, that is mg min m^{-3} - this may be divided by the 'time' in minutes to give the equivalent concentration in mg m^{-3} or µg ℓ^{-1}. The 24 h mortality is deaths from the three animals exposed in each group.

TABLE 1

RESULTS FROM ONE MINUTE EXPOSURES OF FERRETS TO HCN

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	636		
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	644		
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10	615	813	2
	305		
	961		

Run No	Body Wts in g	Ct	24 h mortality
11	530	760	3
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	612		
12	697	786	2
	843		
	679		
13	561	898	3
	541		
	664		
14	505	723	3
	585		
	574		
15	695	778	2
	450		
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16	651	794	1
	539		
	685		
17	490	798	3
	524		
	695		
18	673	848	2
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	575		
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	500		
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20	754	763	3
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TABLE 2

RESULTS FROM FIVE MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
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6	897 973 853	1129	0
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8	975 588 984	1575	1
9	637 625 1058	1313	1
10	963 823 1006	1968	2
11	536 972 669	2000	3
12	940 952 1001	2034	1
13	1259 1266 1103	2199	3
14	787 724 645	2199	3
15	710 722 654	2131	1
16	678 977 666	2100	3
17	685 858 722	2131	3
18	758 1266 901	2131	2
19	724 1056 446	2100	3
20	787 786 966	2165	2

TABLE 3

RESULTS FROM 25 MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
1	812 633 594	2014	1
2	505 721 605	2535	2
3	553 620 563	2980	3
4	488 625 543	2951	2
5	695 764 650	2988	3
6	685 438 825	2320	3
7	672 438 759	2427	3
8	565 658 455	2320	3
9	436 571 503	2298	3
10	603 645 713	2500	3

Run No	Body Wts in g	Ct	24 morta
11	437 503 506	2427	3
12	640 656 573	2157	3
13	639 645 508	2253	2
14	631 807 612	2342	3
15	548 582 587	2838	3
16	737 567 754	2689	3
17	660 699 737	2155	[illegible]
18	649 598 660	2120	[illegible]
19	769 597 648	2095	

The respective mean and standard deviation for the animal body weights were:

	Mean wt g	SD g
1 minute	685	155
5 minutes	743	186
25 minutes	620	99

The results for the LCt_{50} and LCt_{90} with their 95% fiducial limits were:

	LCt_{50}	Limits	LCt_{90}	Limits
1 minute	670	360 → 730	910	820 → 1910
5 minutes	1600	1210 → 1800	2390	2090 → 3450
25 minutes	1740	1440 → 2109	2600	2142 → 3156

Cts to be read as milligram minutes per cubic metre.

The equations for the regression lines for the dose-response curves are:

1 minute $Y - 5.579 = 9.666\ (x - 2.885)$

5 minutes $Y - 5.512 = 7.382\ (x - 3.273)$

25 minutes $Y - 5.967 = 7.315\ (x - 3.372)$

where Y is the probit of the desired % response and x is the log of Ct.

More importantly, the limits on the percentage kill have been estimated for any particular dose, whereas the classical probit analysis results give the limits for the dose for any percentage kill. These limits allow one to postulate the dose required to 'at least' kill any percentage of the population with a probability in this case of P = 0.975.

Exposure Time	80% ←	minimum kill	→ 90%
1 minute	1025)	mg min m^{-3}	(1450
5 minutes	2575)		(3450
25 minutes	2775)		(3625

Full plots of the values of the minimum probable kill versus Ct are in Fig 3.

PART 2 EXPOSURE OF BADGERS TO HCN

Animals

Four badgers were available for experiment from the Ministry of Agricultur station at Tangley Place. These were captive wild animals obtained for the programme.

METHODS

Two variants from the ferret equipment obtained. A larger chamber, of approximately 0.3 m^3 volume, was used with an air flow of 1,000 $\ell\ min^{-1}$ for the lowest concentrations, down to 250 $\ell\ min^{-1}$ for the two highest ones.

An addition, also, was the use of video-recording equipment to obtain a permanent film of the exposures.

Animals were exposed singly.

Exposure No 1. Badger weight 10.7 kg.

This exposure lasted 30 mins, giving a Ct of 2560 mg min m^{-3}, or a concentration of 85 $\mu g\ \ell^{-1}$.

The sequence of events during the exposure was, zero plus;

1 minute	The respiration appeared affected.
3 minutes	Animal was restless.
6 minutes	Showed major signs of intoxication, gasping, vomiting, swaying about.
23 minutes	Collapsed.

On withdrawal from the chamber at approximately zero plus one hour the animal was unconscious. It regained consciousness at about plus 3 hours and was returned to Tangley Place four days later in good health.

Exposure No 2. Badger weight 9.8 kg.

Exposure lasted 30 minutes, giving a Ct of 5575 mg min m^{-3} or a concent of 186 $\mu g\ \ell^{-1}$.

There were no obvious signs until 27 minutes after the beginning of the exposure, then vomiting, gasping, and staggering occurred.

On withdrawal at approximately zero plus one hour after the end of the exposure the animal was semi-conscious, but was returned with badger No 1 i normal condition.

Exposure No 3. Badger weight 8.8 kg.

Exposure lasted 21 minutes. At about 17 minutes from the start of the exposure the badger had ceased breathing and was dead upon withdrawal from the chamber.

Ct = 6560 mg min m^{-3}

Conc. = 312 µg ℓ^{-1}

Exposure No 4. Badger weight 8.8 kg.

Ct = 4020 mg min m^{-3}

Conc. = 335 µg ℓ^{-1}

At 12 minutes from the beginning of the exposure the animal ceased breathing and the gas flow was stopped. Respiration started spontaneously within one to two minutes. The animal was taken back to Tangley Place unwell but recovering.

Video-records of all four exposures and the three immediate post exposure periods are available.

DISCUSSION

The sole reason for using ferrets, which are in good supply, was as an exposure model for badgers, which are not easily available. It is therefore necessary to be able to extrapolate from ferrets to badgers.

The apparent toxicity of hydrogen cyanide by inhalation decreases as the time of exposure lengthens and this difficulty tends to confound the problem. However, it is possible to make some guess as to what should happen when badgers are exposed, and to test whether this is at odds with the short series of experiments.

Starting with the proposition that for any dose rate expressed as mass of hydrogen cyanide/mass of tissue/unit time, the absolute toxicity of cyanide is the same for the two species, then;

1. The dose/kg for any one exposure time will be governed by the volume inhaled/unit time, ie the respiratory minute volume.

2. The apparent difference in toxicity will be due to any differences in respiratory minute volume in the two species.

The relationship between body weight and minute volume is not unity, but has the form:

minute volume ∝ (body weight)$^{0 \cdot 7}$

(Rubner, M. 1883.
Über den Einfluss der Körpergrösse auf Stoff - und Kraftwechs
Z. Biol., 19, 535 - 62.)

The mean weight of the badgers was 10 kilogrammes whilst that of was 700 grammes, a ratio of 14:1, this predicates a minute volume rat approximately 6.5:1. The badger will, therefore, need to inspire a cc twice as high as a ferret to maintain the same inhaled dose/kilogramme

Because of the non-linear relationship between Ct and time of exp is not possible to say that a badger would survive for twice the time inhaling a similar concentration to a ferret.

Taking that the probable 90% kill for 5 minutes in ferrets is 345 and for 25 minutes is 3625, the equivalent figures for badgers would b 7500.

Similarly the LCt_{50} and LCt_{90} may also be multiplied.

	Ferrets			Badgers	
Time	LCt_{50}	LCt_{90}		LCt_{50}	LCt_{90}
1 min	670	910		(1300	2000
25 min	1600	2390	approximately	(3000	5000
25 min	1740	2600		(3500	5500

In fact for two 30 minutes exposures at 2560 and 5575 Ct units re both badgers survived.

At a 17 minute exposure with a Ct of 6560 one badger died, and on survived a Ct of 4000 over 10 minutes. These results support the very figures in the right hand column.

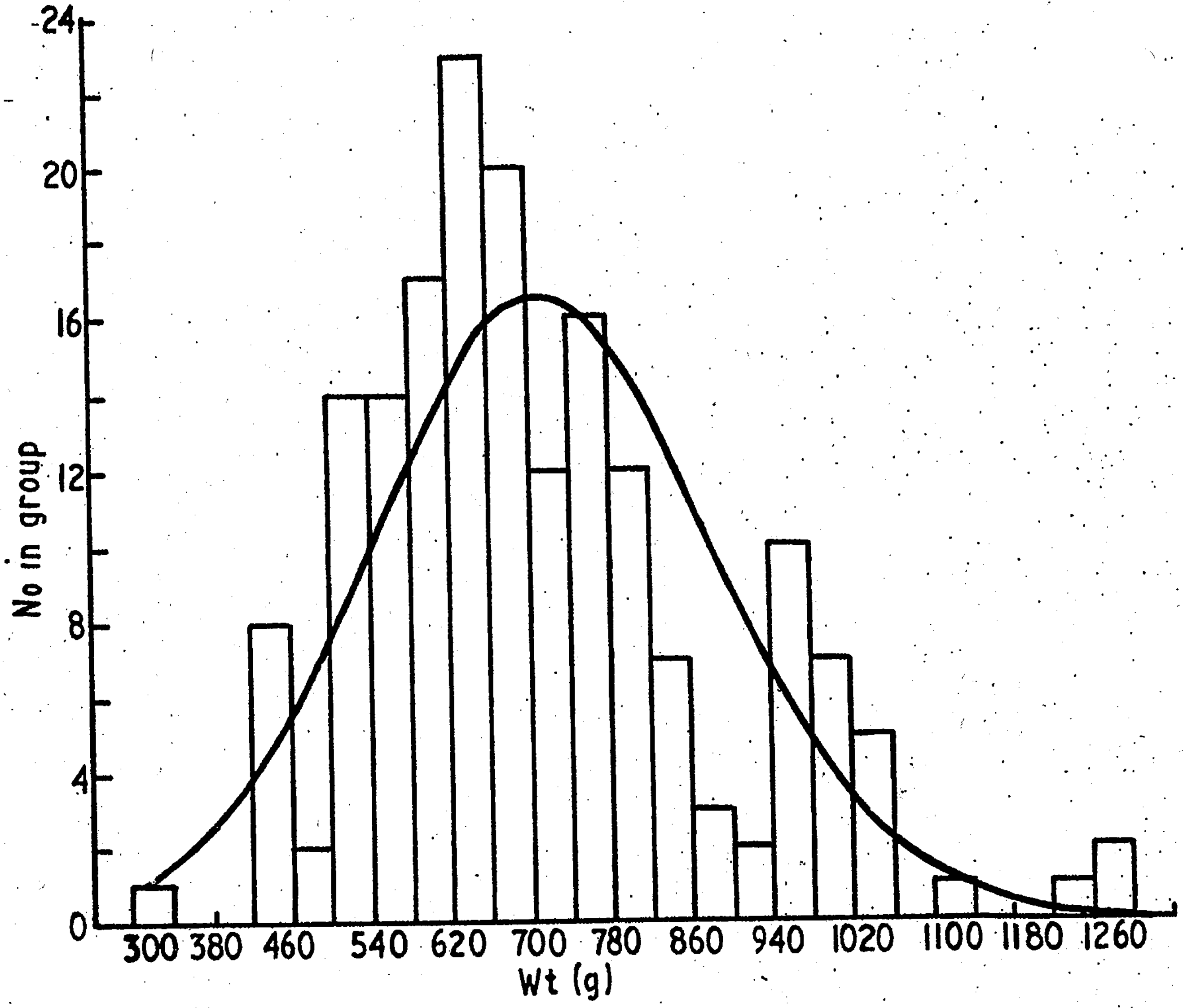
CONCLUSIONS

Using the figures found for the various Cts for ferrets exposed t cyanide, it appears both from extrapolation and from real exposure of that a Ct to obtain 90% kill with reasonable certainty would need to b 7000 mg min m^{-3} for a 30 minute exposure, or a concentration of approx 230 $\mu g\ \ell^{-1}$.

Appendix 1. Table 1.

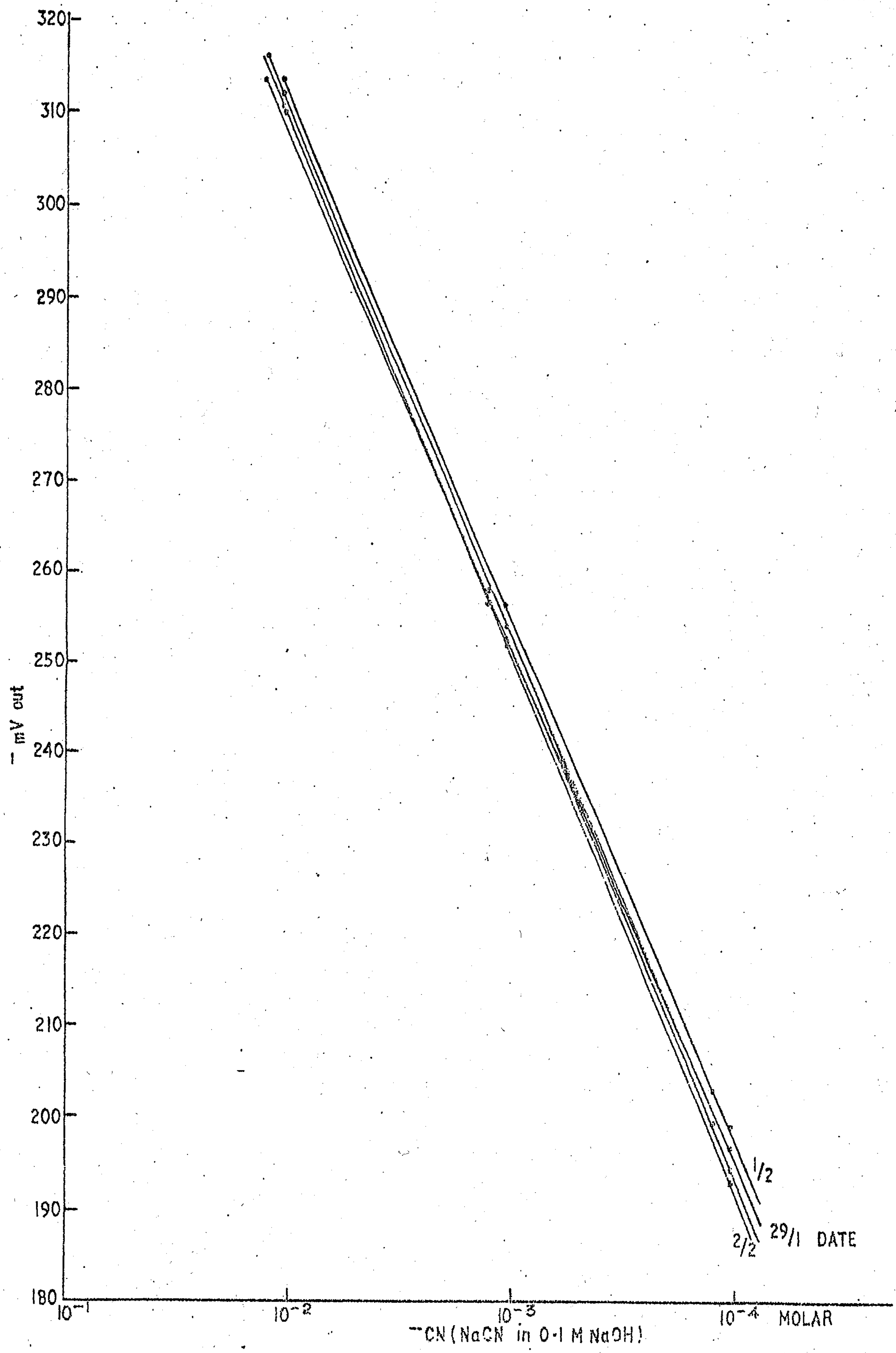
LIST OF FERRET BODY WT (g)

305	541	604	651
424	543	605	654
436	548	612	656
437	548	612	658
438	553	615	660
438	558	615	660
446	561	620	660
458	563	622	664
455	564	625	666
488	565	625	669
490	567	631	672
500	571	633	673
503	575	636	678
503	576	637	679
505	582	639	685
505	585	639	685
506	587	640	685
508	588	644	685
513	592	645	695
514	594	645	695
515	597	645	695
524	598	648	696
530	600	648	697
536	601	649	699
539	603	650	702
710	776	933	1266
713	778	940	1266
715	784	952	
720	786	961	
721	787	963	
722	787	965	
722	794	966	
724	799	972	
724	804	973	
737	807	975	
737	808	977	
741	812	981	
743	817	984	
747	817	987	
752	823	1001	
754	825	1006	
754	829	1009	
756	843	1010	
758	853	1027	
758	855	1040	
759	858	1050	
764	868	1056	
769	884	1058	
770	897	1103	
776	901	1259	



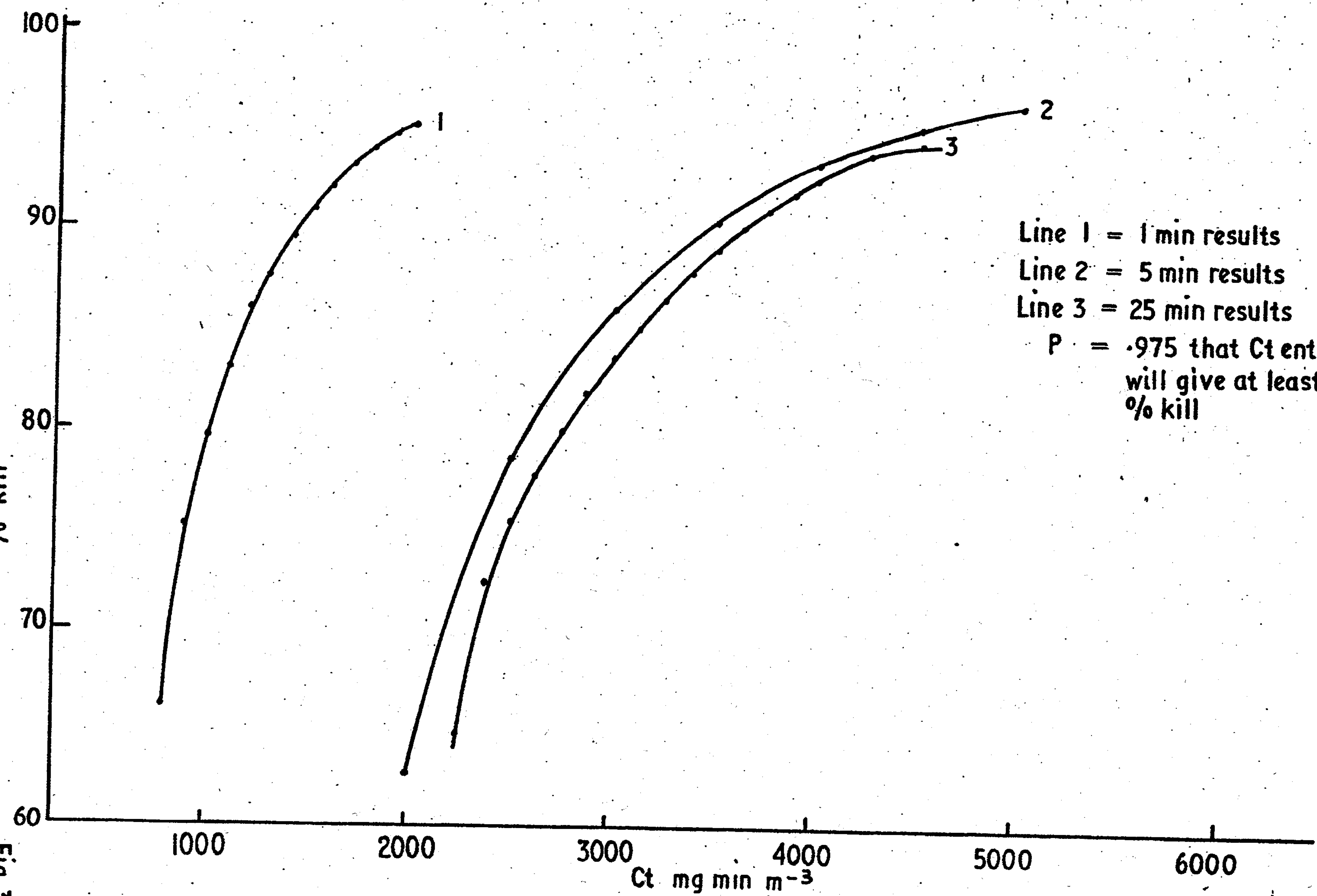
ANIMAL BODY WEIGHTS

Appendix I. Fig. 1



CALIBRATION CURVES FOR CYANIDE ESTIMATION

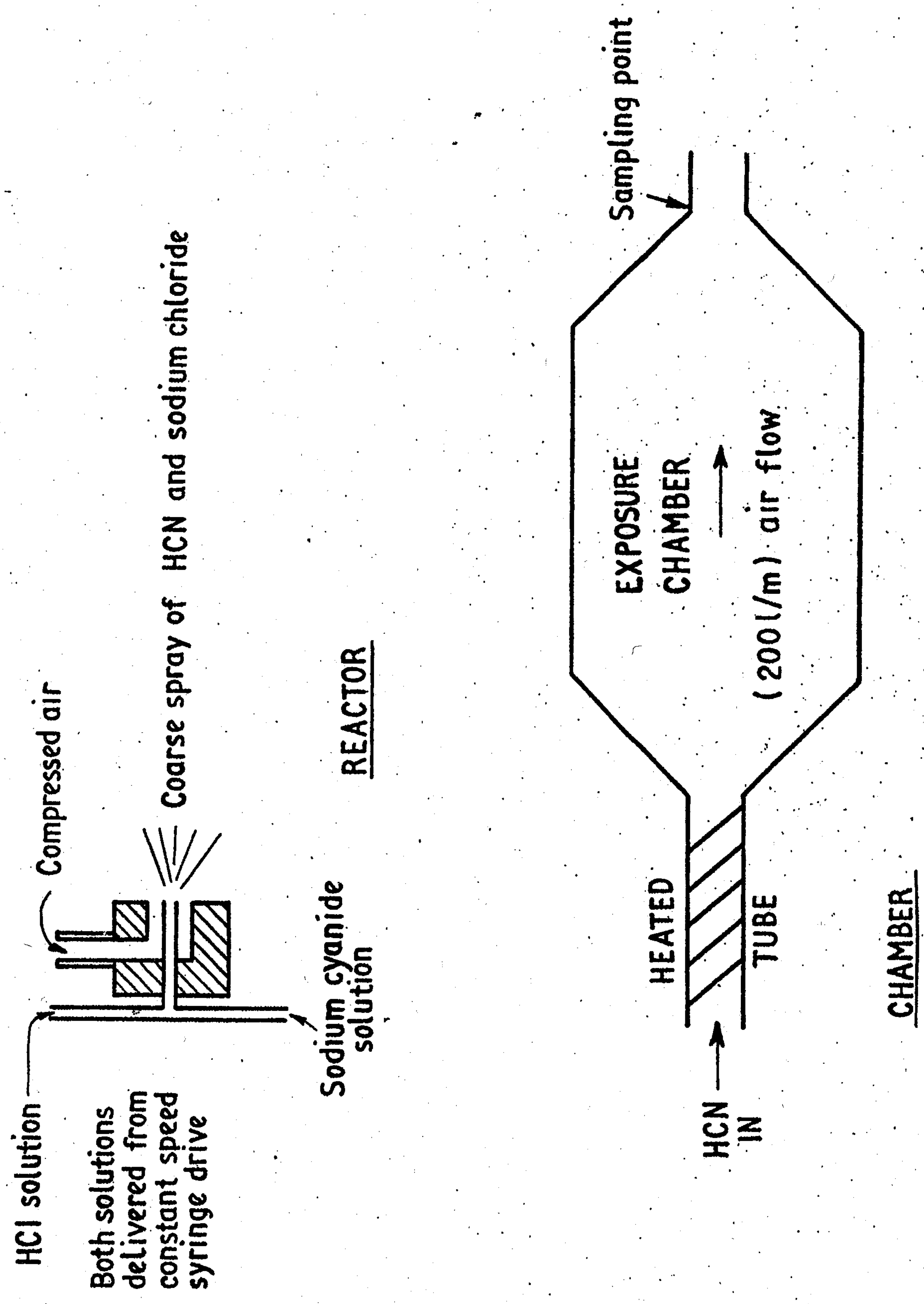
Fig. 2.



MINIMUM PROBABLE KILL FOR VARYING Cts

Fig. 3.

Commercial in Confidence


HCl solution
Both solutions delivered from constant speed syringe drive
Compressed air
Coarse spray of HCN and sodium chloride
Sodium cyanide solution
REACTOR
Sampling point
EXPOSURE CHAMBER
(200 l/m) air flow
HEATED TUBE
HCN IN
CHAMBER

PTA/15/123

THE TOXICITY OF HYDROGEN CYANIDE BY INHALATION TO FERRETS AND BADGERS

Chemical Defence Establishment,
Porton Down, Salisbury, Wilts.

PTA/15/123

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March 1982

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177 female ferrets were obtained from a commercial supplier. Although the animals were especially bred for the contract it was apparent from visual inspection that there were two different groups of animals involved.

This impression was borne out by an examination of all the body weights, Appendix 1, Table 1, from which the histogram, Appendix 2, Fig 1 is derived. It can be seen that there is a significant number of large animals which are outliers, from the main population. The numerical results of a simple analysis were as follows:

From all the animals used:-

Mean body weight	711.2 g
Standard deviation	174.3 g

From the same figures, less the lightest animal and the twenty-seven heaviest ones a new set of statistics was derived. Comparison of the two sets showed that the suspicion of these being a significantly bi-model population was well founded. In particular the values for skewness were:

For all 177 animals	0.754
For the 149 animals	0.005

This abnormality in the experimental animals may account for some of the scatter in the results.

METHODS

Hydrogen cyanide was generated by reacting 3.0 mol ℓ^{-1} sodium cyanide in 0.1 mol ℓ^{-1} sodium hydroxide with 3.1 mol ℓ^{-1} hydrochloric acid. All reagents used were of analytical reagent grade. See Fig 1.

As may be seen from the diagram the two solutions were supplied at a consta rate by a common syringe drive. The resultant mix of HCN, sodium chloride and water was sprayed into the exposure air flow through a heated tube into the exposure chamber of approximately 125 litres capacity. Because of the particle size of the salt spray the droplets evaporated and precipitated within the heated portion of the tube. The air flow through the chamber was monitored using the pressure drop across a calibrated orifice plate and was set at 200 ℓ min^{-1}.

Sampling of the atmosphere was carried out by drawing gas concentrations from the chamber, for the period of complete exposures, through bubblers containing 0.1 mol ℓ^{-1} sodium hydroxide at a nominal rate of 1.0 ℓ min^{-1}. The individual sampling flow rates were measured and used in calculating the concentrations. Estimation of the cyanide in the bubbler was performed using a CN^- specific ion electrode, referring the results to a calibration curve prepared freshly each day, Fig 2. Recoveries of HCN varied between 90-95% of theory. Animals were exposed in individual cages, using groups of three animals for each exposure. Twenty groups of three animals were used for the one and five minute exposures, nineteen groups for the twenty-five minute ones.

Statistical analyses of the results were carried out using methods described generally in 'Probit Analysis' by D J Finney, 3rd edition, 1971 and more particularly in Sections 4.6, 4.7 and 10.2.

RESULTS

The results are listed as Tables 1, 2 and 3, referring to one, five and twenty-five minute runs respectively.

The entry headings are to be read as, body weight in grammes, Ct in concentration time, that is mg min m^{-3} - this may be divided by the 'time' in minutes to give the equivalent concentration in mg m^{-3} or µg ℓ^{-1}. The 24 h mortality is deaths from the three animals exposed in each group.

TABLE 1

RESULTS FROM ONE MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
1	817 576 660	1158	3
2	794 981 1040	919	3
3	1010 1009 758	618	1
4	804 601 752	618	1
5	715 965 868	786	3
6	696 548 636	707	1
7	741 639 644	751	2
8	884 615 784	833	1
9	685 776 743	833	3
10	615 305 961	813	2
11	530 424 612	760	3
12	697 843 679	786	2
13	561 541 664	898	3
14	505 585 574	723	3
15	695 450 817	778	2
16	651 539 685	794	1
17	490 524 695	798	3
18	673 564 575	848	2
19	648 500 622	767	2
20	754 829 592	763	3

TABLE 2

RESULTS FROM FIVE MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
1	575 1050 776	3806	3
2	808 604 702	3282	3
3	558 1027 720	2396	3
4	600 778 858	2034	1
5	987 747 933	1541	2
6	897 973 853	1129	0
7	770 756 799	1510	2
8	975 588 984	1575	1
9	637 625 1058	1313	1
10	963 823 1006	1968	2
11	536 972 669	2000	3
12	940 952 1001	2034	1
13	1259 1266 1103	2199	3
14	787 724 645	2199	3
15	710 722 654	2131	1
16	678 977 666	2100	3
17	685 858 722	2131	3
18	758 1266 901	2131	2
19	724 1056 446	2100	3
20	787 786 966	2165	2

TABLE 3

RESULTS FROM 25 MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
1	812, 633, 594	2014	1
2	505, 721, 605	2535	2
3	553, 620, 563	2980	3
4	488, 625, 543	2951	2
5	695, 764, 650	2988	3
6	685, 438, 825	2320	3
7	672, 438, 759	2427	3
8	565, 658, 455	2320	3
9	436, 571, 503	2298	3
10	603, 645, 713	2500	3

Run No	Body Wts in g	Ct	24 morta
11	437, 503, 506	2427	3
12	640, 656, 573	2157	3
13	639, 645, 508	2253	2
14	631, 807, 612	2342	3
15	548, 582, 587	2838	3
16	737, 567, 754	2689	3
17	660, 699, 737	2155	[illegible]
18	649, 598, 660	2120	[illegible]
19	769, 597, 648	2095	[illegible]

The respective mean and standard deviation for the animal body weights were:

	Mean wt g	SD g
1 minute	685	155
5 minutes	743	186
25 minutes	620	99

The results for the LCt_{50} and LCt_{90} with their 95% fiducial limits were:

	LCt_{50}	Limits	LCt_{90}	Limits
1 minute	670	360 → 730	910	820 → 1910
5 minutes	1600	1210 → 1800	2390	2090 → 3450
25 minutes	1740	1440 → 2109	2600	2142 → 3156

Cts to be read as milligram minutes per cubic metre.

The equations for the regression lines for the dose-response curves are:

1 minute $Y - 5.579 = 9.666\ (x - 2.885)$

5 minutes $Y - 5.512 = 7.382\ (x - 3.273)$

25 minutes $Y - 5.967 = 7.315\ (x - 3.372)$

where Y is the probit of the desired % response and x is the log of Ct.

More importantly, the limits on the percentage kill have been estimated for any particular dose, whereas the classical probit analysis results give the limits for the dose for any percentage kill. These limits allow one to postulate the dose required to 'at least' kill any percentage of the population with a probability in this case of P = 0.975.

Exposure Time	80% ←	minimum kill	→ 90%
1 minute	1025)	mg min m^{-3}	(1450
5 minutes	2575)		(3450
25 minutes	2775)		(3625

Full plots of the values of the minimum probable kill versus Ct are in Fig 3.

PART 2 EXPOSURE OF BADGERS TO HCN

Animals

Four badgers were available for experiment from the Ministry of Agricultur station at Tangley Place. These were captive wild animals obtained for the programme.

METHODS

Two variants from the ferret equipment obtained. A larger chamber, of approximately 0.3 m^3 volume, was used with an air flow of 1,000 ℓ min^{-1} for the lowest concentrations, down to 250 ℓ min^{-1} for the two highest ones.

An addition, also, was the use of video-recording equipment to obtain a permanent film of the exposures.

Animals were exposed singly.

Exposure No 1. Badger weight 10.7 kg.

This exposure lasted 30 mins, giving a Ct of 2560 mg min m^{-3}, or a concentration of 85 μg $ℓ^{-1}$.

The sequence of events during the exposure was, zero plus;

1 minute	The respiration appeared affected.
3 minutes	Animal was restless.
6 minutes	Showed major signs of intoxication, gasping, vomiting, swaying about.
23 minutes	Collapsed.

On withdrawal from the chamber at approximately zero plus one hour the animal was unconscious. It regained consciousness at about plus 3 hours and was returned to Tangley Place four days later in good health.

Exposure No 2. Badger weight 9.8 kg.

Exposure lasted 30 minutes, giving a Ct of 5575 mg min m^{-3} or a concent of 186 μg $ℓ^{-1}$.

There were no obvious signs until 27 minutes after the beginning of the exposure, then vomiting, gasping, and staggering occurred.

On withdrawal at approximately zero plus one hour after the end of the exposure the animal was semi-conscious, but was returned with badger No 1 i normal condition.

Exposure No 3. Badger weight 8.8 kg.

Exposure lasted 21 minutes. At about 17 minutes from the start of the exposure the badger had ceased breathing and was dead upon withdrawal from the chamber.

Ct = 6560 mg min m^{-3}
Conc. = 312 µg ℓ^{-1}

Exposure No 4. Badger weight 8.8 kg.

Ct = 4020 mg min m^{-3}
Conc. = 335 µg ℓ^{-1}

At 12 minutes from the beginning of the exposure the animal ceased breathing and the gas flow was stopped. Respiration started spontaneously within one to two minutes. The animal was taken back to Tangley Place unwell but recovering.

Video-records of all four exposures and the three immediate post exposure periods are available.

DISCUSSION

The sole reason for using ferrets, which are in good supply, was as an exposure model for badgers, which are not easily available. It is therefore necessary to be able to extrapolate from ferrets to badgers.

The apparent toxicity of hydrogen cyanide by inhalation decreases as the time of exposure lengthens and this difficulty tends to confound the problem. However, it is possible to make some guess as to what should happen when badgers are exposed, and to test whether this is at odds with the short series of experiments.

Starting with the proposition that for any dose rate expressed as mass of hydrogen cyanide/mass of tissue/unit time, the absolute toxicity of cyanide is the same for the two species, then;

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The relationship between body weight and minute volume is not unity, but has the form:

minute volume $\propto$ (body weight)$^{0.7}$

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Using the figures found for the various Cts for ferrets exposed t cyanide, it appears both from extrapolation and from real exposure of that a Ct to obtain 90% kill with reasonable certainty would need to b 7000 mg min m^{-3} for a 30 minute exposure, or a concentration of approx 230 $\mu g\ \ell^{-1}$.

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505	582	639	685
505	585	639	685
506	587	640	685
508	588	644	685
513	592	645	695
514	594	645	695
515	597	645	695
524	598	648	696
530	600	648	697
536	601	649	699
539	603	650	702
710	776	933	1266
713	778	940	1266
715	784	952	
720	786	961	
721	787	963	
722	787	965	
722	794	966	
724	799	972	
724	804	973	
737	807	975	
737	808	977	
741	812	981	
743	817	984	
747	817	987	
752	823	1001	
754	825	1006	
754	829	1009	
756	843	1010	
758	853	1027	
758	855	1040	
759	858	1050	
764	868	1056	
769	884	1058	
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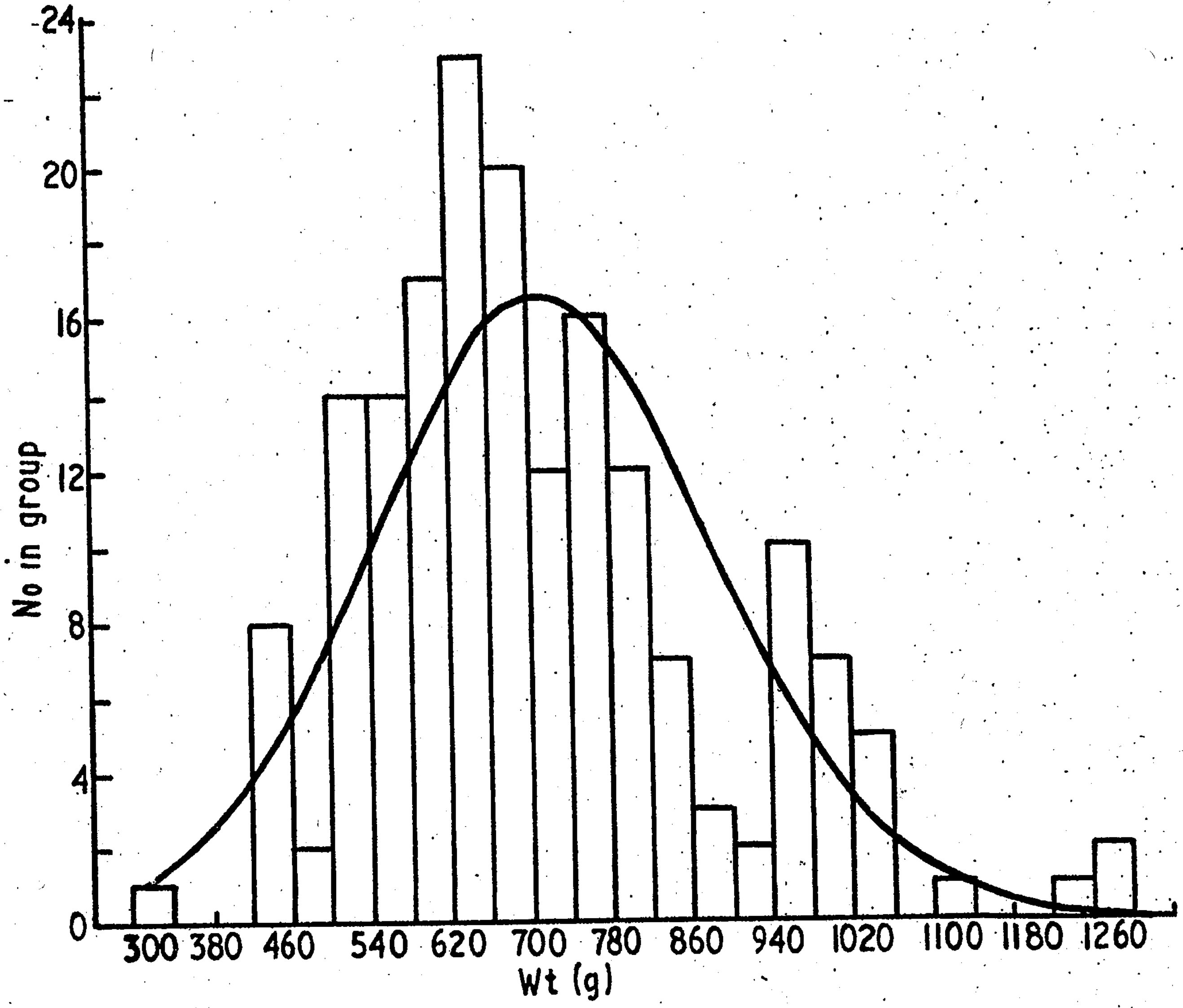

ANIMAL BODY WEIGHTS

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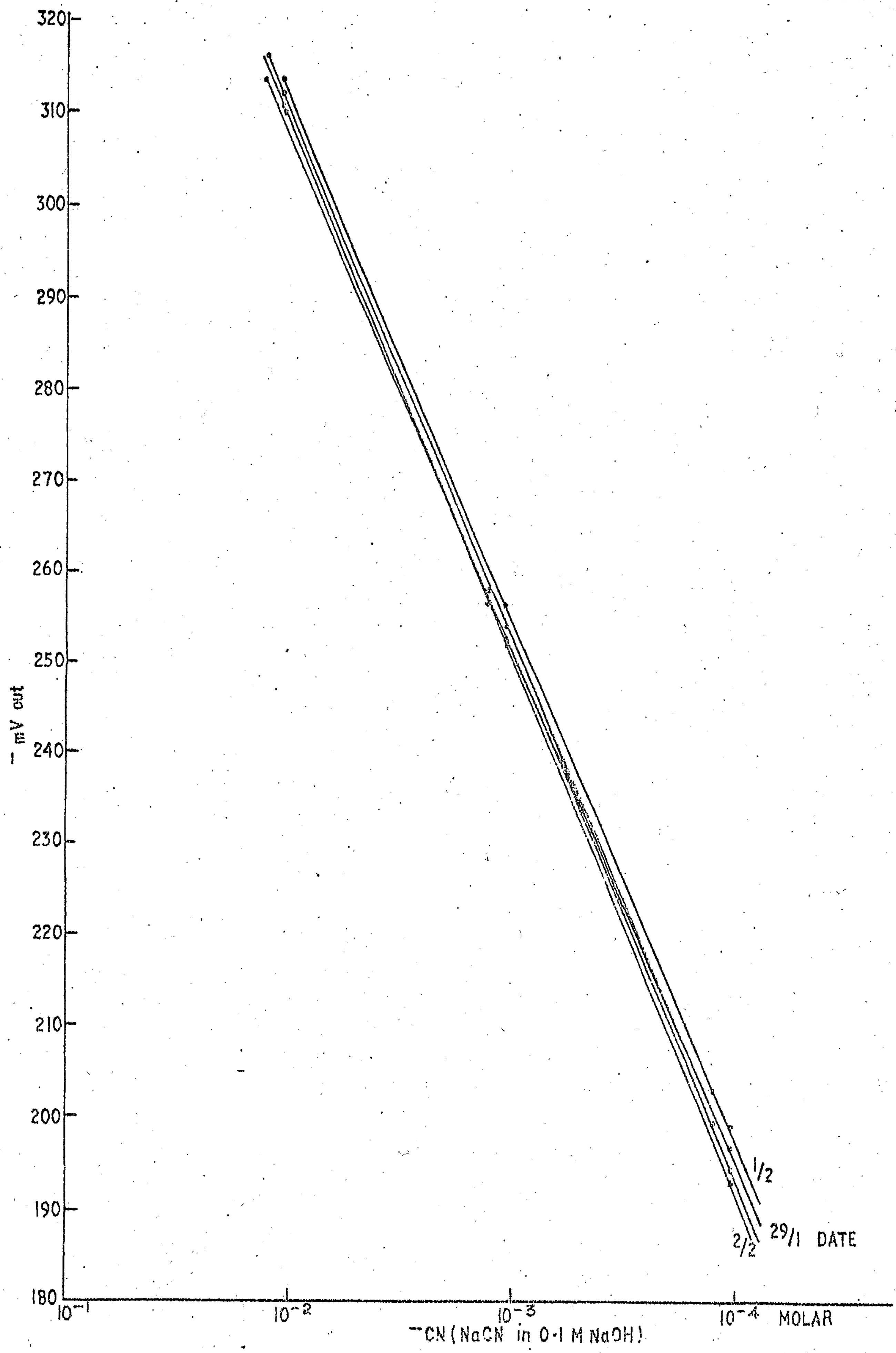
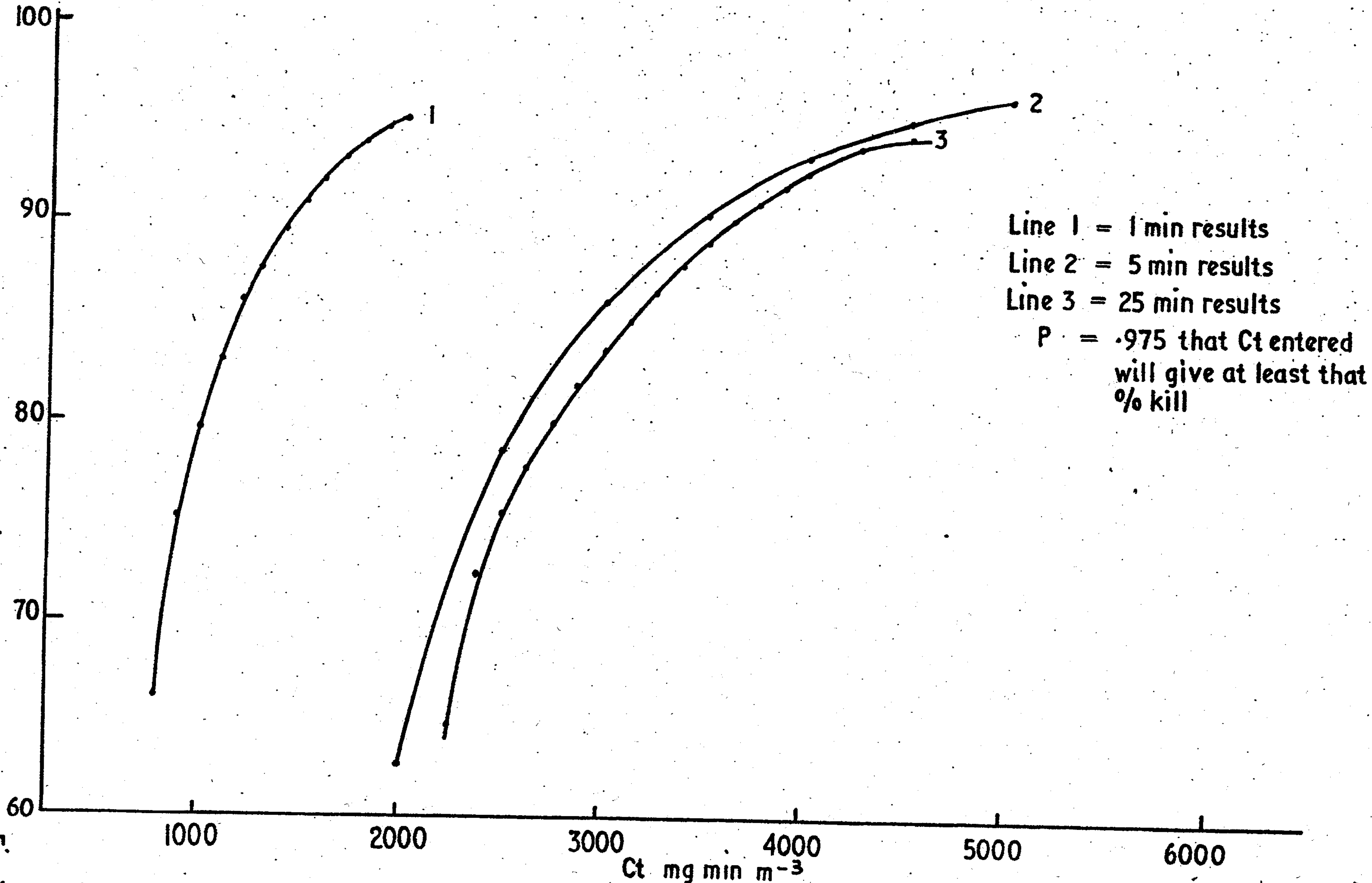


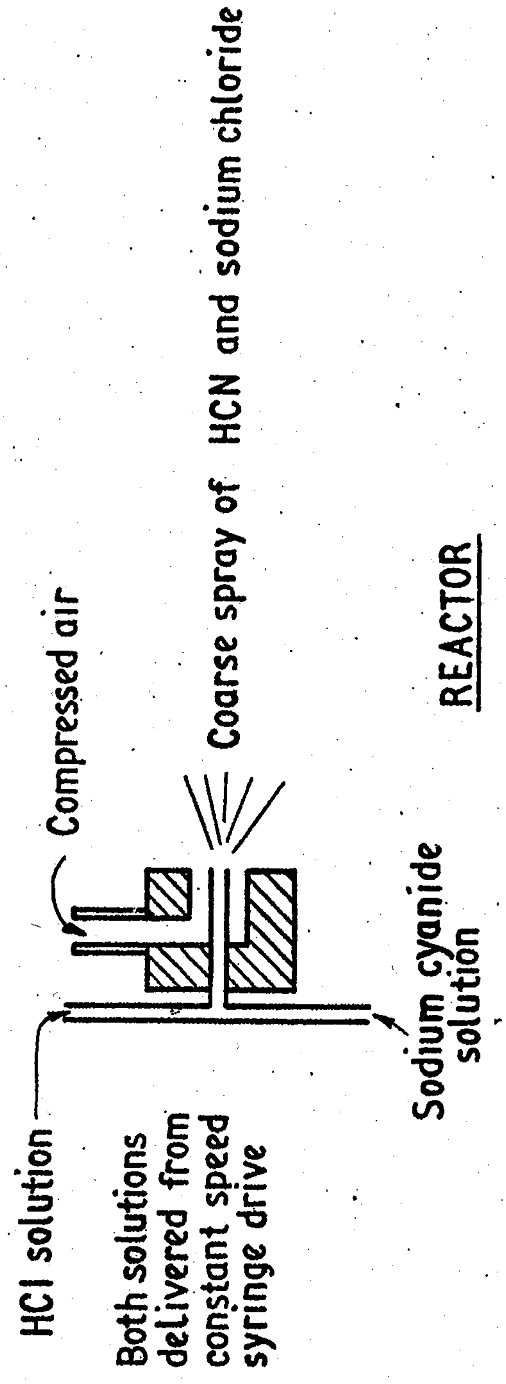
CALIBRATION CURVES FOR CYANIDE ESTIMATION

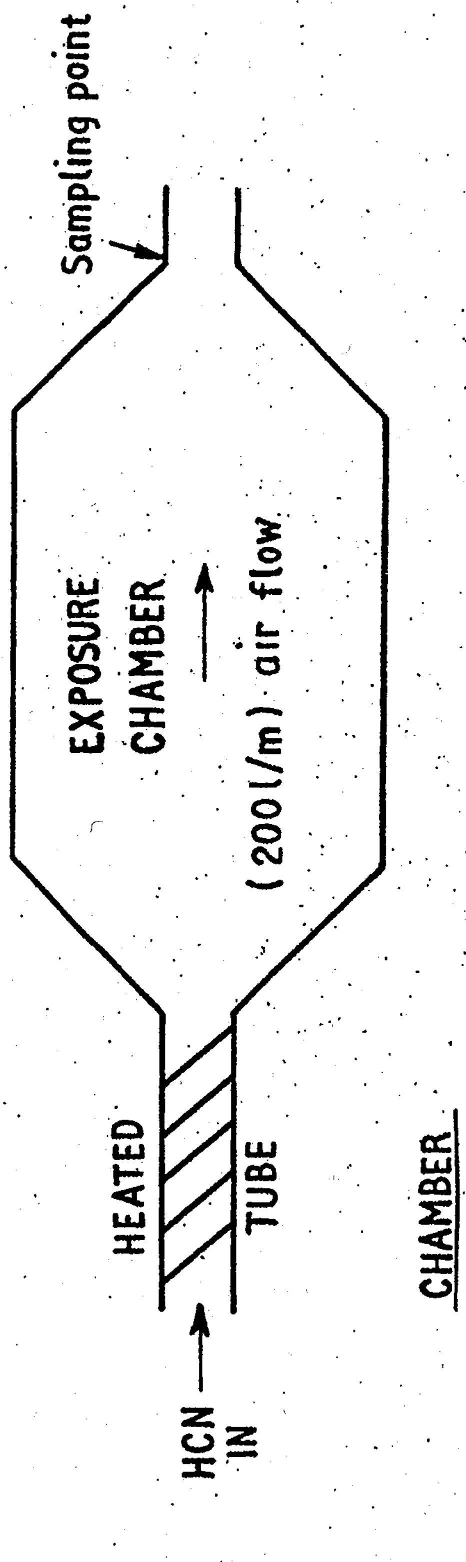
Fig. 2.



MINIMUM PROBABLE KILL FOR VARYING Cts

Fig.3.


HCl solution
Both solutions delivered from constant speed syringe drive
Compressed air
Coarse spray of HCN and sodium chloride
Sodium cyanide solution
REACTOR


HCN IN
HEATED TUBE
EXPOSURE CHAMBER
(200 l/m) air flow
Sampling point
CHAMBER

[redacted]

PTA/15/123

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TABLE 2

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3	558 1027 720	2396	3
4	600 778 858	2034	1
5	987 747 933	1541	2
6	897 973 853	1129	0
7	770 756 799	1510	2
8	975 588 984	1575	1
9	637 625 1058	1313	1
10	963 823 1006	1968	2

Run No	Body Wts in g	Ct	24 h mortality
11	536 972 669	2000	3
12	940 952 1001	2034	1
13	1259 1266 1103	2199	3
14	787 724 645	2199	3
15	710 722 654	2131	1
16	678 977 666	2100	3
17	685 858 722	2131	3
18	758 1266 901	2131	2
19	724 1056 446	2100	3
20	787 786 966	2165	2

TABLE 3

RESULTS FROM 25 MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
1	812 633 594	2014	1
2	505 721 605	2535	2
3	553 620 563	2980	3
4	488 625 543	2951	2
5	695 764 650	2988	3
6	685 438 825	2320	3
7	672 438 759	2427	3
8	565 658 455	2320	3
9	436 571 503	2298	3
10	603 645 713	2500	3

Run No	Body Wts in g	Ct	24 h mortality
11	437 503 506	2427	3
12	640 656 573	2157	3
13	639 645 508	2253	2
14	631 807 612	2342	3
15	548 582 587	2838	3
16	737 567 754	2689	3
17	660 699 737	2155	2
18	649 598 660	2120	[illegible]
19	769 597 648	2095	[illegible]

The respective mean and standard deviation for the animal body weights were:

	Mean wt g	SD g
1 minute	685	155
5 minutes	743	186
25 minutes	620	99

The results for the LCt_{50} and LCt_{90} with their 95% fiducial limits were:

	LCt_{50}	Limits	LCt_{90}	Limits
1 minute	670	360 → 730	910	820 → 1910
5 minutes	1600	1210 → 1800	2390	2090 → 3450
25 minutes	1740	1440 → 2109	2600	2142 → 3156

Cts to be read as milligram minutes per cubic metre.

The equations for the regression lines for the dose-response curves are:

1 minute $Y - 5.579 = 9.666\,(x - 2.885)$

5 minutes $Y - 5.512 = 7.382\,(x - 3.273)$

25 minutes $Y - 5.967 = 7.315\,(x - 3.372)$

where Y is the probit of the desired % response and x is the log of Ct.

More importantly, the limits on the percentage kill have been estimated for any particular dose, whereas the classical probit analysis results give the limits for the dose for any percentage kill. These limits allow one to postulate the dose required to 'at least' kill any percentage of the population with a probability in this case of P = 0.975.

Exposure Time	80% ← minimum kill → 90%		
1 minute	1025)	$mg\ min\ m^{-3}$	(1450
5 minutes	2575)		(3450
25 minutes	2775)		(3625

Full plots of the values of the minimum probable kill versus Ct are in Fig 3.

PART 2 EXPOSURE OF BADGERS TO HCN

Animals

Four badgers were available for experiment from the Ministry of Agricultur station at Tangley Place. These were captive wild animals obtained for the programme.

METHODS

Two variants from the ferret equipment obtained. A larger chamber, of approximately 0.3 m^3 volume, was used with an air flow of 1,000 $\ell\ min^{-1}$ for the lowest concentrations, down to 250 $\ell\ min^{-1}$ for the two highest ones.

An addition, also, was the use of video-recording equipment to obtain a permanent film of the exposures.

Animals were exposed singly.

Exposure No 1. Badger weight 10.7 kg.

This exposure lasted 30 mins, giving a Ct of 2560 mg min m^{-3}, or a concentration of 85 $\mu g\ \ell^{-1}$.

The sequence of events during the exposure was, zero plus;

1 minute	The respiration appeared affected.
3 minutes	Animal was restless.
6 minutes	Showed major signs of intoxication, gasping, vomiting, swaying about.
23 minutes	Collapsed.

On withdrawal from the chamber at approximately zero plus one hour the animal was unconscious. It regained consciousness at about plus 3 hours and was returned to Tangley Place four days later in good health.

Exposure No 2. Badger weight 9.8 kg.

Exposure lasted 30 minutes, giving a Ct of 5575 mg min m^{-3} or a concent of 186 $\mu g\ \ell^{-1}$.

There were no obvious signs until 27 minutes after the beginning of the exposure, then vomiting, gasping, and staggering occurred.

On withdrawal at approximately zero plus one hour after the end of the exposure the animal was semi-conscious, but was returned with badger No 1 i normal condition.

Exposure No 3. Badger weight 8.8 kg.

Exposure lasted 21 minutes. At about 17 minutes from the start of the exposure the badger had ceased breathing and was dead upon withdrawal from the chamber.

Ct = 6560 mg min m^{-3}
Conc. = 312 µg ℓ^{-1}

Exposure No 4. Badger weight 8.8 kg.

Ct = 4020 mg min m^{-3}
Conc. = 335 µg ℓ^{-1}

At 12 minutes from the beginning of the exposure the animal ceased breathing and the gas flow was stopped. Respiration started spontaneously within one to two minutes. The animal was taken back to Tangley Place unwell but recovering.

Video-records of all four exposures and the three immediate post exposure periods are available.

DISCUSSION

The sole reason for using ferrets, which are in good supply, was as an exposure model for badgers, which are not easily available. It is therefore necessary to be able to extrapolate from ferrets to badgers.

The apparent toxicity of hydrogen cyanide by inhalation decreases as the time of exposure lengthens and this difficulty tends to confound the problem. However, it is possible to make some guess as to what should happen when badgers are exposed, and to test whether this is at odds with the short series of experiments.

Starting with the proposition that for any dose rate expressed as mass of hydrogen cyanide/mass of tissue/unit time, the absolute toxicity of cyanide is the same for the two species, then;

1. The dose/kg for any one exposure time will be governed by the volume inhaled/unit time, ie the respiratory minute volume.

2. The apparent difference in toxicity will be due to any differences in respiratory minute volume in the two species.

The relationship between body weight and minute volume is not unity, but has the form:

$$\text{minute volume} \propto (\text{body weight})^{0.7}$$

(Rubner, M. 1883.
Über den Einfluss der Körpergrösse auf Stoff - und Kraftwechs
Z. Biol., 19, 535 - 62.)

The mean weight of the badgers was 10 kilogrammes whilst that of
was 700 grammes, a ratio of 14:1, this predicates a minute volume rat
approximately 6.5:1. The badger will, therefore, need to inspire a cc
twice as high as a ferret to maintain the same inhaled dose/kilogramme

Because of the non-linear relationship between Ct and time of exp
is not possible to say that a badger would survive for twice the time
inhaling a similar concentration to a ferret.

Taking that the probable 90% kill for 5 minutes in ferrets is 345
and for 25 minutes is 3625, the equivalent figures for badgers would b
7500.

Similarly the LCt_{50} and LCt_{90} may also be multiplied.

	Ferrets			Badgers	
Time	LCt_{50}	LCt_{90}		LCt_{50}	LCt_{90}
1 min	670	910		1300	2000
25 min	1600	2390	approximately	3000	5000
25 min	1740	2600		3500	5500

In fact for two 30 minutes exposures at 2560 and 5575 Ct units re
both badgers survived.

At a 17 minute exposure with a Ct of 6560 one badger died, and on
survived a Ct of 4000 over 10 minutes. These results support the very
figures in the right hand column.

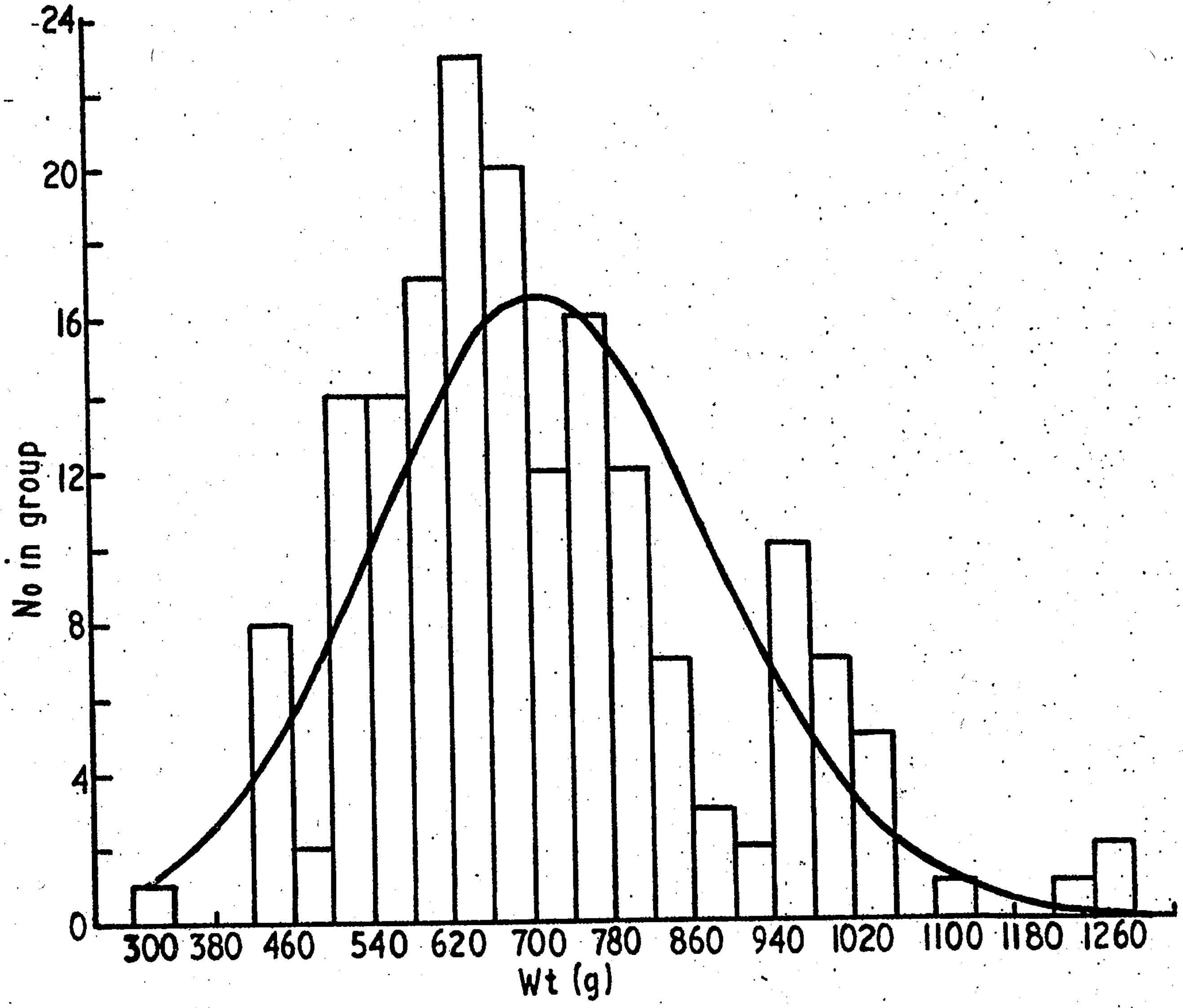
CONCLUSIONS

Using the figures found for the various Cts for ferrets exposed t
cyanide, it appears both from extrapolation and from real exposure of
that a Ct to obtain 90% kill with reasonable certainty would need to b
7000 mg min m^{-3} for a 30 minute exposure, or a concentration of approx
230 $\mu g\ \ell^{-1}$.

Appendix 1. Table 1.

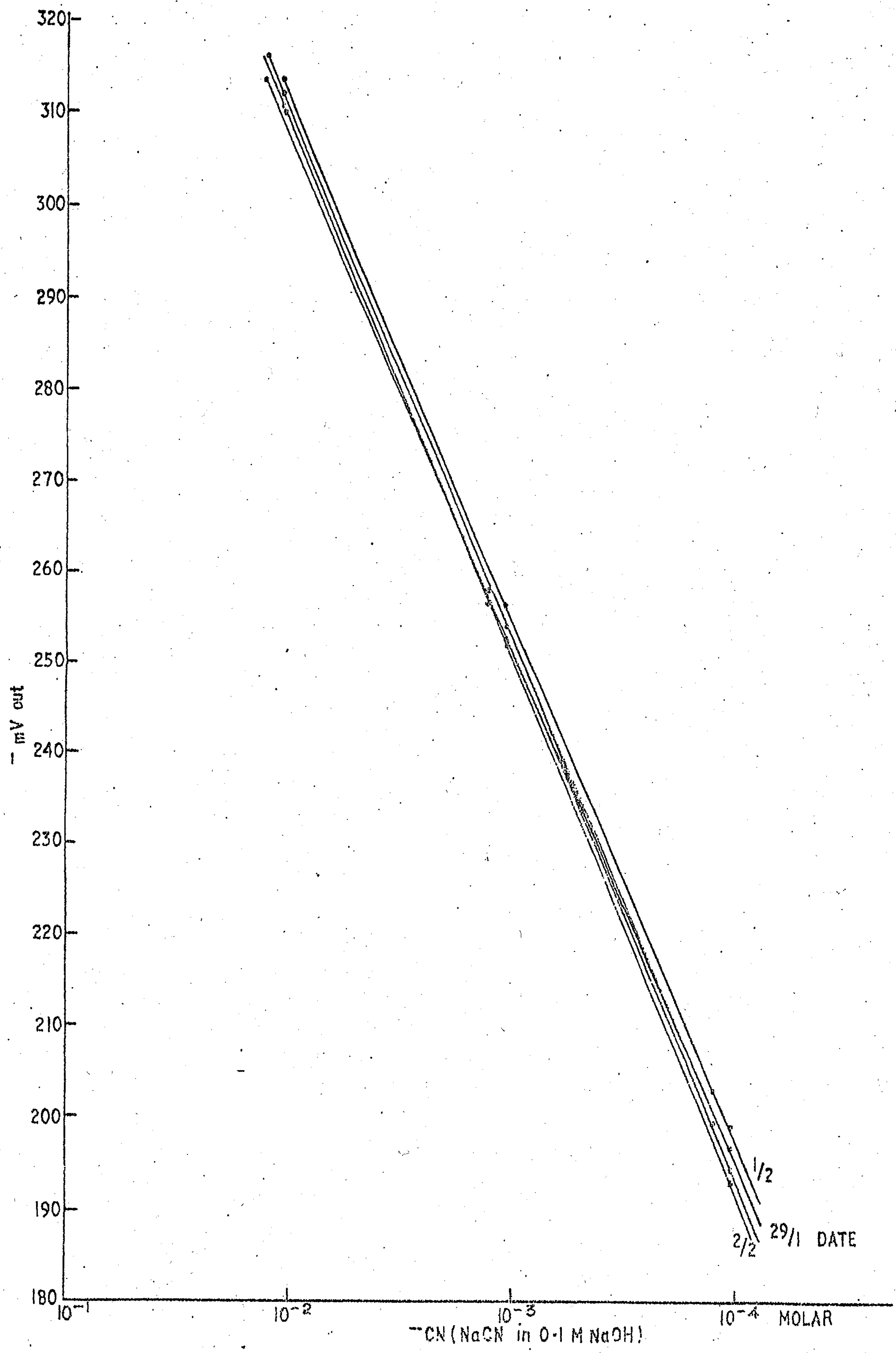
LIST OF FERRET BODY WT (g)

305	541	604	651
424	543	605	654
436	548	612	656
437	548	612	658
438	553	615	660
438	558	615	660
446	561	620	660
458	563	622	664
455	564	625	666
488	565	625	669
490	567	631	672
500	571	633	673
503	575	636	678
503	576	637	679
505	582	639	685
505	585	639	685
506	587	640	685
508	588	644	685
513	592	645	695
514	594	645	695
515	597	645	695
524	598	648	696
530	600	648	697
536	601	649	699
539	603	650	702
710	776	933	1266
713	778	940	1266
715	784	952	
720	786	961	
721	787	963	
722	787	965	
722	794	966	
724	799	972	
724	804	973	
737	807	975	
737	808	977	
741	812	981	
743	817	984	
747	817	987	
752	823	1001	
754	825	1006	
754	829	1009	
756	843	1010	
758	853	1027	
758	855	1040	
759	858	1050	
764	868	1056	
769	884	1058	
770	897	1103	
776	901	1259	



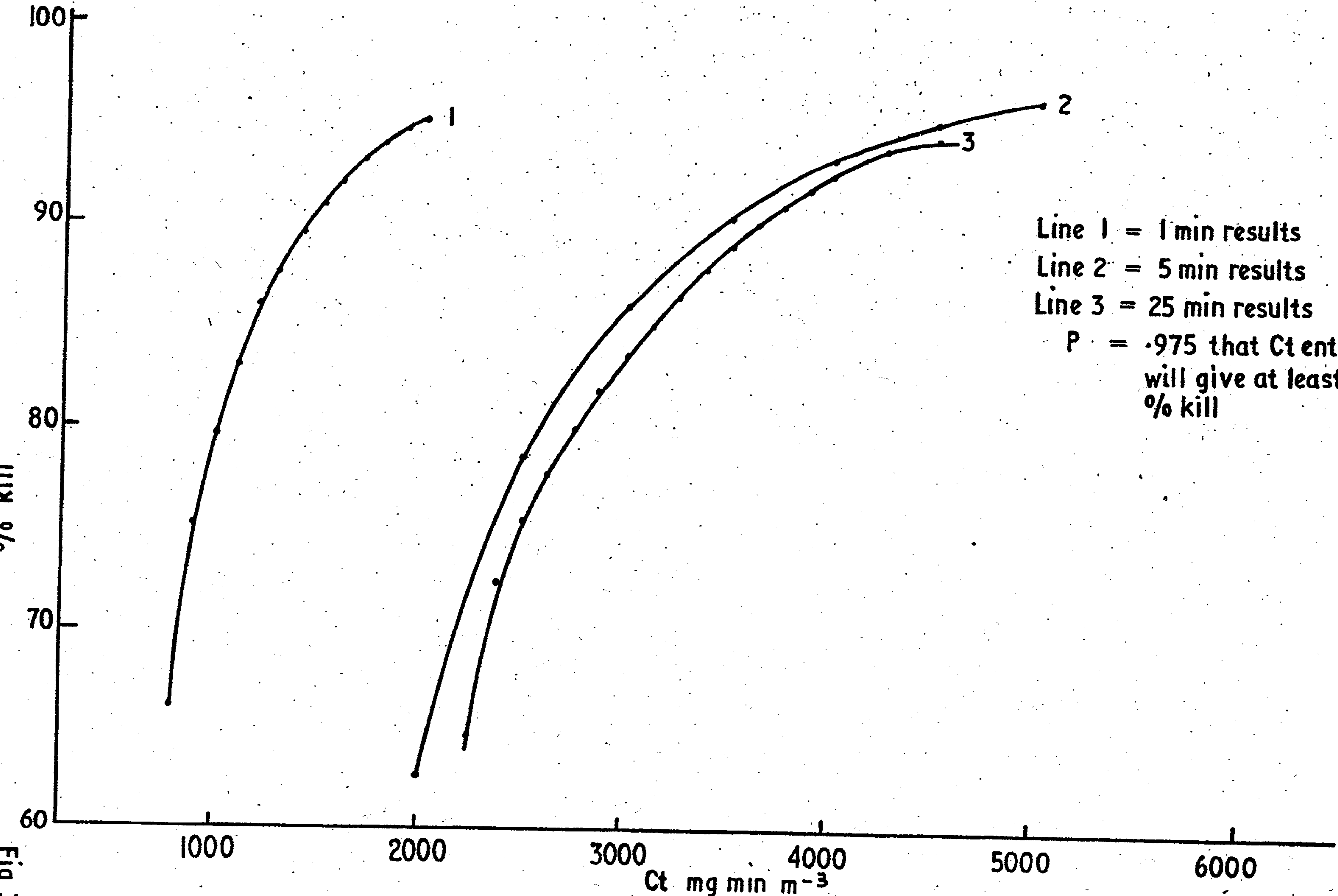
ANIMAL BODY WEIGHTS

Appendix I. Fig. 1



CALIBRATION CURVES FOR CYANIDE ESTIMATION

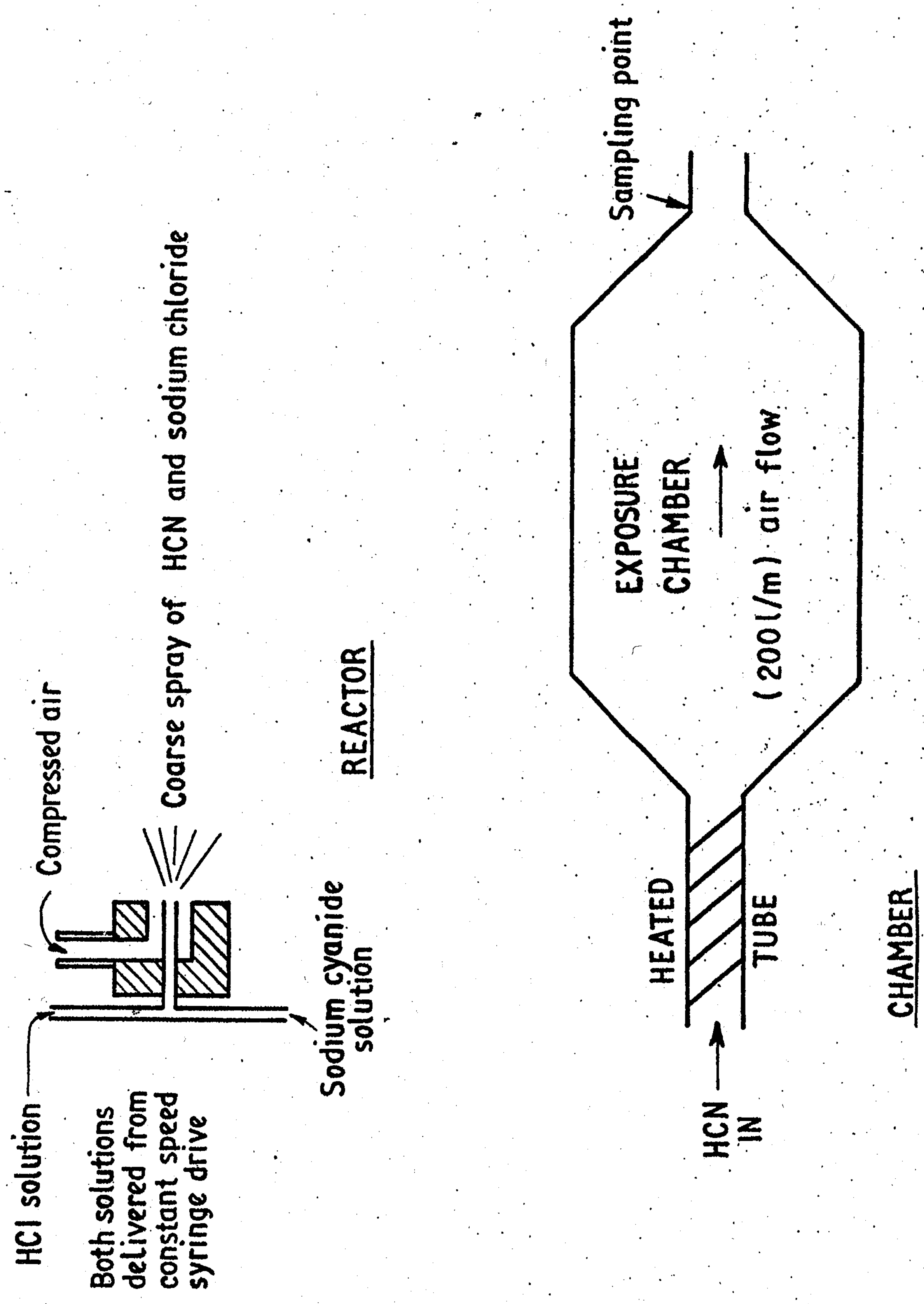
Fig. 2.



MINIMUM PROBABLE KILL FOR VARYING Cts

Fig.3.

Commercial in Confidence


HCl solution
Both solutions delivered from constant speed syringe drive
Compressed air
Coarse spray of HCN and sodium chloride
Sodium cyanide solution
REACTOR
HCN IN
HEATED TUBE
EXPOSURE CHAMBER
(200 l/m) air flow
Sampling point
CHAMBER

[redacted]

COMMERCIAL-IN-CONFIDENCE

PTA/15/123

CAVE

CDE

THE TOXICITY OF HYDROGEN CYANIDE BY INHALATION TO FERRETS AND BADGERS

Chemical Defence Establishment,
Porton Down, Salisbury, Wilts.

COMMERCIAL-IN-CONFIDENCE

PTA/15/123

THE TOXICITY OF HYDROGEN CYANIDE BY INHALATION TO FERRETS AND BADGERS

INTRODUCTION

In order to give some idea of the toxicity of hydrogen cyanide to badgers and the 'humaneness' of this method of killing when badgers are gassed in the tuberculosis eradication campaign, CDE has been asked to carry out some experiments on these animals. Because of the scarcity of badgers, it was suggested by Lord Zuckerman that ferrets be used as a model for the quantitative toxicity testing. What follows is a report which in Part 1 defines the LCt_{50}, LCt_{90} and minimum concentrations to kill 80% and 90% of ferrets exposed, and in Part 2 describes the short series of badgers exposed to HCN and the extrapolation of the ferret results to badgers.

March 1982

PART 1 EXPOSURE OF FERRETS TO HCN

Animals

177 female ferrets were obtained from a commercial supplier. Although the animals were especially bred for the contract it was apparent from visual inspection that there were two different groups of animals involved.

This impression was borne out by an examination of all the body weights, Appendix 1, Table 1, from which the histogram, Appendix 2, Fig 1 is derived. It can be seen that there is a significant number of large animals which are outliers, from the main population. The numerical results of a simple analysis were as follows:

From all the animals used:-

Mean body weight	711.2 g
Standard deviation	174.3 g

From the same figures, less the lightest animal and the twenty-seven heaviest ones a new set of statistics was derived. Comparison of the two sets showed that the suspicion of these being a significantly bi-model population was well founded. In particular the values for skewness were:

For all 177 animals	0.754
For the 149 animals	0.005

This abnormality in the experimental animals may account for some of the scatter in the results.

METHODS

Hydrogen cyanide was generated by reacting 3.0 mol ℓ^{-1} sodium cyanide in 0.1 mol ℓ^{-1} sodium hydroxide with 3.1 mol ℓ^{-1} hydrochloric acid. All reagents used were of analytical reagent grade. See Fig 1.

As may be seen from the diagram the two solutions were supplied at a consta rate by a common syringe drive. The resultant mix of HCN, sodium chloride and water was sprayed into the exposure air flow through a heated tube into the exposure chamber of approximately 125 litres capacity. Because of the particle size of the salt spray the droplets evaporated and precipitated within the heated portion of the tube. The air flow through the chamber was monitored using the pressure drop across a calibrated orifice plate and was set at 200 ℓ min^{-1}.

Sampling of the atmosphere was carried out by drawing gas concentrations from the chamber, for the period of complete exposures, through bubblers containing 0.1 mol ℓ^{-1} sodium hydroxide at a nominal rate of 1.0 ℓ min^{-1}. The individual sampling flow rates were measured and used in calculating the concentrations. Estimation of the cyanide in the bubbler was performed using a CN^- specific ion electrode, referring the results to a calibration curve prepared freshly each day, Fig 2. Recoveries of HCN varied between 90-95% of theory. Animals were exposed in individual cages, using groups of three animals for each exposure. Twenty groups of three animals were used for the one and five minute exposures, nineteen groups for the twenty-five minute ones.

Statistical analyses of the results were carried out using methods described generally in 'Probit Analysis' by D J Finney, 3rd edition, 1971 and more particularly in Sections 4.6, 4.7 and 10.2.

RESULTS

The results are listed as Tables 1, 2 and 3, referring to one, five and twenty-five minute runs respectively.

The entry headings are to be read as, body weight in grammes, Ct in concentration time, that is mg min m^{-3} - this may be divided by the 'time' in minutes to give the equivalent concentration in mg m^{-3} or μg ℓ^{-1}. The 24 h mortality is deaths from the three animals exposed in each group.

TABLE 1

RESULTS FROM ONE MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
1	817	1158	3
	576		
	660		
2	794	919	3
	981		
	1040		
3	1010	618	1
	1009		
	758		
4	804	618	1
	601		
	752		
5	715	786	3
	965		
	868		
6	696	707	1
	548		
	636		
7	741	751	2
	639		
	644		
8	884	833	1
	615		
	784		
9	685	833	3
	776		
	743		
10	615	813	2
	305		
	961		
11	530	760	3
	424		
	612		
12	697	786	2
	843		
	679		
13	561	898	3
	541		
	664		
14	505	723	3
	585		
	574		
15	695	778	2
	450		
	817		
16	651	794	1
	539		
	685		
17	490	798	3
	524		
	695		
18	673	848	2
	564		
	575		
19	648	767	2
	500		
	622		
20	754	763	3
	829		
	592		

TABLE 2

RESULTS FROM FIVE MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
1	575 1050 776	3806	3
2	808 604 702	3282	3
3	558 1027 720	2396	3
4	600 778 858	2034	1
5	987 747 933	1541	2
6	897 973 853	1129	0
7	770 756 799	1510	2
8	975 588 984	1575	1
9	637 625 1058	1313	1
10	963 823 1006	1968	2
11	536 972 669	2000	3
12	940 952 1001	2034	1
13	1259 1266 1103	2199	3
14	787 724 645	2199	3
15	710 722 654	2131	1
16	678 977 666	2100	3
17	685 858 722	2131	3
18	758 1266 901	2131	2
19	724 1056 446	2100	3
20	787 786 966	2165	2

TABLE 3

RESULTS FROM 25 MINUTE EXPOSURES OF FERRETS TO HCN

Run No	Body Wts in g	Ct	24 h mortality
1	812	2014	1
	633		
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2	505	2535	2
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	605		
3	553	2980	3
	620		
	563		
4	488	2951	2
	625		
	543		
5	695	2988	3
	764		
	650		
6	685	2320	3
	438		
	825		
7	672	2427	3
	438		
	759		
8	565	2320	3
	658		
	455		
9	436	2298	3
	571		
	503		
10	603	2500	3
	645		
	713		

Run No	Body Wts in g	Ct	24 morta
11	437	2427	[illegible]
	503		
	506		
12	640	2157	[illegible]
	656		
	573		
13	639	2253	[illegible]
	645		
	508		
14	631	2342	[illegible]
	807		
	612		
15	548	2838	[illegible]
	582		
	587		
16	737	2689	[illegible]
	567		
	754		
17	660	2155	[illegible]
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The respective mean and standard deviation for the animal body weights were:

	Mean wt g	SD g
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The results for the LCt_{50} and LCt_{90} with their 95% fiducial limits were:

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Cts to be read as milligram minutes per cubic metre.

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Exposure Time	80% ← minimum kill		→ 90%
1 minute	1025)	$mg\ min\ m^{-3}$	(1450
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25 minutes	2775)		(3625

Full plots of the values of the minimum probable kill versus Ct are in Fig 3.

PART 2 EXPOSURE OF BADGERS TO HCN

Animals

Four badgers were available for experiment from the Ministry of Agricultur station at Tangley Place. These were captive wild animals obtained for the programme.

METHODS

Two variants from the ferret equipment obtained. A larger chamber, of approximately 0.3 m^3 volume, was used with an air flow of 1,000 $\ell\ min^{-1}$ for the lowest concentrations, down to 250 $\ell\ min^{-1}$ for the two highest ones.

An addition, also, was the use of video-recording equipment to obtain a permanent film of the exposures.

Animals were exposed singly.

Exposure No 1. Badger weight 10.7 kg.

This exposure lasted 30 mins, giving a Ct of 2560 mg min m^{-3}, or a concentration of 85 $\mu g\ \ell^{-1}$.

The sequence of events during the exposure was, zero plus;

1 minute	The respiration appeared affected.
3 minutes	Animal was restless.
6 minutes	Showed major signs of intoxication, gasping, vomiting, swaying about.
23 minutes	Collapsed.

On withdrawal from the chamber at approximately zero plus one hour the animal was unconscious. It regained consciousness at about plus 3 hours and was returned to Tangley Place four days later in good health.

Exposure No 2. Badger weight 9.8 kg.

Exposure lasted 30 minutes, giving a Ct of 5575 mg min m^{-3} or a concent of 186 $\mu g\ \ell^{-1}$.

There were no obvious signs until 27 minutes after the beginning of the exposure, then vomiting, gasping, and staggering occurred.

On withdrawal at approximately zero plus one hour after the end of the exposure the animal was semi-conscious, but was returned with badger No 1 i normal condition.

Exposure No 3. Badger weight 8.8 kg.

Exposure lasted 21 minutes. At about 17 minutes from the start of the exposure the badger had ceased breathing and was dead upon withdrawal from the chamber.

Ct = 6560 mg min m^{-3}
Conc. = 312 μg ℓ^{-1}

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At 12 minutes from the beginning of the exposure the animal ceased breathing and the gas flow was stopped. Respiration started spontaneously within one to two minutes. The animal was taken back to Tangley Place unwell but recovering.

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DISCUSSION

The sole reason for using ferrets, which are in good supply, was as an exposure model for badgers, which are not easily available. It is therefore necessary to be able to extrapolate from ferrets to badgers.

The apparent toxicity of hydrogen cyanide by inhalation decreases as the time of exposure lengthens and this difficulty tends to confound the problem. However, it is possible to make some guess as to what should happen when badgers are exposed, and to test whether this is at odds with the short series of experiments.

Starting with the proposition that for any dose rate expressed as mass of hydrogen cyanide/mass of tissue/unit time, the absolute toxicity of cyanide is the same for the two species, then;

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The relationship between body weight and minute volume is not unity, but has the form:

minute volume $\propto$ (body weight)$^{0\cdot 7}$

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Über den Einfluss der Körpergrösse auf Stoff - und Kraftwechs

Z. Biol., 19, 535 - 62.)

The mean weight of the badgers was 10 kilogrammes whilst that of was 700 grammes, a ratio of 14:1, this predicates a minute volume rat approximately 6.5:1. The badger will, therefore, need to inspire a cc twice as high as a ferret to maintain the same inhaled dose/kilogramme

Because of the non-linear relationship between Ct and time of exp is not possible to say that a badger would survive for twice the time inhaling a similar concentration to a ferret.

Taking that the probable 90% kill for 5 minutes in ferrets is 345 and for 25 minutes is 3625, the equivalent figures for badgers would b 7500.

Similarly the LCt_{50} and LCt_{90} may also be multiplied.

	Ferrets			Badgers	
Time	LCt_{50}	LCt_{90}		LCt_{50}	LCt_{90}
1 min	670	910		(1300	2000
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In fact for two 30 minutes exposures at 2560 and 5575 Ct units re both badgers survived.

At a 17 minute exposure with a Ct of 6560 one badger died, and on survived a Ct of 4000 over 10 minutes. These results support the very figures in the right hand column.

CONCLUSIONS

Using the figures found for the various Cts for ferrets exposed t cyanide, it appears both from extrapolation and from real exposure of that a Ct to obtain 90% kill with reasonable certainty would need to b 7000 mg min m^{-3} for a 30 minute exposure, or a concentration of approx 230 $\mu g\ \ell^{-1}$.

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LIST OF FERRET BODY WT (g)

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505	582	639	685
505	585	639	685
506	587	640	685
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513	592	645	695
514	594	645	695
515	597	645	695
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721	787	963	
722	787	965	
722	794	966	
724	799	972	
724	804	973	
737	807	975	
737	808	977	
741	812	981	
743	817	984	
747	817	987	
752	823	1001	
754	825	1006	
754	829	1009	
756	843	1010	
758	853	1027	
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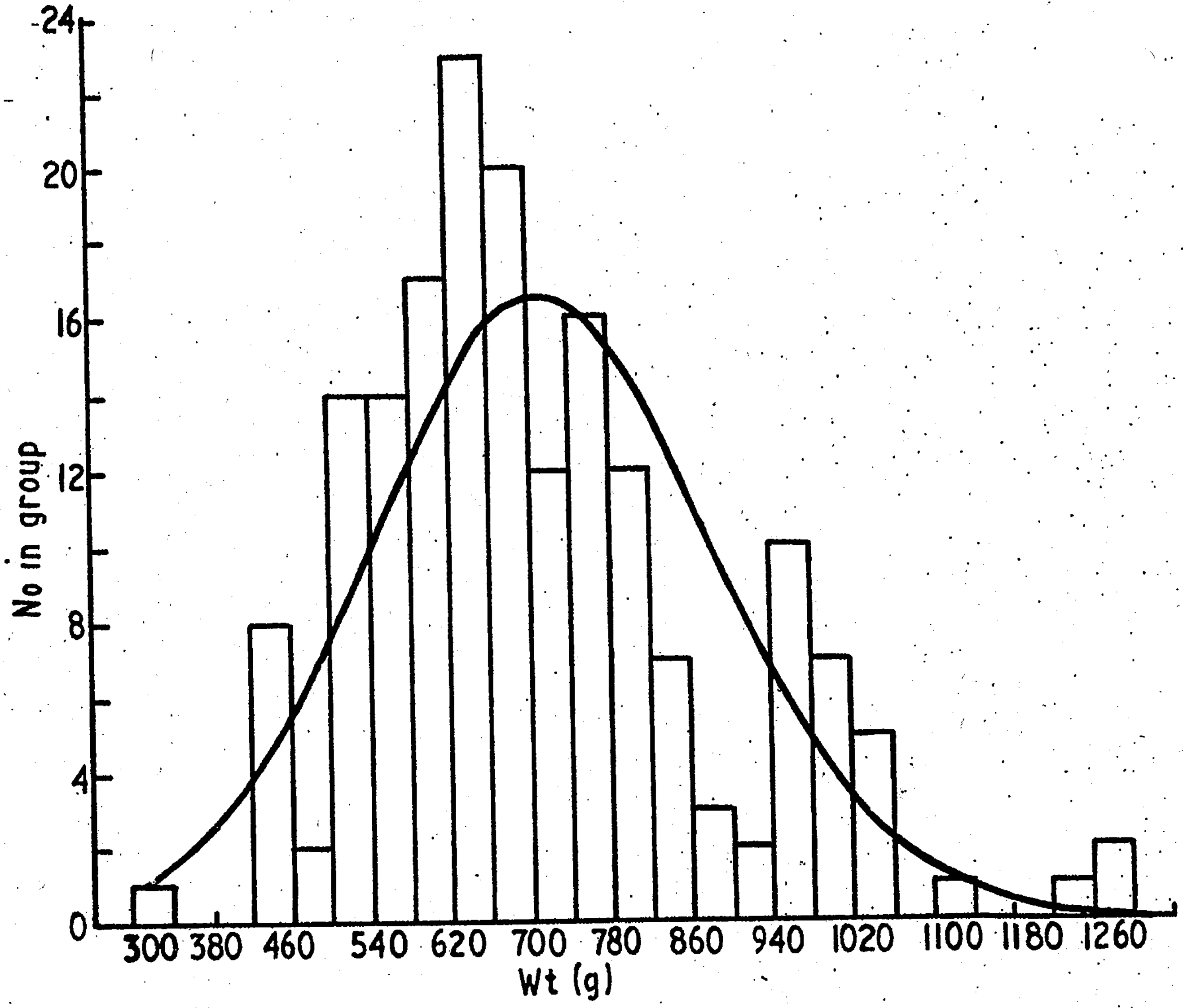
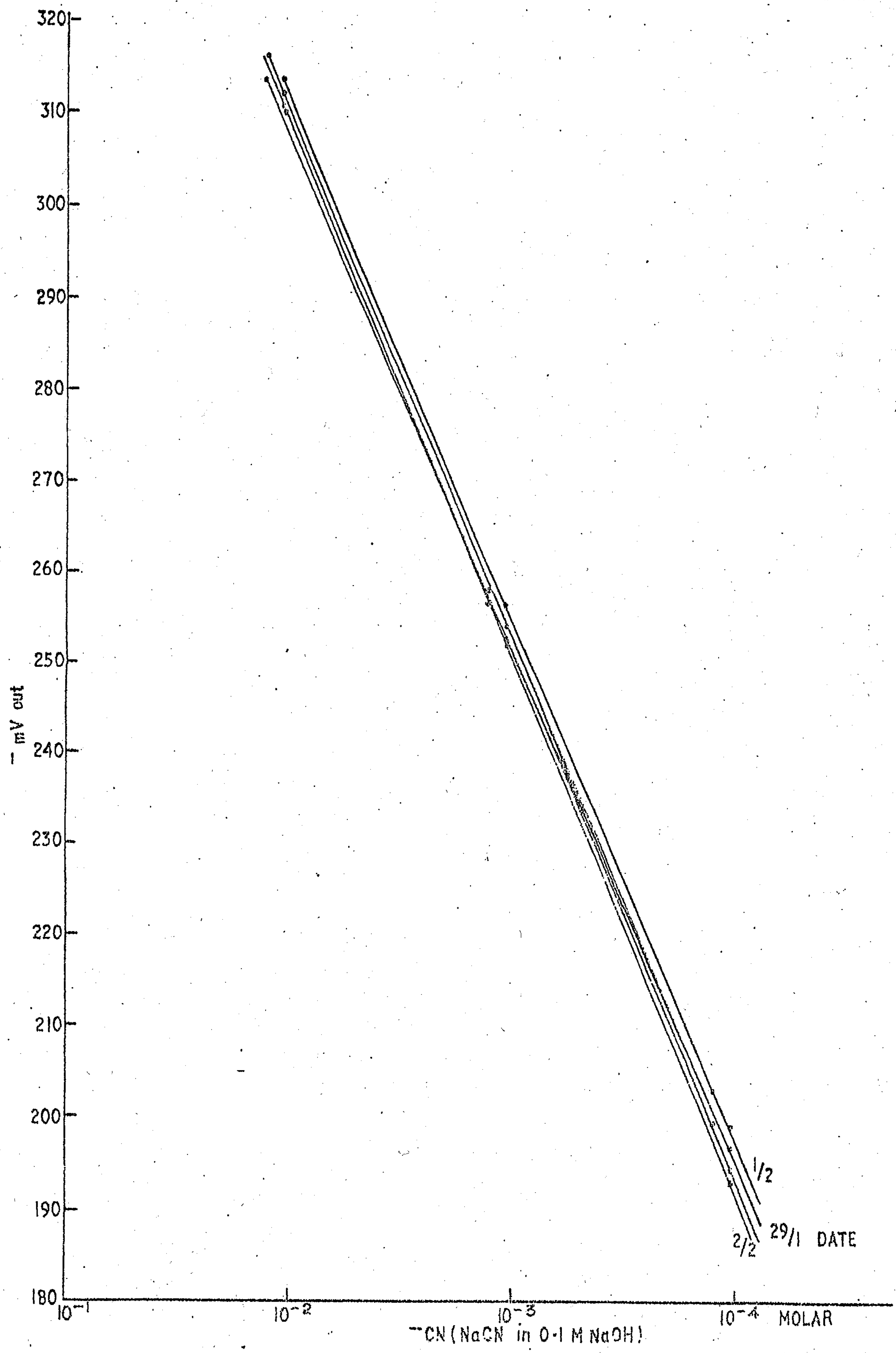
759	858	1050	
764	868	1056	
769	884	1058	
770	897	1103	
776	901	1259	



ANIMAL BODY WEIGHTS

Appendix I. Fig. 1



CALIBRATION CURVES FOR CYANIDE ESTIMATION   Fig.2.

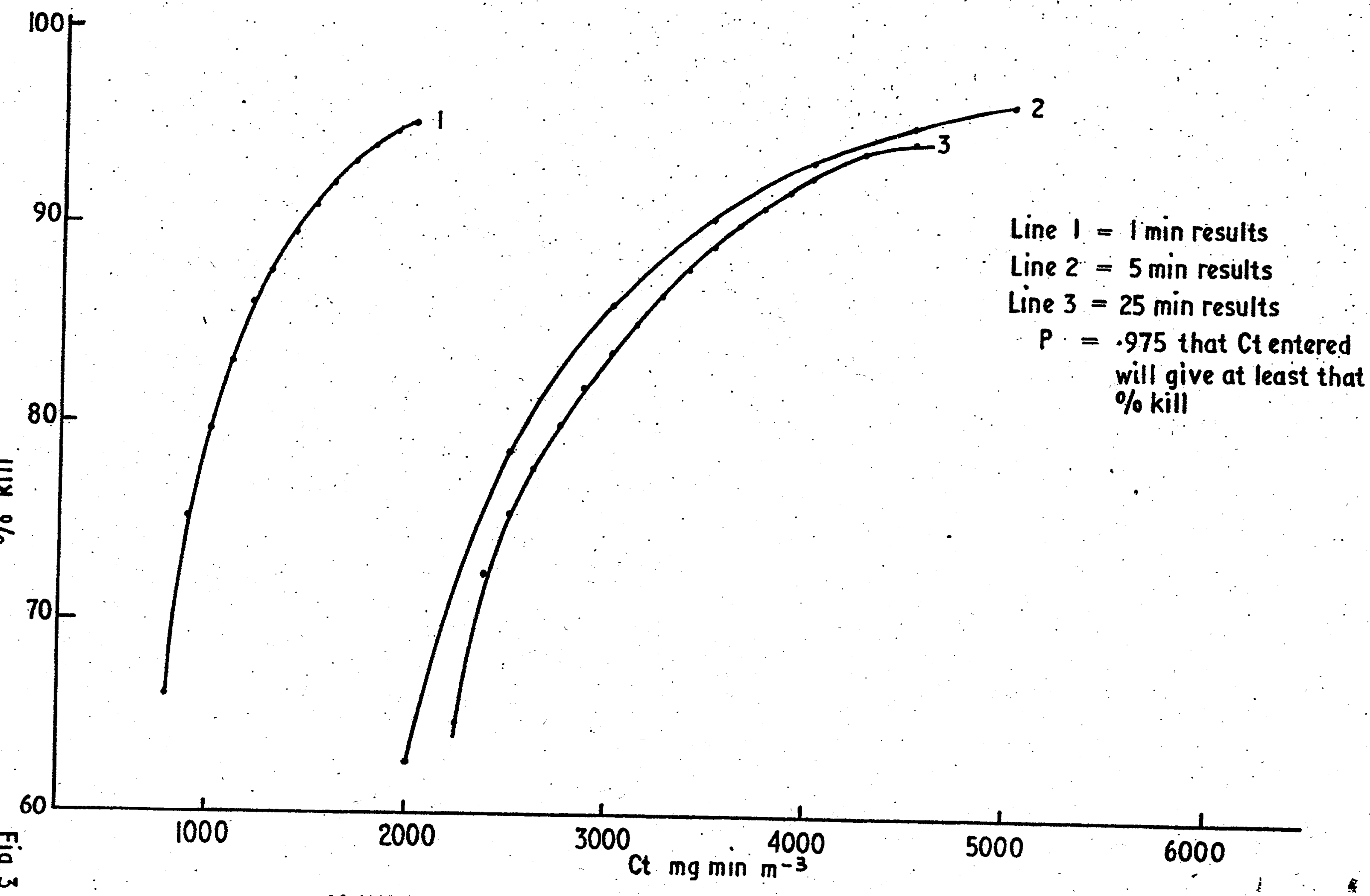
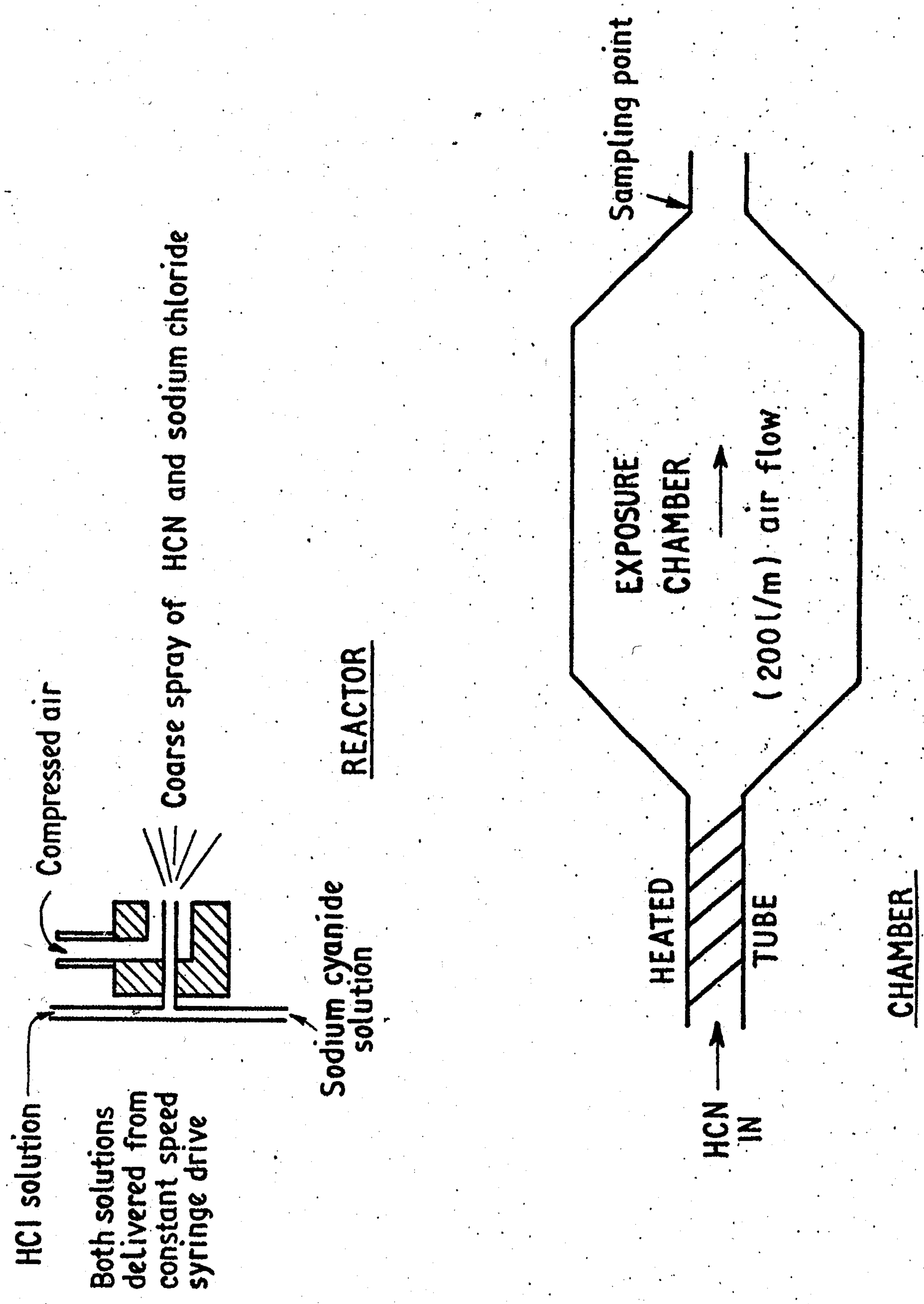


Fig. 3.

MINIMUM PROBABLE KILL FOR VARYING Cts

Commercial in Confidence



HCl solution
Both solutions delivered from constant speed syringe drive
Compressed air
Coarse spray of HCN and sodium chloride
Sodium cyanide solution
REACTOR
Sampling point
EXPOSURE CHAMBER
(200 l/m) air flow
HEATED TUBE
HCN IN
CHAMBER