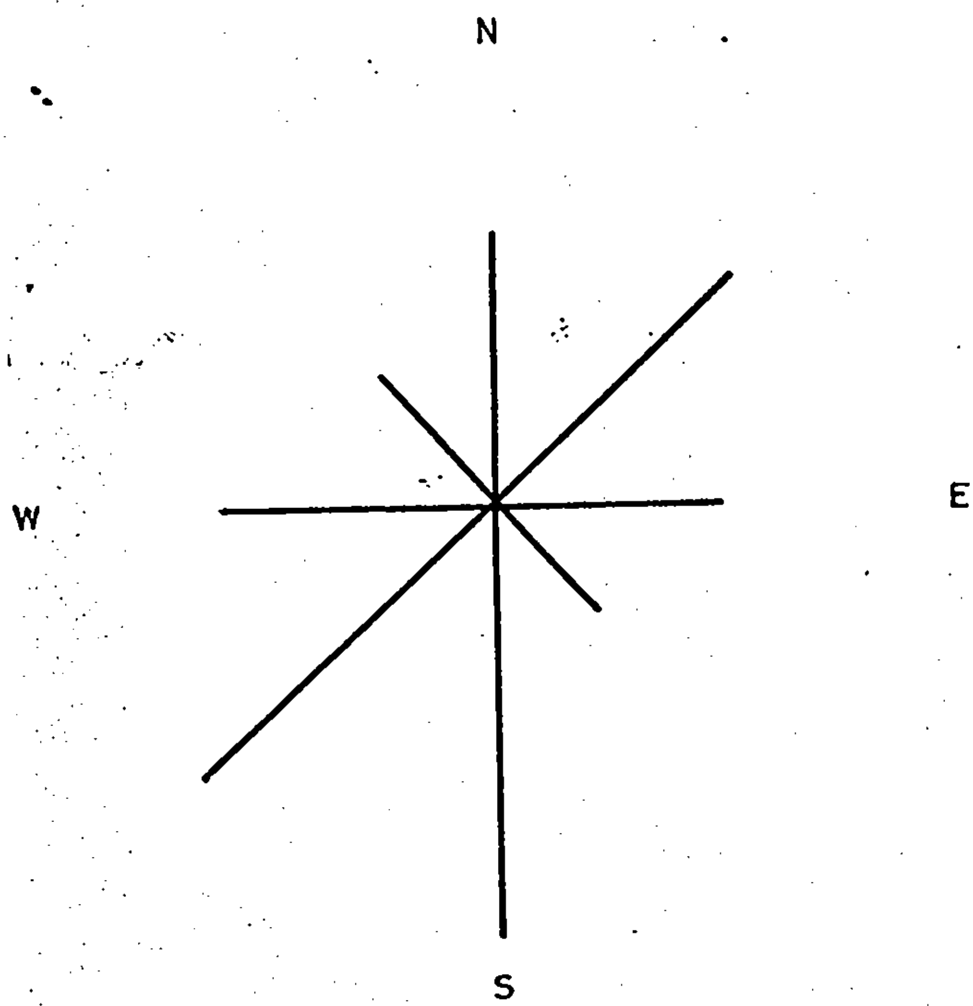


FRONTISPIECE

WIND-ROSE FOR HOLYHEAD 1977.

PROJECT 524

Fluoride in Predatory Mammals

Preliminary Report July 1978

INTRODUCTION

The main background to this work is explained in the Project Plan. Its aim is to conduct a pilot survey into the occurrence of fluoride in two species of predatory mammal and their prey in relation to an industrial emission source, the aluminium reduction plant of Anglesey Aluminium at Holyhead, subsequently referred to as 'the smelter'.

It is already known that fluoride is one of the unwelcome by-products in the manufacture of aluminium and that it is widely distributed from the source by the prevailing winds. On the west coast of Britain these are mainly from the south and west. The frontispiece shows a wind-rose for Holyhead for 1977. Eight sites were chosen at 5 Km intervals along transects running NE and SE from the smelter and one site to the west.

Collection of material was begun in February 1977. Each species or group of species will be dealt with separately. Since this is an interim report whose main virtue should be readability, the following features apply:

1. No references are given since it is assumed that the reader will not have access to them. They will be referred to as 'published work'.
2. Concentrations of fluoride are usually reported as microgrammes per gramme (written $\mu g g^{-1}$). Here they will be referred to as ppm (parts per million) except in calculations. All analyses were done by Jack Parkinson and his team at Merlewood.

3. The transect sampling sites will be called Site 1 ...... etc. For my convenience they are also named in the tables.
4. Common names of animals are used throughout.

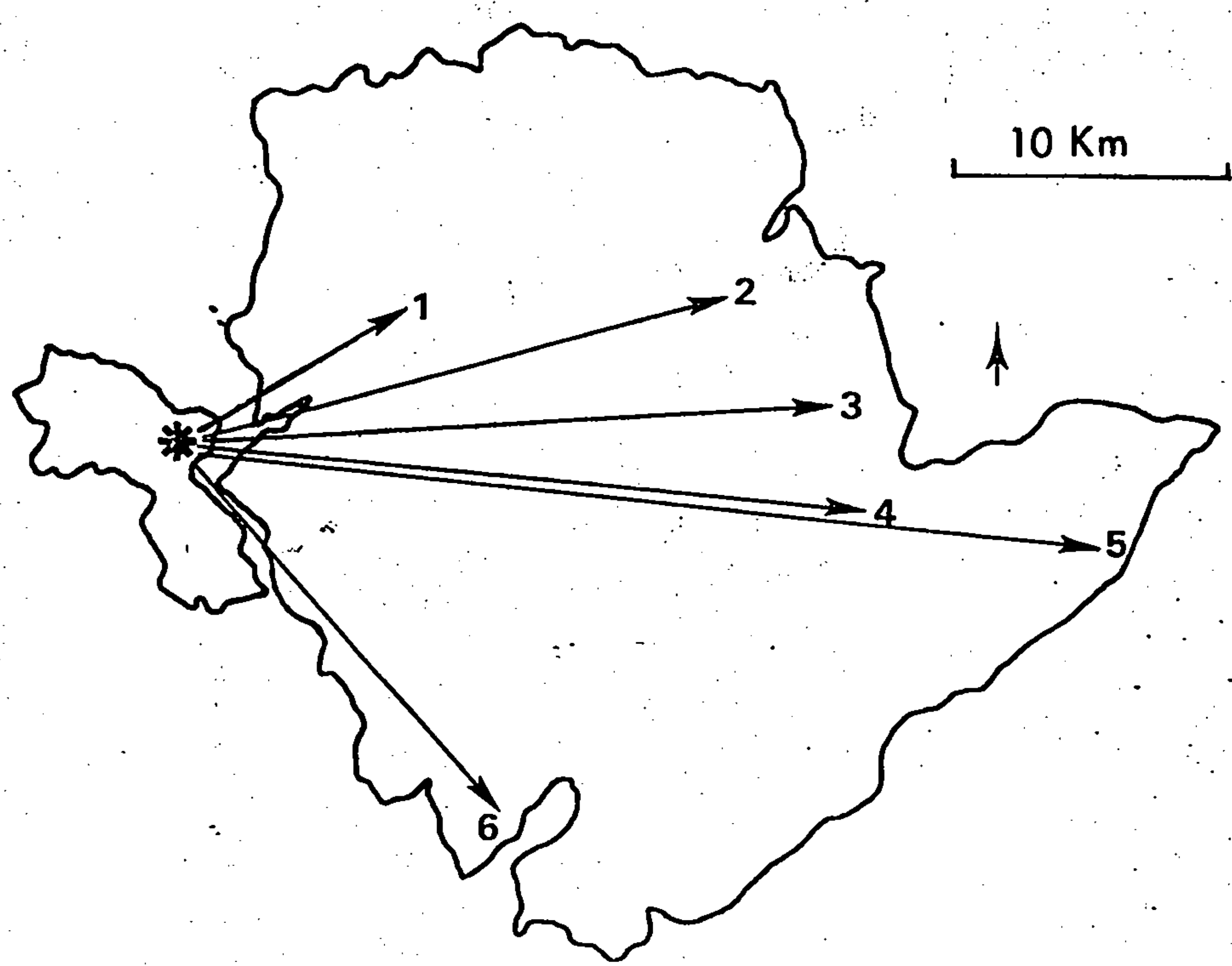


FIG. 1 FOX

SITE			FLUORIDE IN PPM IN BONE 1975-76		
NO.	NAME	DIST.(KM)	MEAN	S.E.	N
1	BRWYNOG	10	635	92	6
2	BODAFON	21	432	33	4
3	MARIANGLAS	25	417	46	12
4	PENTRAETH	30	504	47	16
5	BEAUMARIS	33	544	99	7
6	BODORGAN	17	517	80	7

CONCENTRATIONS OF FLUORIDE IN FOX BONE FROM DIFFERENT SITES ON ANGLESEY

A. FOXES

The weasel was originally the species to have been studied but early in the investigation it was found that captures were likely to be too few to be of any use. I decided to make use of fox material instead which was already being collected from various parts of Wales, including Anglesey, as part of a rabies research (My thanks to former colleagues Gwyn Lloyd, Bob Page and John Woods for their willing assistance).

Following a published account, I asked for and received the dried angular apophysis from the rear angle of the jaw bone (dentary) for all those animals for which the skull had already been prepared (for 1975-76 this was 128 foxes). In addition, forelegs have been received from another 40 animals from 1977-78 for which I intend measuring bone fragility prior to fluoride determination. Additional material will continue to be supplied as long as it is required together with information on ageing, determined by examination of tooth sections (My particular thanks to Bob Page who did these for me). Because foxes are not collected systematically they come from geographically scattered locations but it has been possible to group some of the results so as to give mean values. Generally these locations do not correspond with the transect sites except in one instance.

Present results are shown in Fig. 1 . Mean values should be viewed with some caution since the samples are not homogenous for age. There are three obvious variables which can influence fluoride concentration in living organisms and it is worth mentioning them here because they apply to all the species studied.

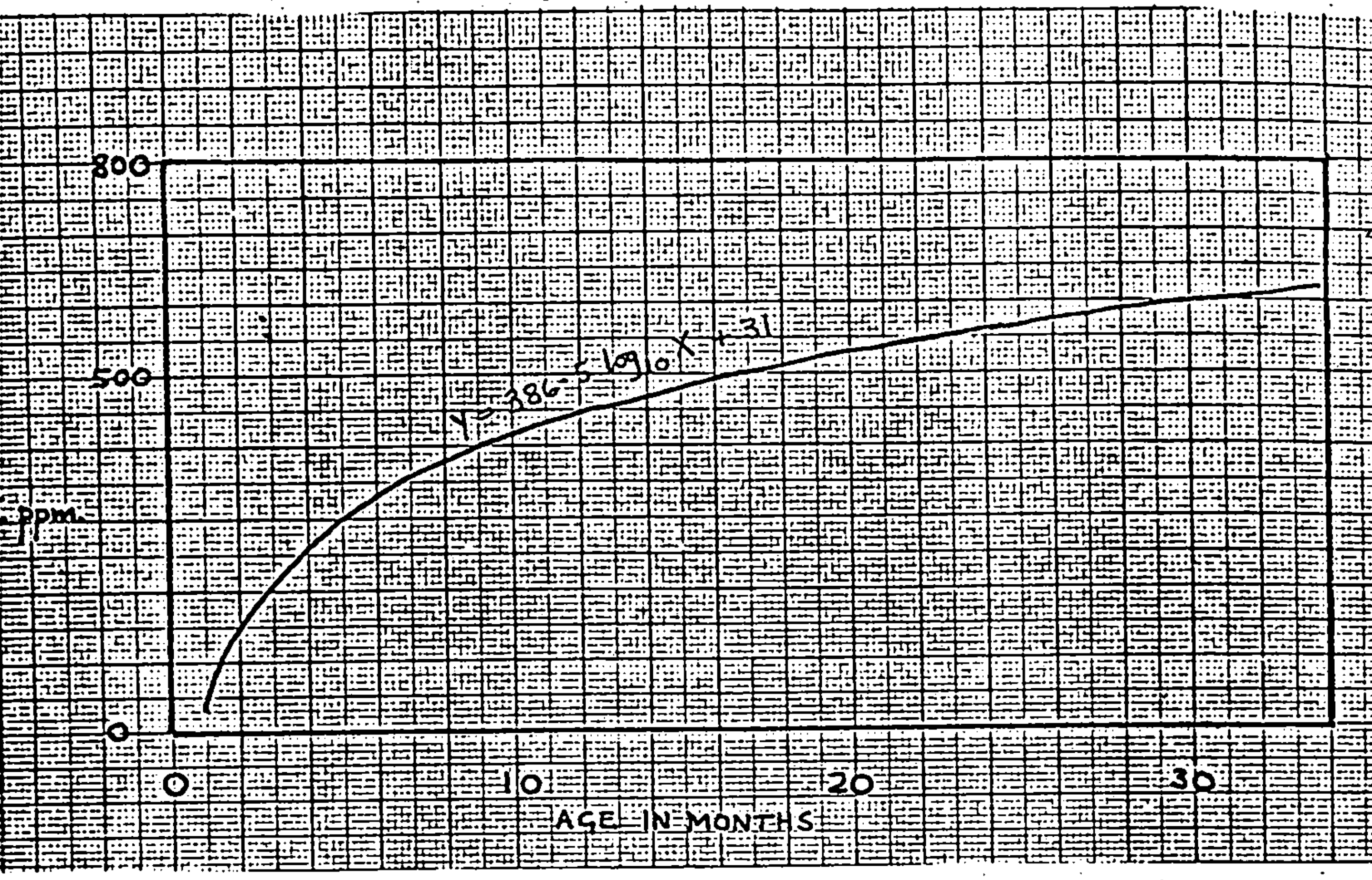


FIG. 1A.   FOX. REGRESSION OF BONE FLUORIDE ON AGE

They are:

(1) Distance and direction of the sampling site from the fluoride source.

(2) Age of the animal (= time of exposure)

(3) Time since emission started (= accumulated fluoride in the ecosystem)

As far as (1) is concerned it seems that the sites from which foxes have been obtained so far are so distant from the smelter that no difference in fluoride content can be demonstrated between them. This is another way of saying that all the samples have been drawn from one population.

Age of an animal (2) is of crucial importance. When fluoride is taken up continuously (and published work shows that this is the case even when environmental fluoride is low) the amount found on analysis is a function of age. If all the fox data from Anglesey are pooled a regression equation is obtained with the following coefficients (Y = fluoride in ppm, X = age in months):

$$Y = 386.5 \log_{10} X + 30.8 \quad (n = 53 \text{ and } r = 0.58)$$

From this equation it is possible to explain the apparent difference between 1975 and 1976 at Site 3 where the mean fluoride values were 397 ppm and 600 ppm respectively ($t = 2.64$, $P<0.02$). The simple explanation of this real difference is that the age composition of the samples was different; mean ages were 11 months and 22 months respectively. If the regression equation is used to predict fluoride values for these ages it gives 433 ppm and 549 ppm which match well with the observed values; so there is no evidence here of a change of fluoride concentration between years (3).

What can be said with certainty about foxes obtained so far is that, although many of them were from sites as much as 30 Km from the smelter, they contained more fluoride than prey animals (voles and mice) only 15 Km from the smelter. If the mean fluoride content of foxes less than a year old (i.e. about the same age as the rodents) is compared with voles and mice from Site 5 the following figures result:

FOXES	MICE	VOLES
383 ppm	210 ppm	126 ppm

The differences are significant at P>0.001. It is to be expected that foxes from much nearer the smelter will produce much higher values.

In order to find control values from unpolluted foxes a sample of fox bones has been obtained from Brecon and sent for analysis.

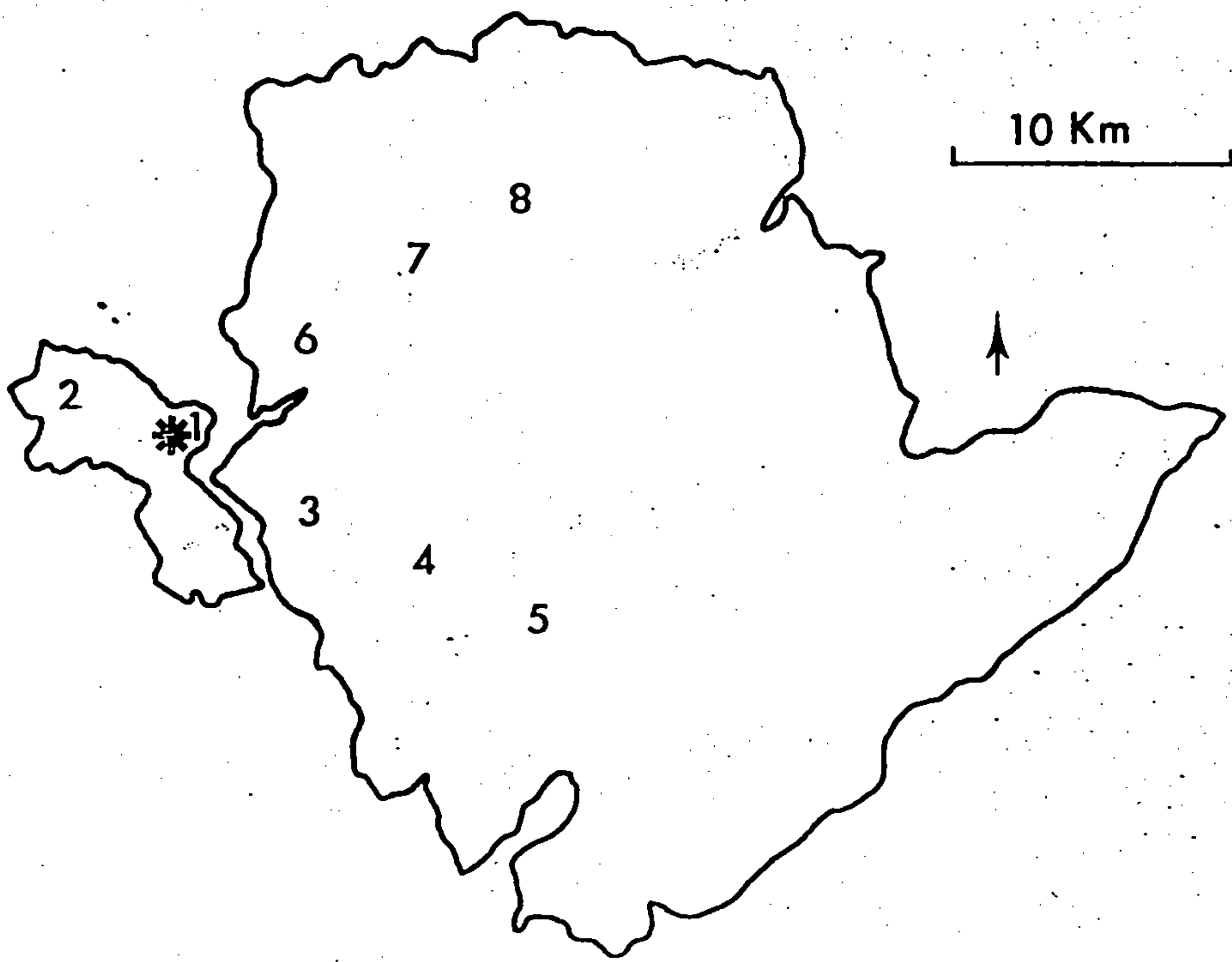


FIG. 2

SYSTEMATIC SAMPLING AREAS FOR SMALL MAMMALS; ALSO MOLE AND EARTHWORMS

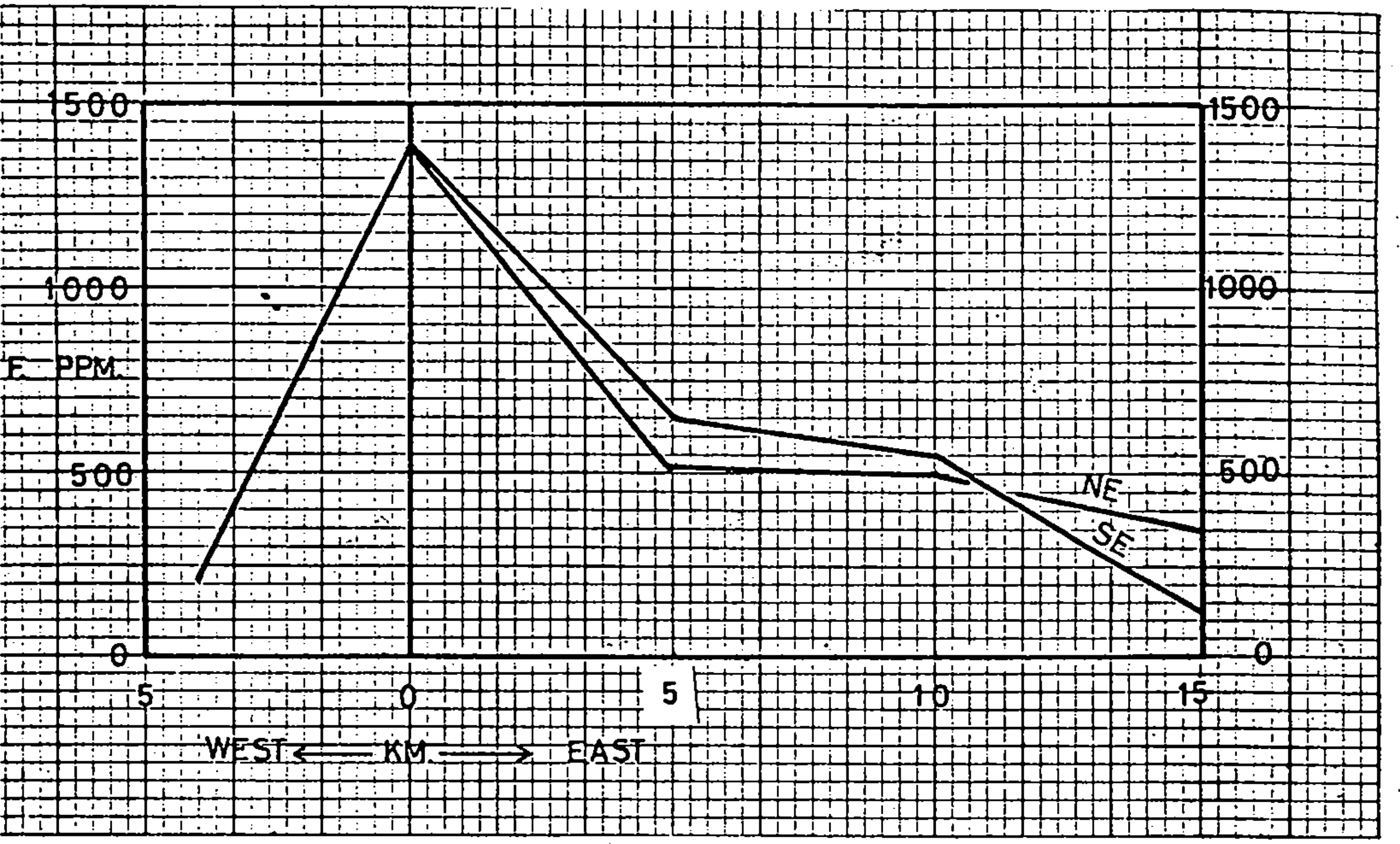


FIG. 2A. FLUORIDE IN VOLE BONE.

FALL OFF IN CONCENTRATION WITH DISTANCE

FROM SMELTER.

SITE				FLUORIDE IN PPM OF DRY WEIGHT		
NO.	NAME	DIST.(KM)	DIR.	MEAN	S.E.	N
1	SMELTER	0	-	1383	392	9
2	PENYBONC	4	W	210	18	4
3	YSBYLLDIR	5	S.E.	652	133	5
4	FACH	10	S.E.	500	30	5
5	PENYGRAIG	15	S.E.	126	10	6
6	DRONWY	5	N.E.	535	100	6
7	BRWYNOG	10	N.E.	450	66	9
8	HAFODOL	15	N.E.	350	17	6

TABLE 1. CONCENTRATIONS OF FLUORIDE IN HOMOGENISED SKELETONS OF FIELD-VOLE FROM DIFFERENT SITES ON ANGLESEY. FEB-MAR. 1977

SITE			FLUORIDE IN PPM OF DRY WEIGHT								
			BONE			SKIN			TISSUE		
NO.	NAME	DIST.(KM)	MEAN	S.E.	N	MEAN	S.E.	N	MEAN	S.E.	N
1.	SMELTER	0	2423	528	21	26	4.9	10	-	-	-
7.	BRWYNOG	10	460	84	4	-	-	-	-	-	-
5.	PENYGRAIG	15	210	39	10	10.5	1.9	5	13.1	7.3	5

TABLE 2(a). CONCENTRATION OF FLUORIDE IN
WOOD-MOUSE, FEB-MAR. 1977

SITE			FLUORIDE IN PPM OF DRY WEIGHT								
			BONE			SKIN			TISSUE		
NO.	NAME	DIST.(KM)	MEAN	S.E.	N	MEAN	S.E.	N	MEAN	S.E.	N
1.	SMELTER	0	1383	392	9	8.2	1.5	6	11.1	4.4	6
6.	DRONWY	5	458	35	5	10.3	2.3	5	7.6	1.1	5
5.	PENYGRAIG	15	126	10	6	10.2	3.1	6	8.9	2.3	6

TABLE 2(b). CONCENTRATION OF FLUORIDE IN
FIELD-VOLE, Feb-Mar. 1977

B. SMALL MAMMALS

Much of the food of foxes (and barn-owls, see Project 525) consists of small mammals mainly the field-vole, wood-mouse, and common shrew. These animals were, therefore, collected during 1977 by trapping in suitable habitats within the 8 sampling sites shown in Fig. 2. Longworth traps baited with grain were set and trapping was continued until a minimum of five of each species per site had been caught (or until two months had elapsed from the start of the session). Trapping was done in four sessions to obtain samples from throughout the year, starting in February, May, August and November. Animals were killed with chloroform.

In the laboratory each animal was weighed, skinned and the reproductive organs removed, weighed and preserved. Total extractable body fat was determined in a sample of 28 mice to examine any relationship of this value to fluoride content (Published work suggested that increased fluoride caused a decrease in body weight. Total fat was chosen to represent body weight since the latter is difficult to measure accurately in field specimens, which are often sodden with water).

All carcases (i.e. fat-extracted and others) were then digested with papain, a proteolytic enzyme, at 40° C for several days and the soft tissue removed from the skeleton as a 'soup' with the help of an ultrasonic cleaner. Skeletons were oven-dried and analysed for every specimen, but for skins and 'soup' only a sub-sample was analysed. The bones were sorted and a selection kept for measuring and age determination. All the remainder were homogenised and a sample analysed from each animal. 'Soup' and skins were freeze-dried before analysis. Unless otherwise stated fluoride values refer to bone. The available results from fluoride analysis of voles and mice are shown in Tables 1 and 2, from which a number of features are apparent.

1. The majority of fluoride found in the body at any time was in the skeleton. (This agrees with findings by other workers: bone acts as a 'sink' for fluoride).
2. There was an obvious gradient in bone fluoride from the smelter outwards. On both transects concentration decreased with distance. (Fig 2A)
3. The amount of fluoride found in skin and soft tissue was minute ranging from about 1% to 10% of that in bone. There was no gradient of fluoride in skin and soft tissue along either transect. The absolute quantity of fluoride in the skin and tissue was virtually the same for all animals which suggests that there may be an upper limit for these tissues above which they cannot function properly. Gradual increases in tissue fluoride are probably compensated for by 'dumping' into the bone. Sudden increases may be lethal. Such animals would not appear in the samples.
4. All values for fluoride from a given site have been pooled regardless of the age and sex of the animals. No satisfactory method of ageing has been found for either species but it has been assumed that the majority of animals were in their first year of life. The occasional animal may have been in its second year and could account for the few exceptionally high fluoride values recorded from the smelter site where the range in mice was found to be 470-8500 ppm (with four individuals having 6300, 6500, 6700 and 8500 ppm). Data from the May/June session

will throw light on this situation since it will contain young animals identifiable by external appearance.

5. Some differences were found in bone fluoride between voles and mice (0.1>P>0.05).

	SITE 1			SITE 5		
	MEAN	S.E.	N	MEAN	S.E.	N
VOLES - F in ppm	2423	528	21	210	39	10
MICE - F in ppm	1383	392	9	126	10	6

TABLE 3 COMPARISON OF BONE FLUORIDE BETWEEN VOLES AND MICE

If the large difference in variances is taken into account the difference between mean fluoride values for voles and mice do not quite reach P = 0.05. If these incipient differences are confirmed by further evidence then it may be conjectured that they are due to differing diets, mice eating more invertebrates than voles do. If fluoride is concentrated at each trophic level (see Section C for confirmation) this could explain these results.

6. Measurements were made on femurs from both voles and mice, since published work shows that fluoride causes enlargement of bone (but not uniformly throughout the body or even within a single bone). A consistent gradient was found along the transects and although the significance level was low for adjacent sites, comparisons between the source and the most distant sites showed marked differences. The measurements made were labelled MAX, MIN, and WING (maximum and minimum widths of femur

and the width of a wing-like process which runs along the proximal part). The results may be set out thus:

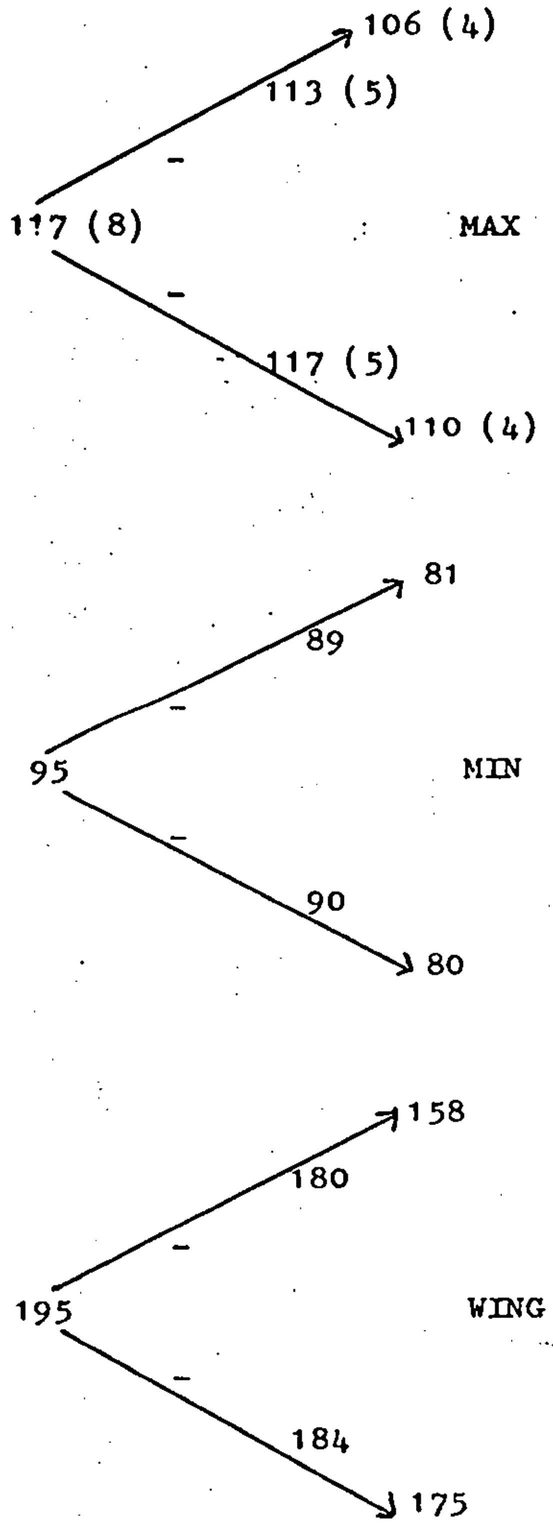


FIG. 3
FEMUR MEASUREMENTS
FIELD-VOLE, MALES.
UNITS 0.01 mm.
(SAMPLE SIZE IN BRACKETS)

Detailed comparison between means of source and distant sites are shown below:

	SMELTER SITE 1			PENYGRAIG SITE 5 (15 Km)			HAFODOL SITE 8 (15 Km)		
	MEAN	S.E.	N	MEAN	S.E.	N	MEAN	S.E.	N
MAX	117	13	8	110	7	4	106	3	4
MIN	95	7	8	80**	5	4	81**	9	4
WING	195	30	8	175	16	4	158*	6	4

TABLE 4. COMPARISON OF FEMUR MEASUREMENTS OF VOLES BETWEEN SMELTER (SITE 1) AND BOTH FURTHEST SITES. (UNITS 0.01 mm. ALL VALUES ROUNDED)

7. The relationship between one of the femur dimensions (MIN) and fluoride concentration was examined in voles from several sites. The correlation coefficient for both sexes was identical ($R = 0.47$, 12 males, 9 females) and whilst this value does not reach significance it is indicative of a relationship.

8. It has not been found possible to establish any relationship between fluoride content and total body fat. Measurement of the latter has been discontinued.

9. Nothing has been deduced from the reproductive organs and it seems unlikely that it will. As with most trapped samples males were in a majority. There were no pregnant females of either species in the Feb./Mar. samples; and no apparent relationship between testes weight and fluoride content in the males of either species.

10. Samples of teeth, from voles and mice whose fluoride content has been determined, have been sent to Dr. B. Berkovitz at Bristol for examination. He has a special interest in dental structure.

C. MOLES

Moles occupy the same position in the food chain as foxes but feed almost entirely on invertebrates principally earthworms. They were collected by trapping with Duffus traps in suitable places within each sampling site, as close as possible to where voles and mice were being trapped. Early observations showed moles to be scarce at the smelter site and absent from Site 2 (Holyhead Mountain). A search of Holyhead Island showed that the nearest area with moles was near Trearddur Bay so that moles from here have had to serve in place of any from the other two sites (except for a single fortuitous capture on the car park opposite the smelter.)

All animals were weighed and their reproductive organs removed, weighed and preserved. Each animal was skinned, the carcase digested in papain and the skeleton cleaned. Some bones were kept for measuring and age determination and the remainder homogenised for analysis. A sub-sample of skins and 'soup' was analysed.

So far results have been received for only a small number of moles:

SITE				MOLES FLUORIDE IN ppm			RODENTS AT SAME SITE (MEAN ONLY)	
NO.	NAME	DISTANCE (Km)	DIRECTION	MEAN	S.E.	N	VOLES	MICE
1.	PENRHOS	0	-	8600	-	1	1383	2423
6.	DRONWY	5	N.E.	1500	153	3	458	-
7.	BRWYNOG	10	N.E.	1270	191	5	432	460

TABLE 5. CONCENTRATION OF FLUORIDE IN PPM OF HOMOGENISED SKELETON. MOLE. FEB.-MAR. 1977.

It is evident that moles contain more fluoride at a given site than either of the two rodents. This is to be expected as they are secondary consumers. The relationship of these figures to those from their principal prey, earthworms, is discussed in the next section.

The femurs from the moles which have been prepared so far have been measured in a manner similar to that for the voles and mice. Only MAX and MIN were measured as there is no 'WING' on the short, stumpy mole femurs. The sample sizes, when divided into sexes and sites are small so far but nevertheless show the same trend as the rodent bones i.e. greater MIN measurements near the smelter and smaller ones further away.

SITE				FEMUR MEASUREMENTS (0.01 mm)					
NO.	NAME	DIST.(KM)	DIR.	MAX			MIN		
				MEAN	SD	N	MEAN	SD	N
2.	TREARDDUR	3	S	221	5.7	4	215	3.3	4
7.	BRWYNOG	10	N.E.	208	13.5	5	204	12.5	5
5.	PENYGRAIG	15	S.E.	208	11.1	5	195**	8.6	5
8.	HAFODOL	15	N.E.	206	3.0	3	201**	6.6	3

TABLE 5A. COMPARISON OF FEMUR MEASUREMENTS OF MOLES FROM SITE NEAREST SMELTER (TREARDDUR) AND THOSE FURTHEST AWAY.

D. EARTHWORMS

Earthworms were sampled by setting out a 30 m. tape at right angles to the boundary of a field where moles had been trapped and digging out a block of turf at 5, 10, 15, 20 and 25 m. Each block was the width of a spade square and as deep as the spade's depth. The blocks were pulled apart by hand on the spot on a plastic sheet and all the worms collected and counted. In the laboratory each sample of worms was weighed and frozen for each block of soil. This gave 5 samples per site which were freeze-dried before analysis for fluoride. The results are shown below.

SITE				FLUORIDE IN PPM		
NO.	NAME	DIST. (Km)	DIR.	MEAN	S.E.	N
1.	SMELTER	0	-	112	16	5
2.	Trearddur	3	S	29	3	5
3.	YSBYLLDIR	5	S.E.	19	1	5
4.	FACH	10	S.E.	35	2	5
5.	PENYGRAIG	15	S.E.	48	2	5
6.	DRONWY	5	N.E.	31	2	5
7.	BRWYNOG	10	N.E.	36	5	5
8.	HAFODOL	15	N.E.	31	6	5

TABLE 6. CONCENTRATION OF FLUORIDE IN PPM.
OF FREEZE-DRIED TISSUE. EARTHWORMS. FEB.-MAR. 1977

These results are probably inconclusive for the following reason. On the assumption that moles ate earthworms whole, complete worms were sent for analysis. Unfortunately, the analytical technique involves ashing the specimens. Not only does this ash the worm's tissues but also any soil in the alimentary tract. This makes available for assay fluoride normally locked up in the inorganic form in soil which is not available to the mole.

It is intended to repeat this investigation using water-soluble fluoride analysis but for the moment it is possible to consider the values as being the maximum figures likely for each site. Comparison of values for those sites for which mole figures are also available enable the following table to be constructed:

		FLUORIDE ppm		
	SITE	MOLES	EARTHWORMS	INCREASE
1.	PENRHOS	8600	112	X77
6.	DRONWY	1500	31	X48
7.	BRWYNOG	1270	36	X35

TABLE 7. COMPARISON OF FLUORIDE CONCENTRATION IN EARTHWORMS AND MOLES

DISCUSSION, SYNTHESIS OF RESULTS, SUGGESTIONS

From the results obtained so far, together with other work carried out at Bangor and published work, it is possible to construct a provisional model of the movement of fluoride in the animals studied.

D. F. Perkins has shown that vegetation (Agropyron sp.) at the smelter site had a mean fluoride value of 269 ppm for the aerial parts of the plants. This was made up of 177 ppm on the surface and 92 ppm in the tissue. Published figures for the dry weight of grass eaten by field-voles in a 24 hr. feeding trial show 2.1 g to be the smallest amount eaten (succulent leaf bases of Dactylis glomerata. The maximum was 13.6 g of Agrostis canina).

If the lower figure of 2.1 g is used to calculate daily intake of fluoride the value is 2.1 x 92 = 193.2 μg/day. Supposing the Feb./Mar. sample of voles to be about six months old they could have eaten 193.2 x 180 = 34,776 μg of fluoride in the course of their lives.

Thirteen vole skeletons had a mean weight of 0.7 g so that with a mean fluoride concentration of 1383 ppm at the smelter site, each skeleton contained about 968 μg. Only about 24 μg would occur in the rest of the body (dry carcase weight 3 g x 8 ppm) so that the overall content would be about 992 μg.

Compared with the amount supposedly ingested this represents a retention rate of 2.8%.

LIVE PLANTS

TISSUE 92 ppm

FIELD-VOLE DAILY INTAKE → 193.2 μg/day

2.1g

6 months (180 days)

FIELD-VOLE

SKELETON 1383 ppm
" WT. 0.7 g } 968 μg.

CARCASE 8 ppm
" WT. 3.0 g } 24 μg

TOTAL IN BODY 992 μg

34,776 μg TOTAL FLUORIDE INGESTED

PROPORTION RETAINED = 2.8%

FIG. 4. PROPOSED PATHWAY OF FLUORIDE IN GRASS & VOLES. 1st MODEL

Published work gives the retention rate for ingested fluoride in laboratory rats as between 42% and 68% at apparently lower dosage rates than have been assumed here. Even if some of the assumptions of this model are adjusted the difference between results is large. For example, if the voles are assumed to be only 90 days old and the plants to contain only 46 ppm (i.e. two parameters are halved) the retention rate would be 11.4%.

Obviously some explanation must be sought for this discrepancy. The immediate possibilities which spring to mind to explain these results are:

1. Voles are highly selective feeders and the initial fluoride figure for vegetation is wrong.
2. Extraction of fluoride from plant material in the gut is inefficient and much of it passes out unchanged.
3. Voles differ markedly from laboratory rats.

To test these ideas some small samples of vole droppings were collected from the smelter site; also some stored grass (stems and leaves) which voles are in the habit of cutting, trimming and stacking in their runs. Unfortunately, the weights of material sent for analysis have an enormous bearing on figures estimated at the lower end of the scale and the only values available are:

MATERIAL		PPM F^-
DROPPINGS	1.	< 36
"	2.	< 46
GRASS	1.	< 69
"	2.	< 10

TABLE 8. FLUORIDE CONCENTRATIONS IN VOLE DROPPINGS AND VOLE-SELECTED GRASS AT THE SMELTER SITE (1)

It would seem that the material selected as food by voles may well be less contaminated by fluoride than that selected for analysis by researchers! If a new value of 10 ppm is used as the starting point for a calculation and extraction is regarded as only 50% efficient, then the retention rate can be nudged into about the right order of size, about 52%. (Some American researchers regard 10 ppm in plants as uncontaminated material i.e. as controls).

LIVE PLANTS $\xrightarrow{\text{FIELD-VOLE}}$

$10\ \mu g g^{-1}$ $2.1\ g \times 10\ \mu g = 21\ \mu g./day$

$\downarrow$ 180 days

TOTAL IN BODY $992\ \mu g$ $3780\ \mu g$ TOTAL INGESTED

PROPORTION RETAINED = 26%

If absorption efficiency is only 50% then retention rate - 52%

FIG.5A. PROPOSED PATHWAY IN GRASS & VOLES. 2nd MODEL

It seems likely that neither of these models is near the truth but they form a useful basis from which to consider further work. Some published work has suggested that species vary in their capacity to cope with fluoride. Thus one worker claimed no change in radiographic appearance, ash content or breaking stress of rat bone on 250 ppm in drinking water for 52 weeks; whilst another found a significant reduction in the strength of femurs of rabbits given 200 ppm for only 8 weeks.

Some of the factors which have been shown to affect absorption of fluoride are the intake of calcium, aluminium (!), vitamin C, dietary fat, and fresh-cut vegetation.

The only laboratory work known to me which is comparable to this investigation, with regard to the species of animal involved, is by two Americans who captured deer-mice and used them as experimental animals. As with most laboratory work the concentration of fluoride given to the animals in their food is stated, but no clue is given as to what the daily intake was. I was surprised at the 'control' figure of 38 ppm which seems very high. Using 2 g dry weight as before, as a normal daily intake the apparent retention is about 11.5% at this concentration. The percentage retained falls, however, as the intake is increased (there is a good negative correlation (r = 0.87, P<0.05) which suggests, like my results, that some factor limits uptake of fluoride and the surplus is excreted.

DIETARY F	FEEDING TIME (DAYS)	ESTIMATED (DOSE µg) (AT 2g/DAY)	AMOUNT FOUND (µg) (SUPPOSE 1g SKELETON)	% RETAINED
38 ppm	56	4256	498	11.7
1065 ppm	42	89460	2830	3.2
1355 "	30	81300	3106	3.8
1936	42	162624	4391	2.7

TABLE 9 ESTIMATED RETENTION RATE OF FLUORIDE IN DEER-MICE FED DIFFERENT CONCENTRATIONS (Adapted from Newman, J.R. & Markey, D. 1976. Fluoride 9 47-53)

Constructing a model for moles is not so easy because of possible errors in the amount of fluoride found in earthworms but if we take 36 ppm as a reasonable figure we can produce the following:

EARTHWORM 36 ppm	50 g/day (published figure) → assume 95% water = 2.5 g x 36 ppm	90 μg/day

↓ assume age at 180 days

MOLE 1500 ppm

SKELETON 3.9 g WT. } 5850 μg

NO VALUES FOR SKIN OR TISSUE

5850 μg	16200 μg
TOTAL FLUORIDE FOUND	TOTAL FLUORIDE INGESTED

PROPORTION RETAINED = 36%

This seems to accord well with published figures for retention and suggests a difference in the way that moles deal with fluoride as compared with rodents.

It is now possible to put together a tentative model for all the species concerned. It seems unlikely that grass will turn out to have a level as low as 10 ppm but assuming that this is so we have the following

$$\begin{tikzcd} \boxed{\text{GRASS 10}} \arrow[r, "\text{x } 12"] \arrow[d, "\text{x } 3"] & \boxed{\text{VOLES 126}} \arrow[r, "\text{x } 3"] & \boxed{\text{FOXES 383}} \\ \boxed{\text{EARTHWORMS 33}} \arrow[d, "\text{x } 38"] & & \\ \boxed{\text{MOLES 1270}} & & \end{tikzcd}$$

Having achieved a synthesis of the results, it seems appropriate at this point to consider some of the questions which need to be answered in order to understand the role of fluoride as a pollutant in mammals.

1. More data are needed from a range of plant material on Anglesey to discover the quantities of fluoride available in the primary producers. This is being done by other people working at Bangor. In addition, differences between growth stages of any particular species need to be known.
2. As a corollary to (1) it is important to find if herbivores (voles and mice) select the material that they eat and if so, how. (The extension of the use of vole-selected grasses may be helpful here).
3. Larger samples of mammals with wider age ranges are needed. Most of these are awaiting either processing or analysis already. Samples are needed to establish background levels in non-polluted areas. These are currently being collected.
4. An accurate assessment is needed of the fate of fluoride in wild mammals under laboratory conditions since published findings do not fall readily into a simple pattern. The use of radio-active fluorine may be necessary, despite its short half-life (F^{18} has a half-life of 1.87 hrs.); or

it may be possible to follow physiological pathways without it.

5. The most important need is to find what ecological effects fluoride is having. There are indications of an effect on bone thickness but this in itself means little if it has no bearing on an animal's ecology. It may be that some of the effects of excess fluoride are too subtle to be detected by present methods; or, alternatively, that effects are so violent that animals are not appearing in trapped samples because of them.

6. There seem to be differences between species of mammal in their reaction to fluoride - there may well be differences between populations too. In the experiment with deer-mice quoted above, 6 out of 7 mice were killed in eight weeks by a daily intake of fluoride which left them with less bone fluoride than many of the voles from the smelter site in this study. Presumably the animals on Anglesey with high bone fluoride have evolved to survive in high levels in the eight years since the smelter began operating. Laboratory experiments with controls from unpolluted areas could throw light on this aspect.

7. If Anglesey Aluminium can reach agreement over terms for development, it proposes to double its production of aluminium. Provisional permission has already been received by the Company. It would be worth continuing to monitor changes in mammals, both predators and prey in view of these changes. (Increased production could start in 1981 at the earliest).

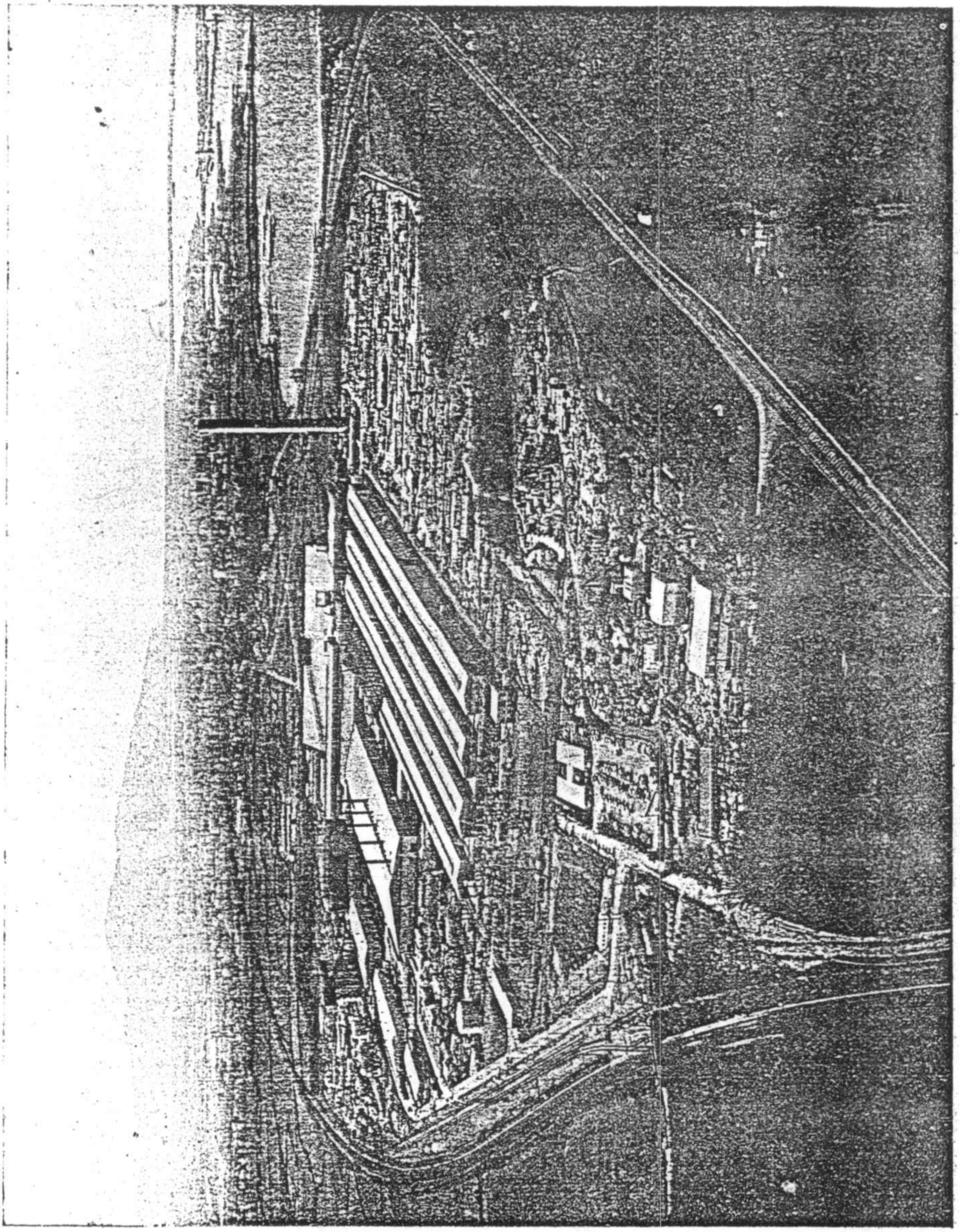
8. It should be possible to use the findings from this work in order to ask the right questions about farm-livestock and human beings on Anglesey and their responses to fluoride (by a curious irony all the current interest in fluoride on Anglesey is in connection with its addition to the water supply).

K. C. WALTON
August 1978

APPENDIX

NOTES ON STATISTICAL TECHNIQUES EMPLOYED

1. The most common statistical test is that which measures the difference between means of a few samples. For this Student's t-test was used, making due allowance for unequal variances when necessary.
2. Where multiple samples were involved (e.g. in the case of groups of foxes from different sites, or earthworms) a single classification analysis of variance was used. In the case of foxes the difference between sites turned out to be non-significant ($F = 1.21$ when $F_{0.05}$ [2.45]). For the earthworms, between site differences were enormous ($F = 19.6$ when $F_{0.01}$ [3.3]). The data were therefore analysed further by multiple comparison among means (SNK test). Most of the variability turned out to be between the smelter site and the other sites. However, a second anova with the smelter site removed showed considerable differences ($F = 6.6$ when $F_{0.01}$ [3.5]). Another SNK test showed that Sites 3 and 5 were responsible for much of the remaining variability.
3. Relationships between two variables (e.g. fluoride and age in foxes) were calculated by regression analysis.



Anglesey Aluminium, Holyhead