F 81/259

No 19..81/7.....

- ☐ NEW PROPOSAL
- ☐ CONTINUING PROJECT
- ☐ FINAL REPORT
- ☒ PROGRESS REPORT

FISHING INDUSTRY RESEARCH TRUST ACCOUNT

TITLE OF PROPOSAL/PROJECT:
AN INVESTIGATION OF THE TOXICITY OF FISH CONTAINING MERCURY AT CONCENTRATIONS IN EXCESS OF PRESENT HEALTH REGULATIONS.

ORGANISATION: UNI OF QLD

PERSON(S) RESPONSIBLE:

FUNDS SOUGHT/GRANTED

YEAR	SOUGHT	GRANTED
1981/82		$ 21,000
1982/83		$ 18,800
1983/84		$ 10,260
1984/85		$ 12,500

RELATED APPLICATIONS:

RECEIVED 8/1/1985 DISTRIBUTED ...../...../19...

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~~FOR~~ Secretary
Fishing Industry Research Committee

F139–12/84

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BUT IN REPLY PLEASE QUOTE:

RES/JAH:JMO

University of Queensland

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FOR ENQUIRIES REGARDING
THIS LETTER TELEPHONE:
377 3784

10th January, 1985

The Secretary
Fishing Industry Research Committee
C/o Department of Primary Industry
Edmund Barton Building
CANBERRA ACT 2600

Dear Sir,

Enclosed are the original plus twelve copies of a report on Dr A. A. Seawright's project "An investigation of the toxicity of fish containing mercury at concentrations in excess of present health regulations".

Yours faithfully,

(S. A. Rayner)
Registrar

Enc.

FIRTA REPORT - DECEMBER 1984

Methylmercury in Shark Flesh - Toxicity for Cats

Introduction

In the past two experiments in this study shark flesh was fed to cats at levels some 10-fold higher than that recommended as suitable for human consumption, i.e. 5 ppm as opposed to 0.5 ppm. Such flesh normally contains selenium in the range of 0.2 to 0.4 ppm. Since Se given in the diet has been shown to exert a protective effect against consumption of potentially toxic levels of methylmercury, the aim of these trials was to determine if such levels were protective against such a high level of mercury. Results indicated that shark flesh containing mercury at 5 ppm and fed daily caused methylmercurialism in the cats almost as rapidly as occurred in animals fed a proprietary diet free from mercury and low in Se - and supplemented to give an intake of these elements similar to that which occurred in shark feeding. In addition supplementation of the diet with mercury and Se at this level caused marked pathological changes in the brain which were evident as early as 45 days from the start of the experiment, although unequivocal clinical signs only became apparent at 71 days. Since this level of intake of methylmercury was abnormally high, it was decided to examine the effects in cats of feeding shark containing lower and rather more realistic levels of mercury similar to those which might occur with regular consumption of commercial shark flesh. In particular, it was decided to determine the rate at which steady state levels of methylmercury in blood occurred in shark fed cats and to observe the level of methylmercury in the brain which corresponded with this level in blood - and to determine if pathological changes were present. In addition, since dietary vitamin E levels interact with Se in the possible protection

effected by the latter against mercury in animals, serial plasma vitamin E levels were determined throughout the experiment. Low plasma vitamin E levels might indicate low vitamin E intake and this might in turn effect the biological availability of Se for the detoxication of mercury. It has been shown also that shark flesh has been shown to contain appreciable levels of arsenic. Arsenic in some forms has been suspected of an interaction with Se which can also effect the relationship the latter element has with mercury in causing mercurialism. Consequently final determinations were made of arsenic, Se and mercury in whole blood in the experimental cats, and content of the elements was measured in various organs at the conclusion of the trial.

Experiment III

In this experiment 13 young cats of either sex were used. One group of 5 cats was given shark flesh and canned food daily in the ratio by weight of 3 to 1. This gave a mean daily intake of methylmercury and selenium of 151 ug and 37 ug/kg/day respectively. Of the remaining two groups of 4 cats, one group was given canned food alone while the other was supplemented as well with methylmercury and sodium selenite at the rates of 170 ug and 26 ug/kg/day respectively.

The shark flesh was derived from a single 4 meter, 15 year old tiger shark caught in Moreton Bay during 1984. The shark was cut up in the fresh state and the muscle stored in 20 kg portions at -20^{0}C. The amount of flesh yielded was 115 kg. A total of 57 samples of the flesh were collected from different areas of the muscle mass and analysed for methylmercury, Se and arsenic giving 1.51 $\pm$ 0.25 ug/g, 0.37 $\pm$ 0.19 ug/g and 7.80 $\pm$ 1.80 ug/g respectively.

Proprietary canned food was shown to contain no mercury and 0.04 ug/g Se. Shark flesh contains both red and white muscle and it was noticed that the red muscle was consistently higher in Se than the white. Accordingly in any particular feed, red and white muscle were present in variable proportions. The Hg, Se and As were determined using the Atomic Absorption Spectrophotometer. No attempt was made to determine the vitamin E in the shark flesh. Vitamin E was determined on freshly collected plasma samples from all cats using an HPLC method.

The quantity of shark flesh available allowed continuous feeding of that group for some 88 days except for one cat which was maintained on the diet for 92 days and then perfused with fixative for microscopic study of the brain. Each of the remaining canned food fed groups were fed for 113 days. The reason these groups were maintained for longer than the shark flesh group was that in the Hg and Se supplemented group, plasma vitamin E levels dropped sharply at 84 days and it was necessary to see if this effect persisted. In addition it was considered desirable to determine if blood mercury levels in this group had reached a plateau. One cat in this group was perfused at 112 days with fixative for microscopic study of the brain. The other canned food fed group was placed in the experiment to serve as a control in relation to plasma vitamin E determinations.

During the study it was the practice to allow the cats out of their individual cages daily for exercise. During this period 3 cats became pregnant, one in the shark fed group and the other in the Hg and Se supplemented group. The foetuses of the shark fed group were recovered at necropsy after 37 days of gestation while the other foetuses were aged 41 and 51 days respectively. Various foetal tissues were assayed for mercury,

selenium and arsenic.

Results

Body weight measured on a weekly basis from the start of the experiment progressively increased in each group as shown in Table 1.Total intake of mercury and selenium for the shark and Hg/Se supplemented groups are also shown.

Table 1

	Initial Mean Body Weight Kg	Final Mean Body Weight Kg	Total Hg Intake ug	Total Se Intake ug
Shark group	1.86 ± 0.26	2.20 ± 0.24	20,779 ± 1910	525 ± 501
Hg & Se Supplemented group	2.04 ± 0.38	2.65 ± 0.38	44,974 ± 6199	7690 ± 942
Non Supplemented group	2.25 ± 0.73	2.84 ± 0.45	0	823 ± 1

The intake of Hg from the shark feeding was less than $\frac{1}{2}$ of that of the supplemented group. The molar ratios of mercury to selenium were 3.95 and 5.84 respectively.

The progressive changes in blood mercury levels are shown in Fig. 1. for the supplemented group maximum Hg levels were reached in about 42 days at 4.5 to 5.0 ug/ml. In the shark fed group on the other hand, blood Hg levels were still rising steadily at 88 days and at that stage had reached mean levels of 2.86 ug/ml. The selenium levels at the beginning of the trial were 0.23, 0.27 and 0.28 for the 3 groups respectively and 0.41, 0.33 and 0.41 at the conclusion of the trial. Initial blood arsenic levels were 0.22, 0.12 and 0.10 ug/ml for the 3 groups respectively and 0.29, 0.05 and 0.05 ug/ml respectively at the conclusion of the trial.

The levels of mercury in the cerebrum, cerebellum, liver and kidney for each of the groups are set out in the Table 2.

Table 2

Tissue Hg Levels (ug/g) (Wet weight)

	Cerebrum	Cerebellum	Liver	Kidney
Shark Group	3.64 ± 0.15	5.62 ± 1.65	26.10 ± 6.66	12.29 ± 2.52
Hg + Se Supple. Group	11.12 ± 2.06	13.27 ± 1.32	93.77 ± 19.86	25.44 ± 4.91
Control Group	.003 ± .004	.008 ± .005	0.024 ± .013	0.024 ± 0.006

In all tissues most if not all Hg is in the organic form with the exception of the liver. The brain Se levels in the shark and Hg + Se supplemented groups were similar but in the liver there was a significantly higher Se level in the Hg and Se supplemented group.

Vitamin E levels in the plasma for the shark fed group decreased from 1082 ug/100 ml at the start of the experiment to 634 ug/100 ml two weeks later and remained around this level for the remainder of the study (88 days). In the canned food control group the plasma vitamin E levels remained at the original level throughout the 113 day period of observations ($965^{\pm}475$ to $1257^{\pm}$ 330 ug/100 ml). Progressive changes in the plasma vitamin E levels for the

groups are set out in Figs. 2 and 3.

In the Hg + Se supplemented group however, two of the cats were in advanced pregnancy at the end of the experiment and in these two animmals the terminal plasma vitamin E levels were elevated (i.e. 1220 to 2025 ug/100 ml and 1327 to 2520 ug/100 ml respectively). The remaining two cats were similar to the unsupplemented canned food controls (742 to 1057 ug/100 ml and 1091 to 1575 ug/100 ml respectively).

Neuropathological examination of the brains of the supplemented cats revealed the presence of widespread necrosis of pyramidal neurones of the cerebrum as previously described for clinically affected animals in earlier experiments. In addition there were scanty pyknotic granule cells in the anteromedial parts of the anterior lobe of the cerebellum. In the shark fed cats there were no lesions in the cerebellum but there were occasional pyknotic pyramidal neurones in the midlaminar regions of the cerebrum.

Studies of the levels of mercury in the pooled organs of the three foetuses from the pregnant shark fed cat revealed 1.36 ug/g wet. wt. in the brains, 0.14 ug/g in the kidneys and 2.68 ug/g in the livers. In the pooled organs of the foetuses from the 41 day Hg + Se cat, there was 5.85 ug/g Hg in the brain, 13.21 ug/g in the kidneys and 12.5 ug/g in the liver. The foetuses from the 51 day pregnant Hg + Se female were examined individually and in the brain there were Hg levels of 6.59, 6.98 and 8.0 ug/g respectively. The kidneys contained 9.39, 9.0 and 10.99 ug/g and the livers 13.95, 14,34 and 15.24 ug/g Hg respectively. High Hg levels in the latter foetuses were found in the skin at 23.36, 24.06 and 25.66 ug/g respectively. The Hg levels in several other organs examined were also high. These observations confirm the vulnerability of the foetuses to intoxication with methylmercury when this

compound is high in the diet of the dam. The content of selenium and arsenic in the foetuses from the shark fed cat were 1.47 and 0.55 ug/g respectively compared with 0.08 and 0.02 ug/g in the tissues of the Hg + Se foetuses indicating that these elements too may readily be absorbed into the foetus from the dietary intake of them by the dam, particularly in the form in which they exist in shark flesh.

Discussion

The intention at the start of the experiment was that the shark fed and the Hg + Se supplemented groups would consume approximately the same amount of Hg and Se respectively. Preliminary analyses of the shark flesh consisting of white muscle only suggested that the mean Hg and Se levels were 1.70 and .26 ug/g respectively. The dose rate of the elements per/kg in cats eating 100 g shark/day would then be 170 and 26 ug of Hg and Se respectively. The dose selected for the Hg and Se supplemented cats were accordingly 170 and 26 ug/kg/day. Subsequent more extensive sampling of the shark including red muscle indicated that the levels of Hg were lower and those of the Se higher than originally considered, namely 1.5 and 0.37 ug/g respectively. Therefore when the trial was in progress the shark fed cats were receiving substantially less Hg and more Se than intended compared with the Hg and Se supplemented cats. In addition and more importantly the consumption of shark flesh was generally lower than expected. The Hg + Se supplemented cats rapidly developed high blood Hg levels and at the end of the trial at 113 days also had brain levels of Hg sufficiently high to be able to cause methylmercurialism. Although there were no clinical signs, moderate typical neuropathological changes were present.

In the shark fed group steady state levels for mercury were not reached

in the 88 day period although this occured in the higher dosed Hg and Se supplemented group. As would have been expected from the total Hg intakes the brain levels of Hg in the shark fed group were less than half those of the supplemented group. Nevertheless, dead neurones could be identified in the cerebral cortex of the cats fed shark. No significant changes were seen in the cerebellum. Clearly the amount of selenium present was not effective in preventing changes due to the methylmercury. Arsenic which is present in sea food as the methylated form, while higher in the shark fed cat tissue than in non arsenic supplemented controls, appeared to be without effect on the animals and in itself would almost certainly constitute no hazard. It was not possible to determine if its presence had any effect on the biological availability of the Se. Moreover, the Hg and Se intakes of both groups were not the same and the animals were killed for study at different times.

This study indicated that feeding shark flesh to cats at 75% of the diet was likely to result in low blood vitamin E levels. The levels of vitamin E which were present after two weeks of feeding were slightly below the normal range for plasma vitamin E concentration in the cat. In both canned food groups plasma vitamin E levels were maintained in the normal range and in some cats actually increased. Despite low plasma vitamin E levels there were no indications of vitamin E deficiency and the body weight of the animals increased during the course of the experiment. It was not possible to show whether the lowered plasma vitamin E levels had any influence on the consequences of the high mercury intake in the shark fed cats since there was insufficient shark available to feed these animals till clinical signs of mercurialism appeared. Also, the mercury + selenium dose rates in the supplemented controls were not sufficiently comparable. An explanation of the fluctuations in the plasma vitamin E levels of the cats in the Hg + Se

supplemented group is not available at this stage. Clearly though, supplementation of potentially toxic doses of methylmercury had no significant effect on plasma vitamin E levels in the cat.

The results of this study indicated that relatively low levels of methylmercury in the cerebral cortex (3 to 4 ppm) due to a regular intake of Hg of 1.5ug/g in the food were associated with necrosis of neurones. It continues to appear that the present approved level of Hg in fish (0.5 ppm) could not justifiably be increased.

R.R. Lauryssen
Chief Investigator 8/1/84.

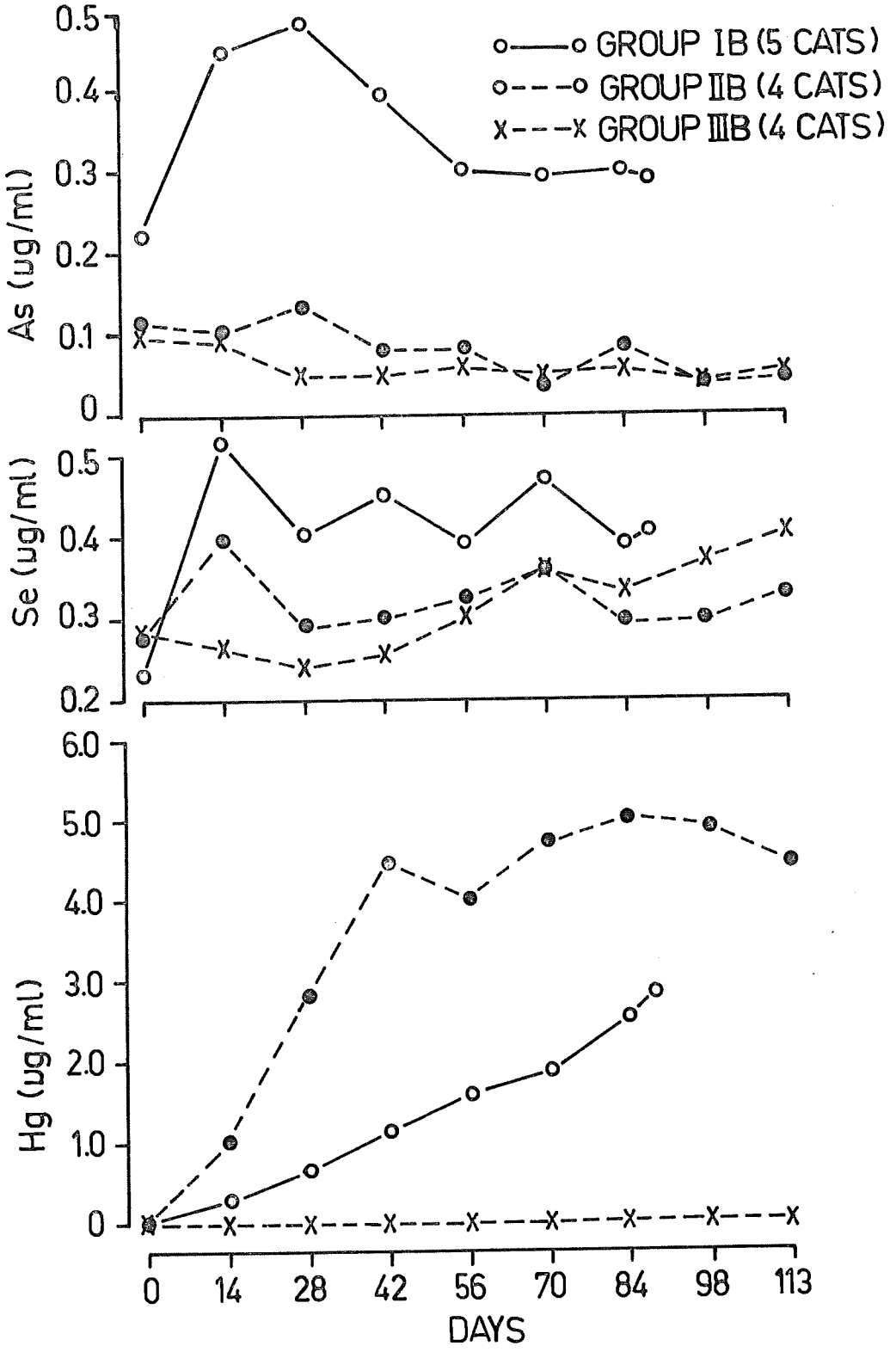

Fig. 1. Progressive mean Hg, Se and As concentrations in whole blood of cats fed Tiger shark diet (Group IB); after daily oral dosing with 170 µg Hg/kg as methylmercuric chloride (CH_3HgCl) and 26 µg Se/kg as sodium selenite (Na_2SO_3) (Group IIB); and control cats fed canned food diet (Group IIIB).

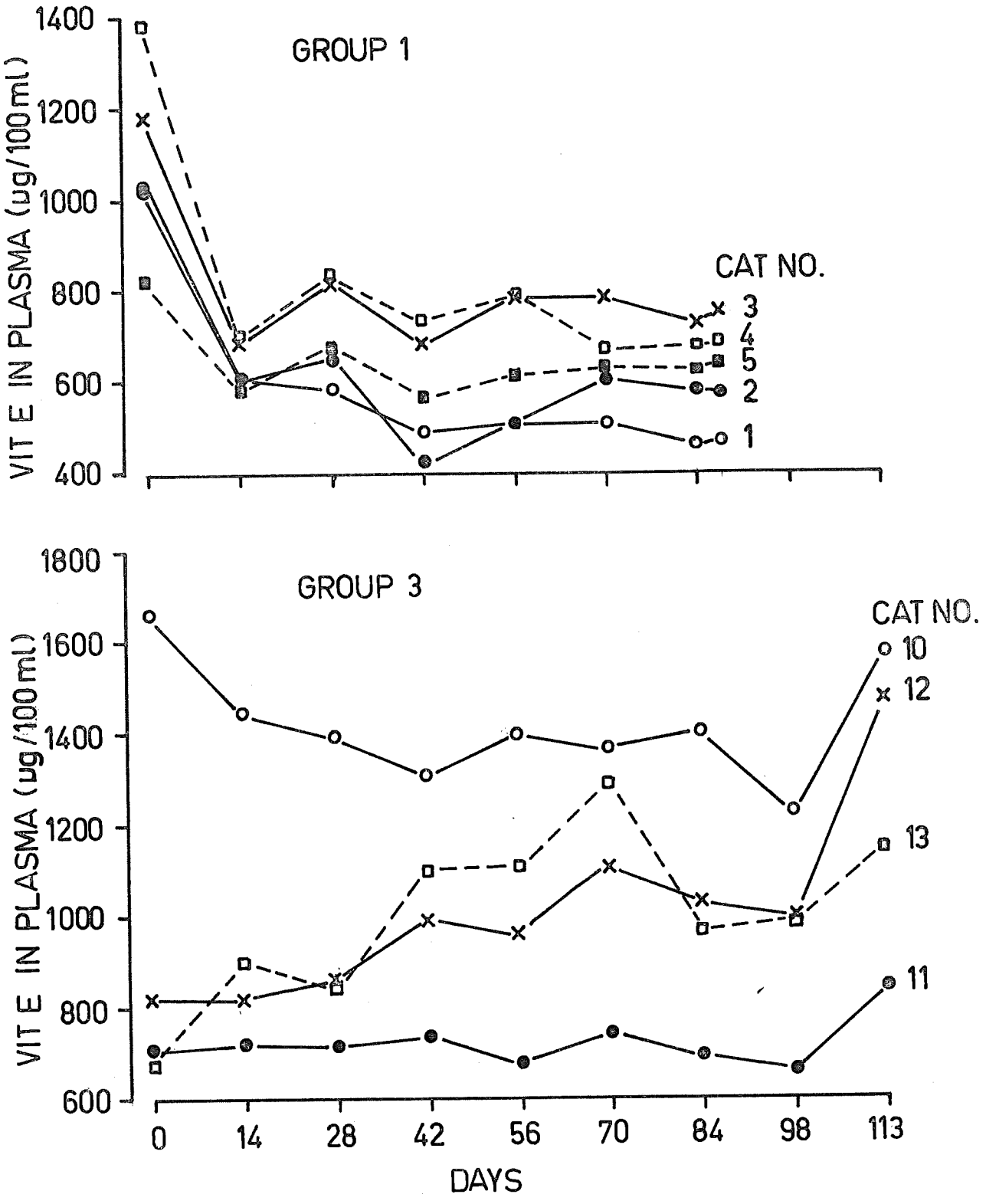

Fig. 2. Vitamin E in plasma of cats fed Tiger shark diet (upper)
Vitamin E in plasma of control cats fed canned food only (lower)

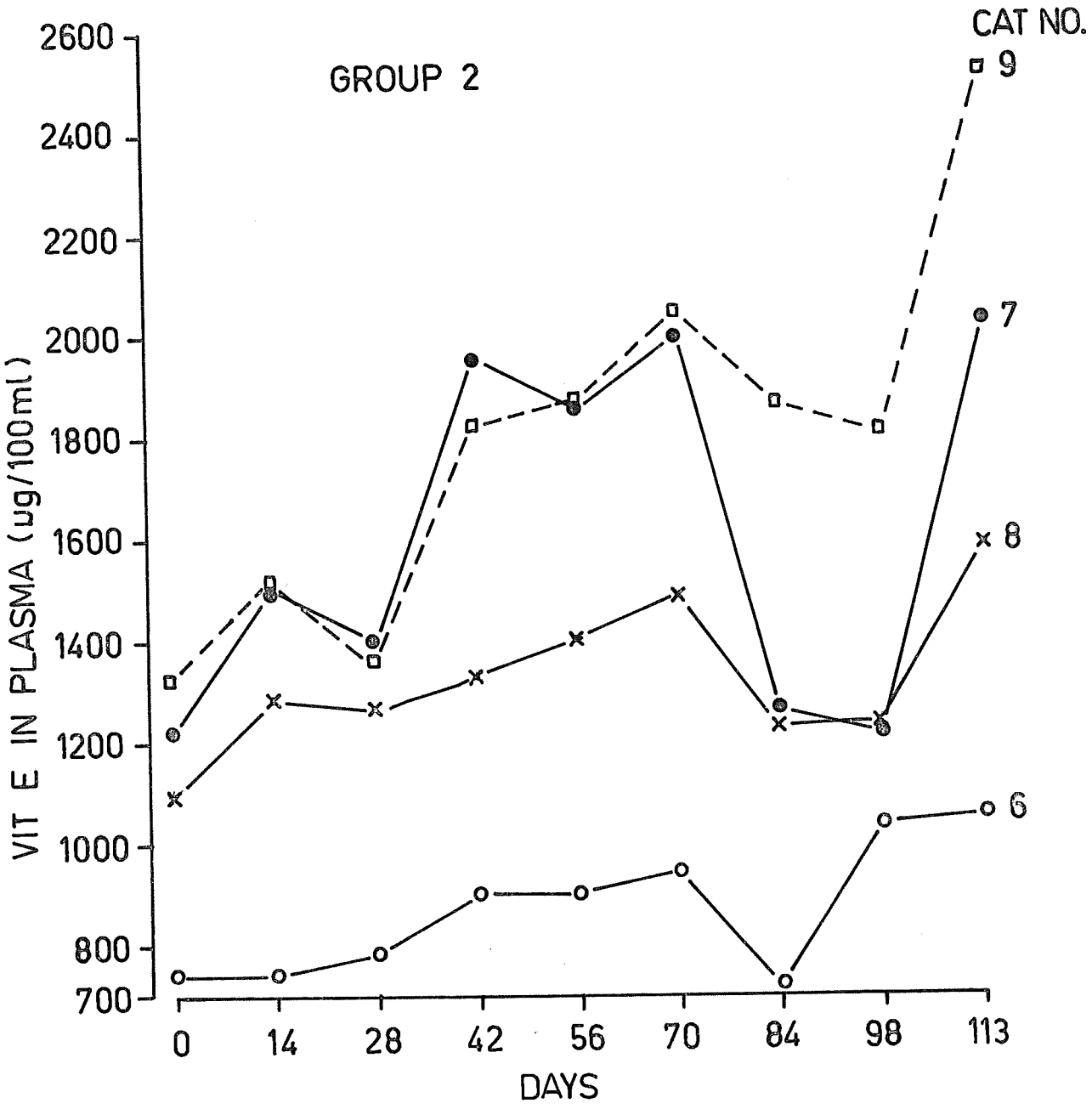

Fig. 3. Vitamin E in plasma of cats orally dosed with CH_3HgCl and Na_2SO_3