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CONTAMINATION OF FRESHWATER BY ^{54}Mn and ^{60}Co

by

C. NICOLAS and R. KIRCHMANN

1974

Report prepared by CEN
Centre d'Etude de l'Energie Nucléaire, Mol - Belgium
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The activity distribution in different organs was studied during both contamination and decontamination periods. In all experiments performed, the external organs retain mostly ^{54}Mn, whilst ^{60}Co is taken up more readily by kidneys and genital tracts. Muscles are relatively little contaminated when comparing activities on a weight basis.

Balance-sheets, metabolic regulating mechanisms and variability in the results are discussed.

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ABSTRACT

The ^{60}Co and ^{54}Mn contamination of minnows, an inhabitant of the Meuse which receives radioactive wastes from nuclear power plants, was investigated. Both radioisotopes enter rapidly in live fish. Maximum ^{60}Co and ^{54}Mn concentrations in fish are reached within a few days after fish-radioisotope contact. Fed fish accumulate more ^{54}Mn and more ^{60}Co, and faster, than starving ones. Decontamination follows several components.

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Contamination of Freshwater Fish by ^{54}Mn and ^{60}Co

The present study was completed in the Radiobiological Department (Phytobiology-Agronomy Section) of the Centre d'Etudes de l'Energie Nucléaire (Mol, Belgium), with the aid of an EURATOM Grant.

CONTAMINATION OF FRESHWATER FISH BY ^{54}Mn AND ^{60}Co

(Nicolas C. and Kirchmann R.)

I. INTRODUCTION

With the building of new Nuclear Power Stations, risks of radiopollution increase every year. For example, radioecological surveys of the river Meuse below the PWR power plant of Chooz demonstrate the existence of several radioisotopes in mud, plants and animals (Cantillon et al., 1969; Micholet-Coté et al., 1973; Merlini M. et Bittel R. 1969; Foulquier L. et al., 1971; Reed J.R., 1971) Fish, one of the human food chain links, contain some radioactivity. ^{60}Co and ^{54}Mn are among the radioisotopes easily detected and they are 2 isotopes of plant and animal oligoelements. Contamination of fish by these 2 radioelements has been little studied up to now.

II. MATERIALS AND METHODS

1. Fish

Minnows (Phoxinus laevis, Cyprinidae) were used as experimental material. They came from an uncontaminated tributory of the Meuse. They were about $1^1/_2$ years of age. The average weight of each individual was 1.67g. They were stocked for 2 weeks prior to an experiment in 2 large aquaria receiving running tap water. They were fed with Tetraphyll Merck, at a rate of 20mg/g wet fish (about the maintainance ration : Berg, 1968).

2. Experiments

a. Contamination period

Three experiments were run in which fish were

1. kept alive and fed
2. kept alive but starved
3. sacrificed just before the beginning of the assay.

For each experiment, several batches of 12 fish were used. Each batch was introduced into a 12 ℓ aquarium consisting of a double plastic bag inside a bucket with holes in it. Its water contained either 5µCi ^{54}Mn or 5µCi ^{60}Co chloride/ℓ. The temperature during the first experiment was (17±1)°C, and during the 2 following ones, (12.5±0.5)°C. Compressed air was injected into the water.

In the case of fed fish, 120 minnows were used for each radioisotope studied. They were fed daily with 20 mg/g fish of tetraphyll Merck. Aquarium water was

filtered on the 7th and on the 11th experimental days for collecting remains (food scraps and faeces) and colonial algae which were processed for radioactivity counting.

In the experiment with fasting fish, 120 minnows were used for each radioisotope studied. Starvation started a week before the beginning of this assay. The aquarium water and radioactive solution were renewed every 4 days.

For the assay with dead fish, 48 minnows were sacrificed 2 hours before being placed in the ^{54}Mn or ^{60}Co contaminated aquaria. To reduce bacterial and fungi growth, streptomycine (50 mg/ℓ) chloramphenicol (25 mg/ℓ) and cycloheximide (25 mg/ℓ) were added to the aquarium water. Water and solution were renewed on the 8th day.

The contamination period lasted 16 days for each experiment.

b. Decontamination

In the experiments using live minnows the fish not sampled during the contamination period were transferred to large aquaria supplied with running tap water. The feeding diet was the same as the one during the contamination period. Decontamination lasted 17 days for fed fish and 33 days for starving fish.

3. Sampling and Counting

Fish and aquarium water were sampled at definite intervals. When sampling, 6 ^{54}Mn and 6 ^{60}Co contaminated fish were removed and blotted with absorbant paper. The samples were then weighed, set in tubes handled for γ spectrometry and fixed with formaline 10 %. Three times 2 mℓ aliquots of aquarium water were removed when sampling fish and were processed for activity measurement. Counting was performed by an automatic γ spectrometer Philips PW4003, equiped with a Na I(Tl) 1"3/4 x 2" type crystal and a 20 ml well. The sensitivity threshold of this apparatus was less than 6 pCi both for ^{54}Mn and ^{60}Co.

Some of the sampled minnows were dissected and studied for ^{60}Co and ^{54}Mn distribution in their organs. The investigated organs were :

1° external organs : gills, skin, fins and head ;
2° internal organs : bones, muscles, digestive tract, liver, kidneys, swimming bladder and genital tract. Each organ was weighed, set in a tube for γ spectrometry measurement, kept in formaline 10 % and counted with the above-mentioned γ spectrometer.

1. In the case of live and fed fish, dissection was performed for organs of fish sacrificed after 1, 4 and 16 days of contamination as well as after 1 and 17 days of decontamination.

2. For live but starving fish, dissection was performed for fish sampled after the above-mentioned time periods as well as after 2 hours of decontamination. For this experiment, fish weredissected after a 33 day decontamination period instead of after 17 days.

3. As for dead fish, their organs were dissected after 8 days of contamination only. In this assay the viscera were difficult to separate one from another and were counted all together.

4. Statistics

The standard deviations to the average of our results were calculated according to the formula :

$$t_{\alpha}^{n-1} \sqrt{\frac{\varepsilon i \; fi \; (xi - \bar{x})^2}{n(n-1)}}$$

where x = variable

$\bar{x}$ = average

f = frequency associated to the variable

n = effective of sample

t = area left to the left of $\alpha/2$

α = risk of error of the first type

t values were obtained from "Tables de Statistiques" (Presses universitaires de Bruxelles).

III. RESULTS

1. Contamination of whole fish by ^{54}Mn

1. Live and fed fish

As soon as the fish areput into contaminated water they accumulate ^{54}Mn (Fig.1). For the first 4 days the activity increase in fish is relatively regular, but from the 5th day onwards the contamination curve becomes irregular. The maximum activity observed is of 70,7 nCi/g wet on day 7.

Fig. 1 also shows that water decontaminates itself rapidly : its activity decreases from 4.992 nCi/mℓ on day zero to 1.050 nCi/ml on day 16. The kinetical curve for water is much more regular than the one for fish.

The concentration ratio * (Fig.1) is higher than 1 from the first day onwards. It reaches 50 both on the 9th and on the 14th day (Table 5). This high value is partly due to the disappearance of ^{54}Mn activity from water. Even when taking this fact into account, this value shows that fish do concentrate ^{54}Mn from water by at least a factor of 10.

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*Concentration ratio stands for activity of fish in nCi/g wet per activity of aquarium water sampled on the same day in nCi/ml.

The remains and the colonial algae collected from all the aquaria on day 7 contained 23.5 µCi ^{54}Mn/g wet. On day 11, the gathered large particles* represented 18.4 µCi/g wet. Their contamination is therefore very high : their concentration ratio being of 8,700 on day 7, and of 47,000 on day 11.

The radioactive balance-sheet of 2 aquaria is reported in Table 1. Out of the 60µCi ^{54}Mn introduced per aquarium, only 36.37 µCi are detected on day 7, and, 12.03 µCi on day 11 in one or other of the inventoried aquarium compartments. Most of the activity measured is in the water. This apparent disappearance of ^{54}Mn will be discussed in the next section. This balance-sheet shows also the heavy ^{54}Mn content of remains and of colonial algae.

Fish decontamination is fast (Fig. 1) during the first 24 hours they are transferred to "cold" water. At this time only 18 % of the fishes' maximum ^{54}Mn content is left (Table 5). But as soon as the 2nd day, the decontamination rate slows down : after 2 and 17 days, respectively 13 and 8 % of the maximum ^{54}Mn activity are still measured. This points out that decontamination follows 2 components at least.

2. Starving fish

The food given to the minnows during the first experiment could ad-absorb radioactivity before being ingested by fish. So an experiment during which fish were not fed was set up. It could indicate too whether fish metabolizing less also accumulate less radioactivity. Minnows can remain in apparent good health without being fed for over 2 months.

^{54}Mn is detected in fish as soon as the first day (Fig. 2). The maximum activity registered corresponds to 46 nCi/g wet on day 14. Thus ^{54}Mn take up by starved fish is quantitatively less, and maximum activity is reached after a longer period of time than when fed fish are assayed. The kinetical curve is more regular for starving fish than for fed ones. The ^{54}Mn concentration ratio in fish is of 0.23 on the first day. It increases irregularly during the contamination period and reaches a maximum of 29 on day 14 (Table 5).

Although during this assay, water and its radioactive solution were changed every 4 days, the water's decontamination is measurable even over such a short time interval (Fig. 2). The only uninvestigated possible ^{54}Mn contaminated compartments in the first experiment are the aquaria walls and air-bubblers.

* large particles stands for remains and colonial algae.

In this second assay, strips of plastic walls were cut and processed for activity counting. Their ^{54}Mn absorption was very high (Table 2). More than half of the ^{54}Mn is absorbed on the tested aquaria walls after 12 days of experiment.

This fact must be kept in mind when assessing the radioactive balance-sheet of an aqarium's content. Table 2 shows that 42.3 µCi out of the initial 59.7 µCi ^{54}Mn are found in either one or other of the studied aqaria compartments. A later experiment showed that air-bubblers can take up the unaccounted for ^{54}Mn. This table shows also that fish do not take up more than 5% of the introduced activity.

Decontamination was studied over a longer time period for starved fish than in the case of fed ones : 33 days instead of 17. During the first 24 hours samples were collected at very short time-intervals (2-6 hours). The sampling schedule was thus modified to try and gather more information on the decontamination components. This is also true for the decontamination study of ^{60}Co contaminated starved fish.

After 2 hours of decontamination the fishes' activity decrease is weak. It is very irregular for the first 24 hours. But after 24 hours (Fig.2) of decontamination only 22% of the maximum ^{54}Mn registered was left (Table 5). Later on the decontamination rate is much slower. After 33 days of decontamination 7% of the maximum ^{54}Mn is still found in fish. This shows again that decontamination follows 2 components at least. The decontamination curves for ^{54}Mn of fish follow the same pattern whether the animals are fed or starved. The absolute value of ^{54}Mn left in starving fish after 33 days of decontamination is of 3.22 nCi/g wet. It is similar to the one registered for fed fish after 17 days of decontamination (4.15 nCi/g wet). It looks as if however much ^{54}Mn a fish contains, when decontamination has reached a certain activity threshold, then it becomes very slow. Perhaps this slow component corresponds to the biological turnover rate.

3. Dead fish

This experiment was designed to determine the percentage of radioactivity absorbed, and the percentage which was adsorbed by fish. No such experiment was found in the literature.

Fig. 3 shows that ^{54}Mn is taken up rapidly by fish. The maximum ^{54}Mn registered is 810 nCi/g wet. It is well over tenfold the maximum amount recorded for live fish. It leads to a concentration ratio of 200 (Table 5). This value was reached on the 16th day. During the assay and for the activity level used there doesn't seem to be any peak of activity for dead fish. This is very different compared to the situation with live fish.

In this assay, the ^{54}Mn level in water is more regular than when live fish were used. This is testified by the balance-sheet (Table 3) : hardly any ^{54}Mn is recorded for the aquarias' walls. Much of the introduced activity has been taken up by fish (20%) as well as by bacteria (20%).

4. Discussion

Dead fish accumulate so much ^{54}Mn because they lack the regulating mechanisms which exist in live fish. Since take up by dead fish is high, and although the water retains most of its activity, the ^{54}Mn concentration ratio reaches 200 on the last day of experiment. This is 4 times higher than for living fish. If the experiment with dead fish had been carried on for a longer period, one would probably have reached higher concentration values.
Some data can be found in the literature concerning work performed with live fish. De Bortoli and al. (1969) found that ^{54}Mn concentration is of the same order of magnitude for 3 species of fish *(Perca fluviatilis, Scardinius erythrophtalmus, Lepomis gibbosus)* sampled from 4 different Italian lakes. Nevertheless the concentration ratio range is relatively wide : from 148 to 659. These authors consider it possible to establish the approximate ^{54}Mn amount in fish, when the ^{54}Mn amount in water is known. This is often difficult for it implies that equilibrium exists between fish and water activities. This equilibrium is rarely reached, since fish have a delayed response to contamination and to decontamination.

Foulquier et al.(1971) used laboratory conditions to study the ^{54}Mn contamination of the carp. The maximum ^{54}Mn level is reached after 9 days in the carp : this is a slightly longer time period than in our assay. This maximum ^{54}Mn level is followed by a state of equilibrium : we do not find this in our investigation. As to desorption, they also find that it follows 2 components : a short one of 1.5 days and a longer one of 47 days. When sediments are added to the aquaria, the carp could ingest only a small amount of ^{54}Mn from them. This would mean that in our investigation the high contamination of fed fish compared to the somewhat lower activity of starved ones is not only a question of ingesting contaminated food, but also because fed fish have a higher metabolism than starving ones.

2. Contamination of whole fish by ^{60}Co

1. Live and fed fish

The contamination curve for ^{60}Co (Fig.4) resembles the one established for ^{54}Mn (Fig.1). The ^{60}Co content of the minnows increases rapidly during the 1st few days. From the 5th day onwards it becomes irregular. Maximum activity is registered on the 4th day : 52.6 nCi/g wet. This is a lower value than in the case of a ^{54}Mn fed fish contamination, but it is reached faster.

The activity of water diminishes slowly, passing from 4.988 nCi ^{60}Co/ml on day 0 to 3.799 nCi/ml on day 16. This decontamination curve for water is quite regular.

The fish concentration ratio values are much lower for ^{60}Co than for ^{54}Mn. This seems normal, since the fish contain less ^{60}Co than ^{54}Mn whereas the water retains more ^{60}Co than ^{54}Mn. Nevertheless, except for the first day of experiment, where it is 0.80, the concentration ratio is always above 1. It's maximum is 11 on day 4 (Table 5).

The remains and the colonial algae sampled in all the aquaria contain 11.10 and 7.00 µCi/g wet respectively on days 7 and 11. The ^{60}Co retention by these large particles is several times less than that of ^{54}Mn. Nevertheless their concentration ratio is still very high : 2,500 and 1,800 respectively on days 7 and 11.

The activity balance-sheet, established for 2 acquaria (Table 1) indicates that most of the ^{60}Co introduced is found in the water. Nearly all of the initial ^{60}Co is detected in one or other of the studied compartments after several days of experiment. The table again shows the high activity content of large "particles".

The results for the decontamination period (Fig.4) show that only 14% of the maximum ^{60}Co registered (on day 4) is left after one day (Table 5). But as soon as the second day of decontamination, the desorption slows down. After 2 and 17 days of decontamination there are respectively 11.9 and 8.6% ^{60}Co left in the fish (compared to maximum values). Thus the decontamination pattern for ^{60}Co is similar to the one for ^{54}Mn. It could mean that decontamination of fish, after incorporation of ^{60}Co, follows at least 2 components too.

2. Live but starving fish

Fish contamination is easily detected after a 24 hour period of contact between fish and radioactive water (Fig.5). The maximum ^{60}Co registered is of 12.9 nCi/g wet on the 7th day. This is 4 times less than for fed fish and after a longer contact period. It is also less but earlier on than for ^{54}Mn contaminated starving fish. Just as for ^{54}Mn, the ^{60}Co curve for starving fish is more regular than for fed ones.

The water retains most of its activity during the whole experiment (Table 2). This means that there is little ^{60}Co loss, and, that there is hardly any activity reported for the aquaria walls. The balance-sheet shows this too (Table 2).

The ^{60}Co concentration ratio in starving fish is 0.43 on the first day. Its highest value is 2.66 on day 7 (Table 5). For the whole of the 16 day period it is just a little over 1. Such a relatively low value is consistent with the fact that starving fish are relatively little contaminated by ^{60}Co, which remains in the water.

The decontamination rate is irregular : peaks of quite high ^{60}Co activity are registered upon transfer of the fish to "Cold" water. After 33 days of decontamination, there is still 26 % of the maximum activity measured during the contamination period (table 5). In this case the supposed 2 components of the decontamination curve are less visible. But in absolute values, the ^{60}Co left in starving fish after 33 days of decontamination (3.46 nCi/g wet) is similar to the ^{60}Co left in fed fish after 17 days of decontamination (3.77 nCi/g wet). This does support the idea of multiple decontamination components, and, that under a certain activity level, ^{60}Co decontamination is very slow. This interpretation is similar to the one given for ^{54}Mn fish decontamination.

3. Dead fish

The contamination curve (Fig. 6) reveals a very similar pattern to the one obtained for ^{54}Mn - dead fish (Fig. 3). The only significant difference is that ^{60}Co is less absorbed than ^{54}Mn. Here too, no activity peak is registered during a 16 day contamination period. The maximum ^{60}Co recorded (460 nCi/g wet) is 9 times that for alive fish (52.6 nCi/g wet).

The water activity is fairly constant. The assessed concentration ratio is 9 as soon as the first day. It reaches 100 on day 16 (Table 5). Table 3 shows that 10 % of the activity introduced is absorbed by fish and 16 % by bacteria. Hardly any ^{60}Co is absorbed by the aquaria walls.

4. Discussion

As in the case of ^{54}Mn fish contamination, this large ^{60}Co uptake by dead fish could be due to the lack of a metabolic regulation mechanism in dead fish. As soon as fish die, they do not excrete any longer. This is further supported by 2 facts. The first one is that casualties during experiments with live fish contain more radioactivity than fish alive when sampling. The second fact is that large particles during these experiments contain a heavy percentage of activity : they don't possess any metabolic regulation mechanism either. This could mean that the difference between live and dead fish lies not only in different accumulation rates between them, but also when reaching a certain activity threshold, most of the radioisotopes are excreted by live fish but not by dead fish. This is supported by the fact that activity peaks are registered for live but not for dead fish. This means too that when feeding fish in an active solution with "cold" food, this food can become radioactive if not ingested immediately. To prevent fish getting contaminated in this way or by eating faeces, grids could be used to separate fish from bottom remains. Another method is to train

certain types of fish to feed straight away on distributed food (Berg, 1968) or from bottles (trouts, Foulquier, personal communication).

Experiments with diluted effluents have been carried out with starved fish, as well as decontaminations in the Meuse of laboratory contaminated starved fish with artificial isotopes. The results (Micholet-Coté et al., 1973) are parallel to the ones obtained in totally artificial conditions.

Radiocobalt fish contamination has been more studied than radiomanganese contamination, although no studies have been reported for dead fish. Lowman (1963) registered a discrimination against ^{57}Co, ^{58}Co and ^{60}Co in the food chain : water → plankton → omnivorous fish → carnivorous fish. Bittel (1968) mentioned that in laboratory assays the ^{60}Co concentration ratios range from 60 to 10.000. This wide range is assigned to : 1 an approximative realization of equilibrium between aquatic species and water, 2 physical factors, metabolism, age of animals and physico-chemical forms of ^{60}Co. He acknowledges that in an aquatic food chain, ^{60}Co undergoes a clear negative discrimation : 10% for omnivorous fish, a few % for carnivorous fish. This doesn't prevent Antonelli et al., (1971) from finding ^{60}Co and ^{58}Co concentration factors in samples of various fish species to be 40 ± 15 in the river Garigliano.

Reed (1971) working with *catfish* and Merlini et Bittel (1969) with *sunfish* and *goldfish* performed experiments closer to ours. Both groups of investigators found a fast ^{60}Co contamination. Reed found that maximum activity was attained after 1 day and Merlini after 6 days of experiment : our value (4 days) belongs to the same range. During the decontamination period, Merlini obtained a linear ^{60}Co diminution, whilst Reed observed a 3 phase (rapid, intermediate, slow) ^{60}Co decrease. Reed observed that the decontamination pattern is similar whether the fish were contaminated with water or food (fed with ^{60}Co injected worms).

3. Contamination of fish organs by ^{54}Mn

To complete this study, the distribution of both ^{54}Mn and ^{60}Co in several fish organs was determined after dissection (see materials and methods).

1. Live and fed fish

All the fish organs studied are radioactive from the 1st day onwards (Fig.7). The contamination level varies from organ to organ. The most contaminated organs (nCi/organ, Fig.7) are the bones and the fins (1.664). Then follow the kidneys (1.126), the head (0.838), the skin (0.518) and the muscles (0.486). The other organs contain little activity. When one considers the weight of the organs counted (results in nCi/g wet, Fig.8), the fins, the bones and the head are the most contaminated organs. The muscles are in this respect much less active. The concentra-

tion ratio differs thus from one organ to another (Fig.9). It is higher than 1 for the external organs.

On days 4 and 16, all the organs, except the liver, contain more ^{54}Mn than on the 1^{st} day (Fig.7). Skin, fins, bones, head, muscles and digestive tract are the most radioactive organs, but none of them is particularly radioactive. The organs are all more contaminated on day 4 than on day 16. These 2 facts tend to show that for the considered activity levels, no minnow's organ can be truely considered to stock ^{54}Mn. Comparing the figures of Fig.7 to the ones of Fig.8, the impact of the organs'weight on their activity content is emphasized again. The concentration ratios are highest on day 4 reaching 227 for fins (Table 5). Muscle concentration ratio on that day is 12.5 only (Fig.9), which is relatively lower than that for some of the other organs.

The outline of the figures established for 1 and 17 days after the beginning of decontamination (Fig.7) resembles that of the contamination period. The most contaminated organs are still the fins, the bones, the head and the muscles. When the organs' weights are taken into account (Fig.8), the difference of ^{54}Mn take up by external and internal organs is stressed again.
On table 4 the percentage activity of the external organs grouped together is reported. On day 4 over 1/3 of the total fish activity is incorporated by the external organs. This value increases, and reaches 61% on the 17^{th} day of decontamination.

2. Live but starving fish

On the first day of contamination (nCi/organ, Fig.10) the activity is detectable for several organs : skin, fins, bones, head, muscles. The viscera contain very little ^{54}Mn. When the organs'weights are taken into account (nCi/g wet, Fig.11), the fins are the most active organs. Then follow the muscles and the head. The concentration ratios (Fig.12) differ widely from one organ to another. They are higher than 1 for fins only.

On days 4 and 16 the organs contain more ^{54}Mn than on day 1 (Fig.10). The most contaminated organs are the fins, the bones, the head and the muscles. On day 4 there is more activity in fed fish than in starving ones. On day 16 it goes the other way round. The ^{54}Mn distribution pattern is similar whether the fish are starved or fed, except that viscera of starving fish contain even less radioactivity than that of fed ones. The difference is especially striking for the digestive tract : this could mean that when counting fed fishes' digestive tracts they contain radioactive remains. When the organs' weights are considered (Fig.11) the most contaminated organs are the fins on day 16 : 104 nCi/g wet,

this leads to a concentration ratio of 157 (Fig.12, Table 5) for them, which is the highest concentration ratio registered for this assay. The viscera (Fig.11), except the swimming bladder containing no radioactivity at all, show a low ^{54}Mn content.

The pattern of ^{54}Mn distribution in fish organs during the decontamination period resembles that of the contamination period (Fig.10). This means that the activity decrease is proportional for the different organs examined. This is supported by Table 4 giving the percentage of total fish activity in the grouped external organs. They contain 2/3 of the whole fish activity. This value is higher than that for fed fish organs, especially during the contamination period.

3. Dead fish

The fish organs analysed all contain ^{54}Mn (Fig.13). Just as for live fish, the most active organs are the fins (227 nCi/organ). The head is very active as well. Gills, bones, muscles and viscera accumulate relatively less ^{54}Mn when the fish are dead than when they are alive. But in absolute values these dead fish organs are just as or even more radioactive than organs from live fish. The same trends are visible when dealing with the dead fish organs' weights (Fig.13). Nevertheless over 2/3 of the activity (Table 4) is accumulated by external organs. This is somewhat more than for live fish.

Concentration ratios are reproduced on Fig.14. They reach 500 for fins : this is more than twice the maximum value for live fish (227 for fed fish on day 4, Table 5). All the concentration ratios of dead fish are well over 1 even for viscera.

4. Discussion

The work performed by Foulquier et al. (1971) on live and fed fish tends to point out that absorption varies from one organ to another. They find the digestive tract to be the critical organ for it retains 36.8% of all the fish activity. In our investigation it contains only from 6.8 to 19.3% ^{54}Mn throughout the experiment. They group the organs on the basis of ^{54}Mn take up : intestine, stomach and gills absorb ^{54}Mn rapidly; skeleton, liver and muscles more slowly. They find that organs taking up ^{54}Mn rapidly desorb it fast too. Comparison with our results is difficult since they assayed on a different fish species (carp) and they don't mention the fishes' food diet. It can only be said that such factors seem to have heavy consequences on ^{54}Mn contamination.

4. Contamination of fish organs by ^{60}Co

1. Live and fed fish

After 1 day of contamination, ^{60}Co is detected in all the studied fish organs (results expressed as nCi/organ, Fig.15). ^{60}Co is more uniformly distributed than

^{54}Mn in these organs. Organs in close contact with water (such as fins and skin) contain little more ^{60}Co than viscera. The most contaminated organs are nevertheless the head (1.023) and the muscles (0.928). This difference in radioactivity distribution is stressed when one takes the organs' weights (Fig.16) into account. The concentration ratio is above 1 for most organs. It is of 2 for digestive tract and fins (Fig.9).

Four days after contamination (maximum contamination of whole fish, Fig.15) shows that there is a larger amount of ^{60}Co activity in all the organs than on the 1st day. Muscles, digestive tract and head contain most of the fish activity. But when the organs'weights are considered (Fig.16), then the kidneys and the genital tract are the most contaminated organs. This is stressed on day 16, especially for kidneys, although the fish as a whole is less radioactive on day 16 than on day 4. Contrary to the case of ^{54}Mn take up, minnows do seem to possess organs which accumulate ^{60}Co preferably (kidneys especially). Concentration ratios are lower for ^{60}Co than for ^{54}Mn (Fig.9). The highest one registered is of 81 for kidneys on day 16 (Table 5).

The different organs decontaminate themselves at similar rates (Fig.15 and 16). The highest activity(nCi^{60}Co g wet),recorded on days 1 and 17 of this period is in the kidneys. The difference in ^{60}Co content between the kidneys and the other organs is large. So the kidneys do seem to concentrate more ^{60}Co than the other organs.

Table 4 shows that external organs retain only 1/3 of the total fish activity. Their ^{60}Co content falls throughout the assay from 39% on the 1st day of contamination to 27% on the 17th day of decontamination. So external organs contain less ^{60}Co than ^{54}Mn, and, in these organs, ^{60}Co content diminishes with time (contrary to ^{54}Mn content).

2. Live but starving fish

All the fish organs are active after 24 hours of contamination (Fig.17). The most contaminated organs are the head (1.400 nCi/organ) and then the muscles. When activity is expressed as nCi/g wet (Fig.18), the ^{60}Co content of the organs is more uniform than in the case of a ^{54}Mn contamination. Gills, fins, bones reach a concentration ratio well over 1, while kidney and head concentration ratios are near 1 (Fig.12).

There is an increase in ^{60}Co content for all the organs studied after 4 and 16 days of contamination (Fig.17). Nevertheless the general pattern of the activity's

distribution resembles that on day 1. All the organs are less contaminated when fish are starving than when they are fed. The viscera accumulate more ^{60}Co than ^{54}Mn. This is even truer when the organs'incorporated activities are compared on a weight basis (Fig.18). The kidneys are then the most contaminated organs (43 nCi/g wet on day 16), followed by the genital tract (31.5 nCi/g wet on day 4). The highest concentration ratio recorded is that of kidneys on day 16 which is of 10.2 (Table 5). This is low compared to values for ^{54}Mn (e.g. : 157 for fins).

During the whole of the decontamination period, the general pattern for ^{60}Co distribution in organs is conservative (Fig.17). This again means that there is a proportional diminution of activity for all the organs. This remains true, when one considers the organs' weights. The kidneys still contain a fair amount of ^{60}Co.

The muscles retain little activity compared to the data obtained with the other experimental designs with live fish. But the starving fishes' external organs show a relatively high ^{60}Co content compared to fed fishes' external organs. This is reflected in Table 4.

3. Dead fish

Just as in the case of live fish, the ^{60}Co distribution (Fig.13) is somewhat more uniform than that of ^{54}Mn. For dead fish, ^{60}Co is more absorbed by the head (77nCi/organ) than by the other organs. The viscera are more contaminated by ^{60}Co than by ^{54}Mn. When the organs' weights are considered (Fig.13) the most contaminated organs are the fins. In this respect, the muscles are relatively less and the viscera more contaminated by ^{60}Co than by ^{54}Mn. Still, nearly 2/3 of the activity is reported for organs in contact with water (Table 4). This is just a little less than for ^{54}Mn contaminated dead fish.

The concentration ratios are well over 1 for all the organs studied : 17 for muscles, 30 for viscera, 168 for fins (Fig.14). The fins have the highest value (Table 5).

4. Discussion

Reed (1971) reports that organs contaminate themselves with ^{60}Co at different rates and to a different extent. The most contaminated organ in his investigation is the gut, although the fish aren't fed during the assays. The gills are very active too. Because of their high activity, Reed considers the gills to play an important part for the ^{60}Co's entry. If this should be so, then when the activity is given with the diet, its entry path differs than when fish are submitted to direct contamination. It can also be stated that entry path and high organ activity may not be related: entry path is more related to activity

turnover in the organ. (Berg 1968) Reed also considers the kidneys to be the target organ for ^{60}Co contamination.

As for Merlini et Bittel (1969) they think that ^{60}Co enters fish not only through gills but also with swallowed water. But it cannot be forgotten that freshwater fish live in a Hypotonic environment; thus they are supposed to drink very little. These authors also find the digestive tract to be very active, as well as the liver. They also consider the kidneys to be the target organ.

5. Variability in the results

Fish sampled on the same day contain varying amounts of ^{60}Co or ^{54}Mn. This renders the concentration ratios difficult to assess. Most authors reviewed find the same difficulty. This variability hampers the interpretation of the results and it must be taken into account when performing monitoring studies. It is necessary to know to what degree a fish sample is reliable when monitoring a river plant. To increase reliability in experimental work one can experiment with larger batches of fish and measure activities with a whole-body counter when available. This variability could be partly related to the metabolic state of fish: starving fish (with lower metabolic rates than fed ones) and dead fish give less variable data than fed ones (e.g.: Table 6) because their metabolic state is more uniform than the one of fed fish. So the variability in the data is not only due to the amount of food the fish ingest. This is also supported by the fact that a very contaminated fish from a batch has all its organs, not only the gut, which are more active than the average fish organs of the same batch. Merlini and Bittel (1969) found this too.

IV. CONCLUSIONS

It can be concluded that :

1. Both ^{54}Mn and ^{60}Co enter rapidly in live fish, and a peak of maximum activity is registered. The maximum concentration ratio is of 50 (in the case of fed fish contaminated by ^{54}Mn). In all cases it is reached within a few days after the fish-radioisotope contact. Whether contamination is performed with ^{54}Mn or ^{60}Co, fed fish take up more activity and faster than starving ones.

2. The radioisotopes' contamination rate and their final concentration varies from one organ to another and with the radioisotope tested. The external organs retain mostly ^{54}Mn, while ^{60}Co is taken up more readily by kidneys and genital tract.

3. Decontamination follows several components. The ^{54}Mn and ^{60}Co organ distribution differs little during the decontamination period from that during the contamination period.

4. The remains and the microorganisms accumulate ^{54}Mn and ^{60}Co very strongly. One must keep this fact in mind when establishing balance-sheets.

5. Dead fish take up ^{54}Mn and ^{60}Co very fast. After 2 weeks of contamination their activity level is more than tenfold than that of live fish. The contamination curve looks different than the one established for live fish : it is more regular, and doesn't show any peak within a 16 day contamination period. The activity of external organs is higher for dead than for live fish. It seems that not only the absorption rates differ for live and dead fish, but also that dead fish lack a metabolic regulating mechanism.

6. Muscles are little contaminated. It is only due to their mass that they contain an important fraction of ^{60}Co or ^{54}Mn. Being of consequence as a human food chain link, they present a particular interest in monitoring studies.

7. The variability in the results stands out as a problem both in research work and in monitoring studies.

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TABLE 1

^{54}Mn and ^{60}Co balance-sheet in aquaria during contamination of fed fish

Isotopes and compartments/ aquarium	Radioactivity / compartment in µCi after	
	7 days contamination	11 days contamination
1) ^{54}Mn :		
- 12 fish	0.64	0.27
- 12 ℓ water	32.36	4.65
- large particles	3.37	7.1
Total	36.37	12.03
Activity introduced	± 60	± 60
2) ^{60}Co :		
- 12 fish	0.28	0.15
- 12 ℓ water	53.73	51.71
- large particles	1.1	4.63
Total	55.15	56.29
Activity introduced	± 60	± 60

TABLE 2

^{54}Mn and ^{60}Co balance-sheet in aquaria during contamination of starving fish

Compartments	Radioactivity/compartment in µCi/aquarium	
	^{54}Mn (after 12 days of contamination)	^{60}Co (after 16 days of contamination
11 live fish	0.439	0.124
2 dead fish	0.259	0.091
12 ℓ water	9.564	53.928
aquarium wall	32.074	1.028
air - bubbler	?	?
Total	42.336	55.171
Activity introduced	59.652	62.124

TABLE 3

^{54}Mn and ^{60}Co balance-sheet on day 8 in aquaria enclosing dead fish

Compartment	Activity in µCi/aquarium after an initial contamination of	
	31.25 µCi ^{54}Mn	30.28 µCi ^{60}Co
12 fish	6.52	3.26
Filtered water	18.76	19.91
Bacteria	6.74	5.06
Total	32.02	28.23

TABLE 4

Activity accumulated by external organs of fish in per cent of total fish activity

Period of	Live and fed fish			Live but starving fish			Dead fish		
	sampling day	^{54}Mn	^{60}Co	sampling day	^{54}Mn	^{60}Co	sampling day	^{54}Mn	^{60}Co
Contamination	1	--	39	1	68	59	8	67	63
	4	36	35	4	61	51			
	16	59	33	16	62	51			
Decontamination	1	56	26	1/12 (=2 hours)	58	56			
	17	61	27	1	64	52			
				33	63	52			

TABLE 5

Main data from ^{54}Mn and ^{60}Co contamination experiments of minnows. The numbers in brackets correspond to the experimental day on which the maximum concentration ratio was observed

Type of data	Fed fish	Starving fish	Dead fish
Maximum concentration ratio in :			
whole fish ^{54}Mn	50 (9,14)	29 (14)	200 (16)
^{60}Co	11 (4)	2.66 (7)	100 (16)
Organ ^{54}Mn	fins : 227 (4)	fins : 149 (16)	fins : 500 (8)
^{60}Co	kidneys : 81 (16)	kidneys : 10.2 (16)	fins : 168 (8)
% decontamination of fish after			
1 day following ^{54}Mn	18	22	-
17 days contamination	8	7	-
1 day following ^{60}Co	14	-	-
17 days contamination	9	26	-

TABLE 6

Variability in ^{54}Mn and ^{60}Co uptake by fish for the different experiments performed

Length of contamination period (in days)	Laboratory conditions applied to fish	Radioactivity measured in nCi/g wet ± t_{α}^{n-1} standard deviation	
		^{54}Mn	^{60}Co
1	live, fed	4 ± 1	6 ± 4
	live, starving	1.1 ± 0.9	2.1 ± 0.5
	dead	130 ± 50	50 ± 30
4	live, fed	50 ± 50	60 ± 90
(8 for dead fish)	live, starving	11.3 ± 5.6	8.0 ± 7.1
	dead	270 ± 50	160 ± 50
16	live, fed	50 ± 40	40 ± 30
	live, starving	2.4 ± 8.3	8.4 ± 3.5
	dead	800 ± 300	400 ± 100

ABBREVIATIONS USED FOR THE FIGURES

G : Gills

S : Skin

F : Fins

B : Bones

M : Muscles

DT : Digestive Tract

L : Liver

K : Kidneys

SB : Swimming Bladder

GT : Genital Tract

H : Head

F : Formaline

Vi : Viscera

Fig. 1

^{54}Mn: Changes in activity of whole fed fish and aquarium water with time

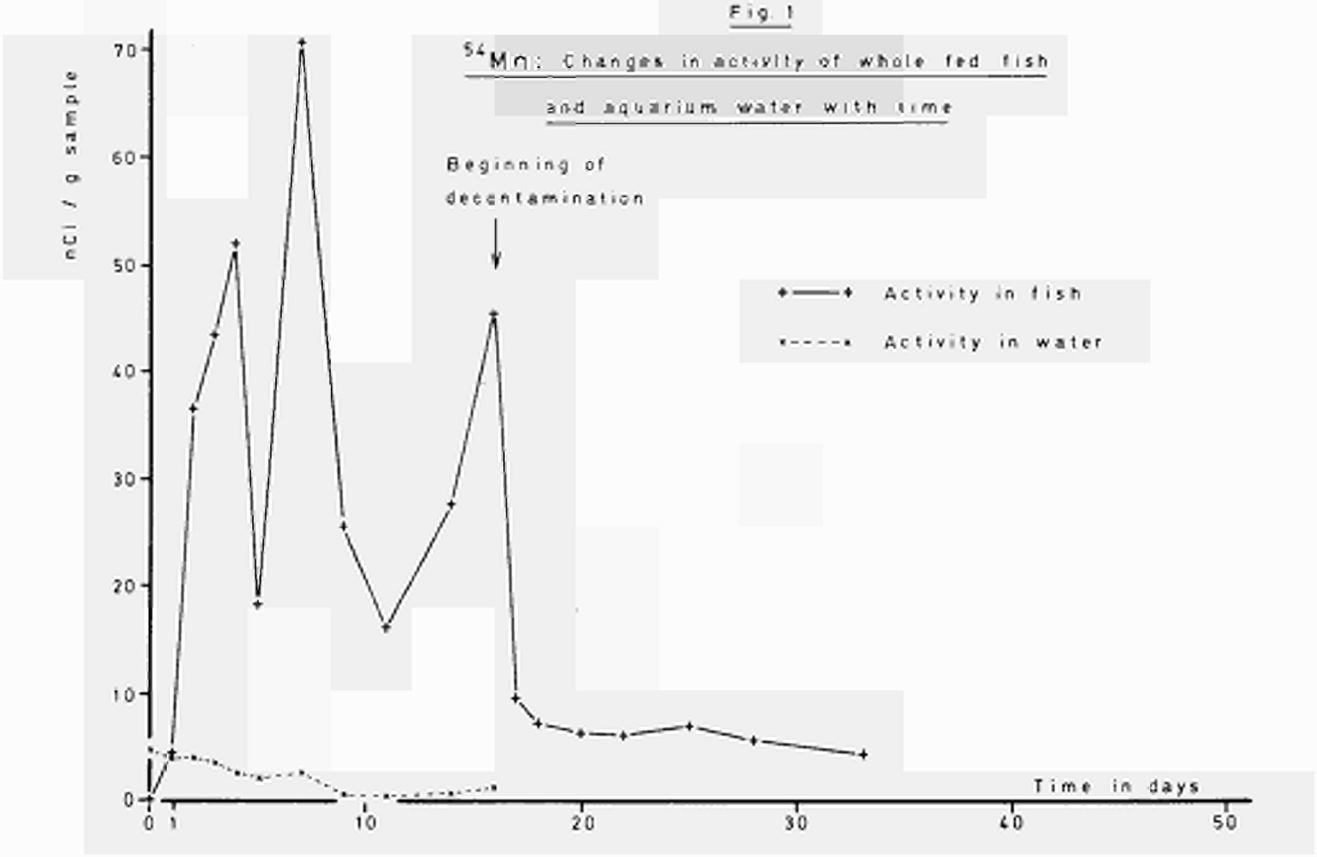


Fig. 2

^{54}Mn: Changes in activity of whole starving fish and aquarium water with time

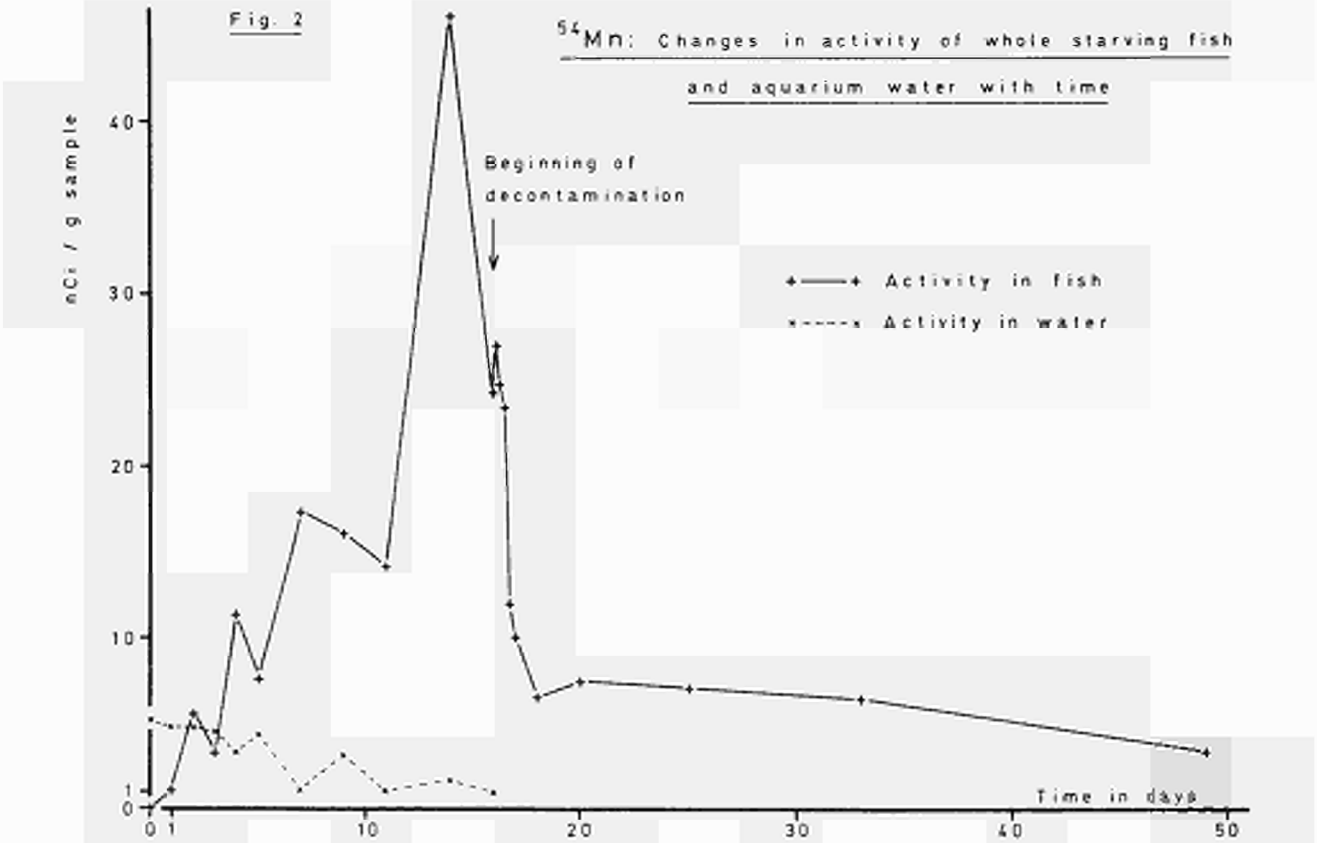


Fig. 3

^{54}Mn: Changes in activity of whole dead fish and aquarium water with time

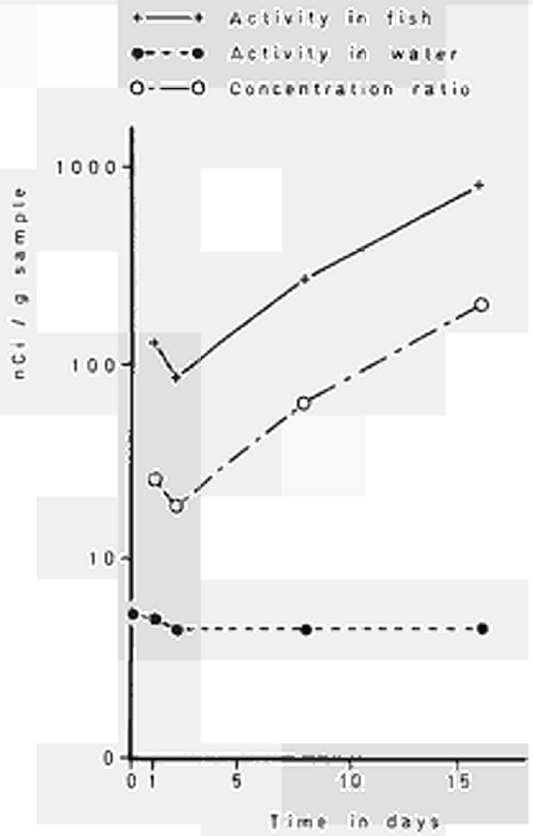


Fig. 4

^{60}Co: Changes in activity of whole fed fish and aquarium water with time

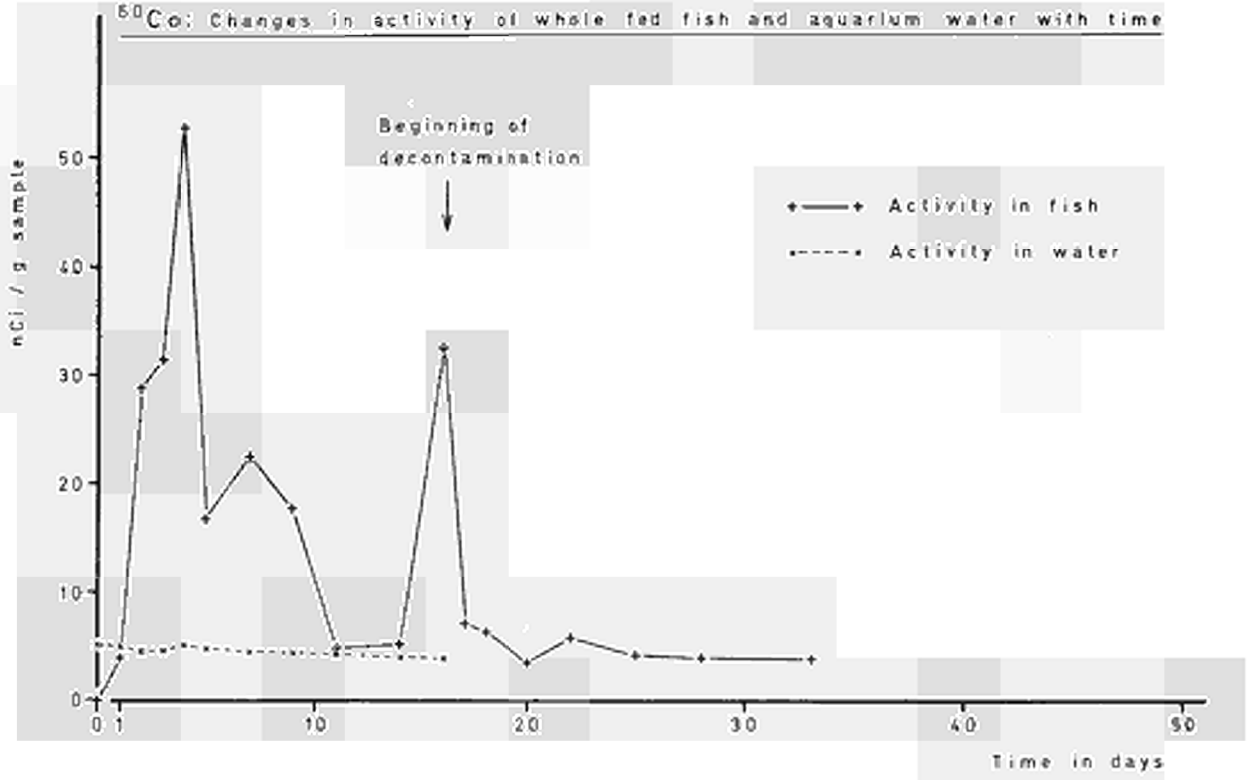


Fig. 5

^{60}Co: Changes in activity of whole starving fish and aquarium water with time

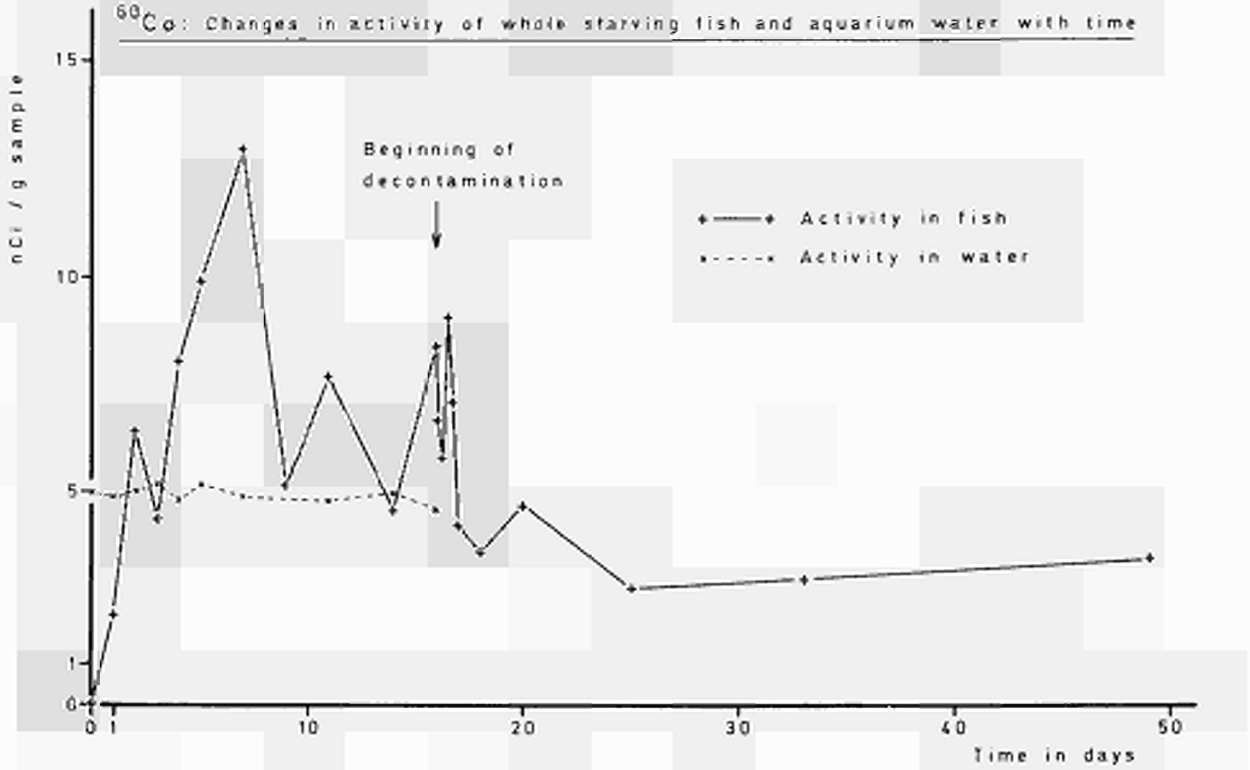


Fig. 6

^{60}Co: Changes in activity of whole dead fish and aquarium water with time

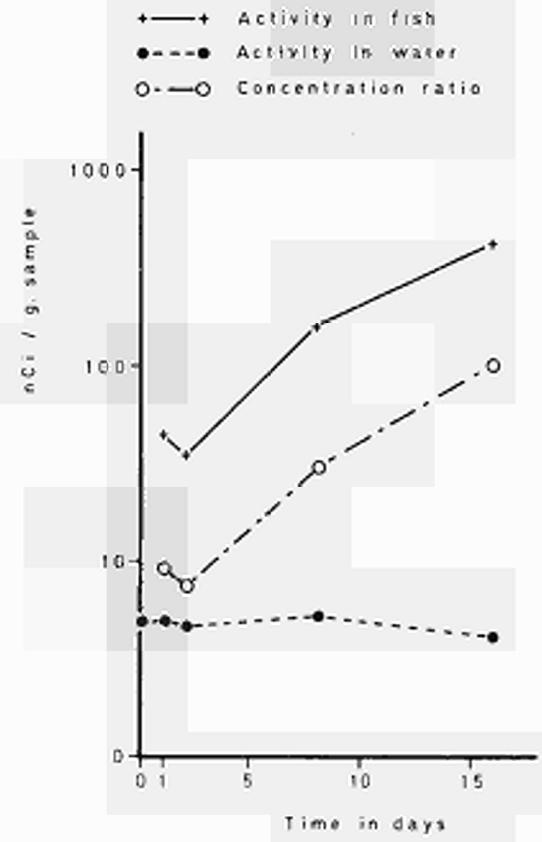


Fig. 7 ^{54}Mn: Radioactivity of fed fish organs measured in nCi / organ

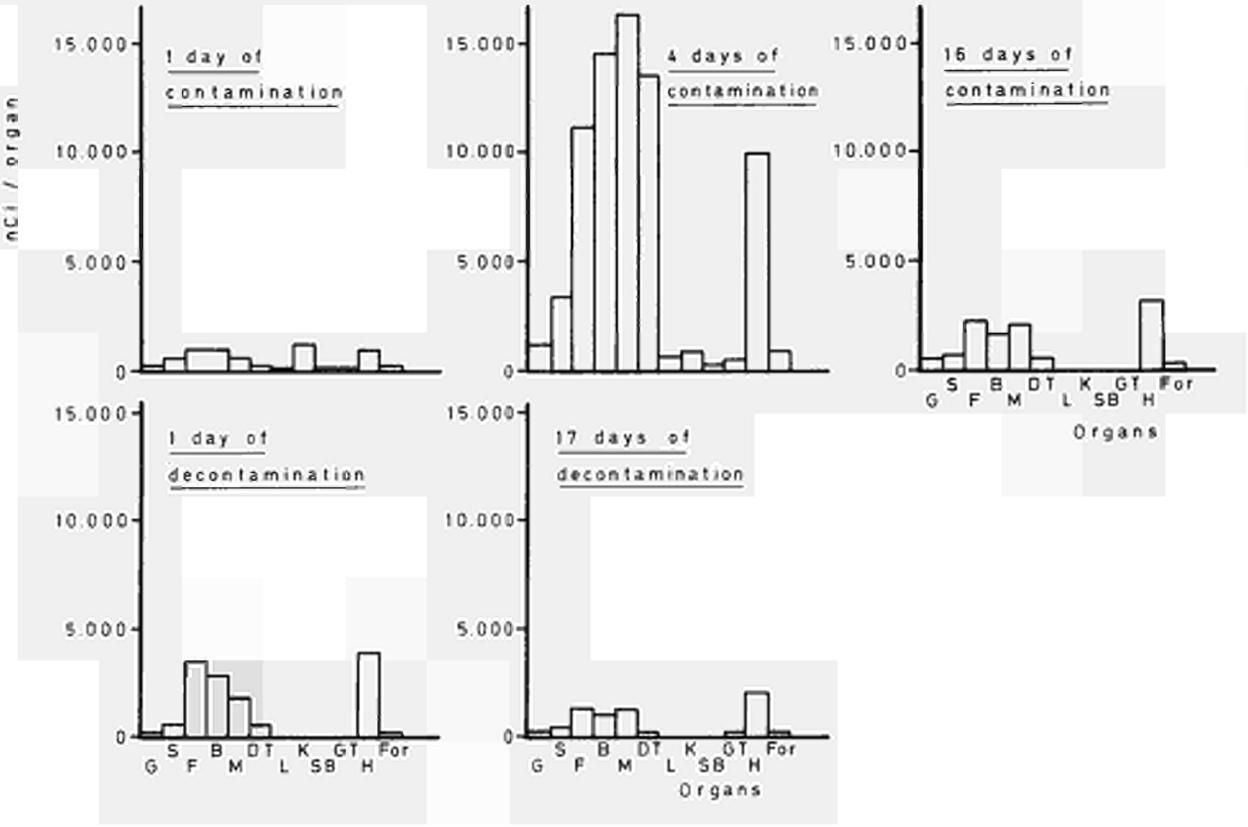


Fig. 8 ^{54}Mn: Activity of fed fish organs measured in nCi / g organ

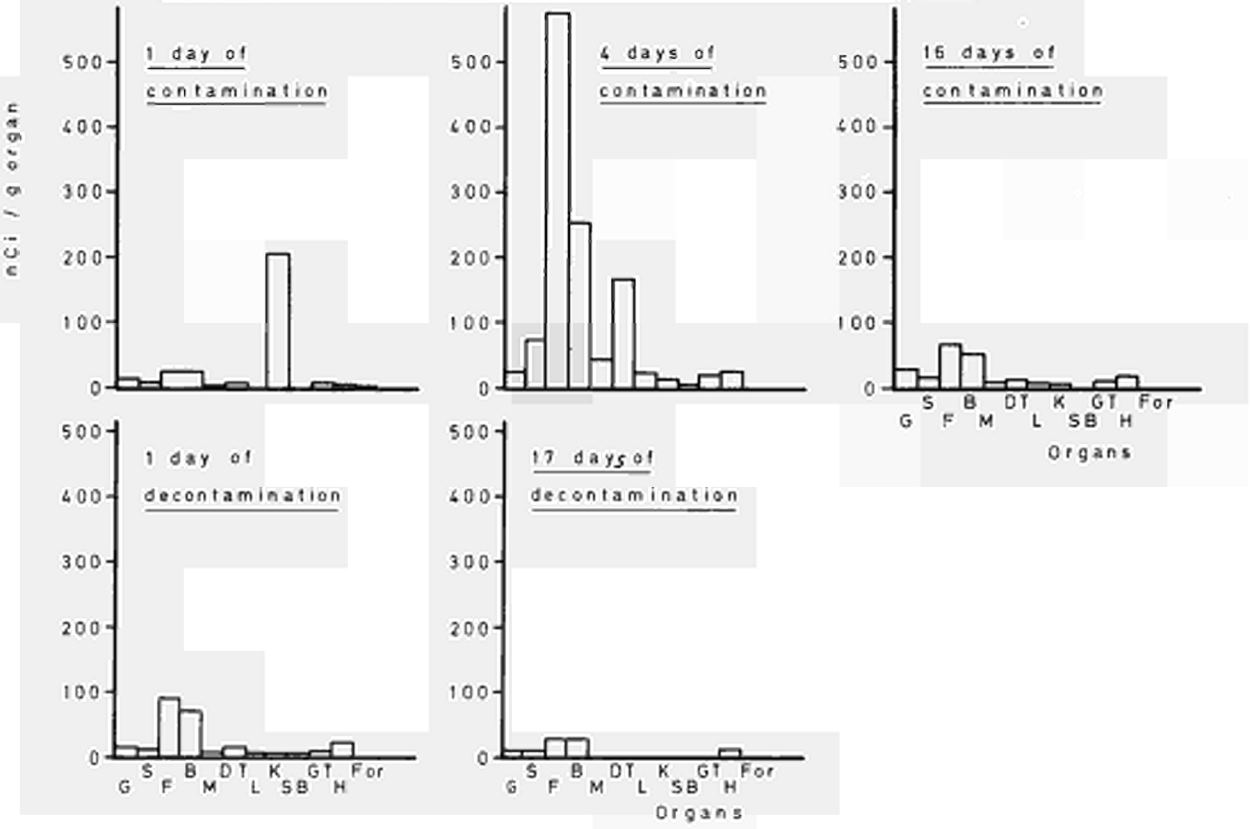


Fig. 9

Concentration ratios of fed fish organs

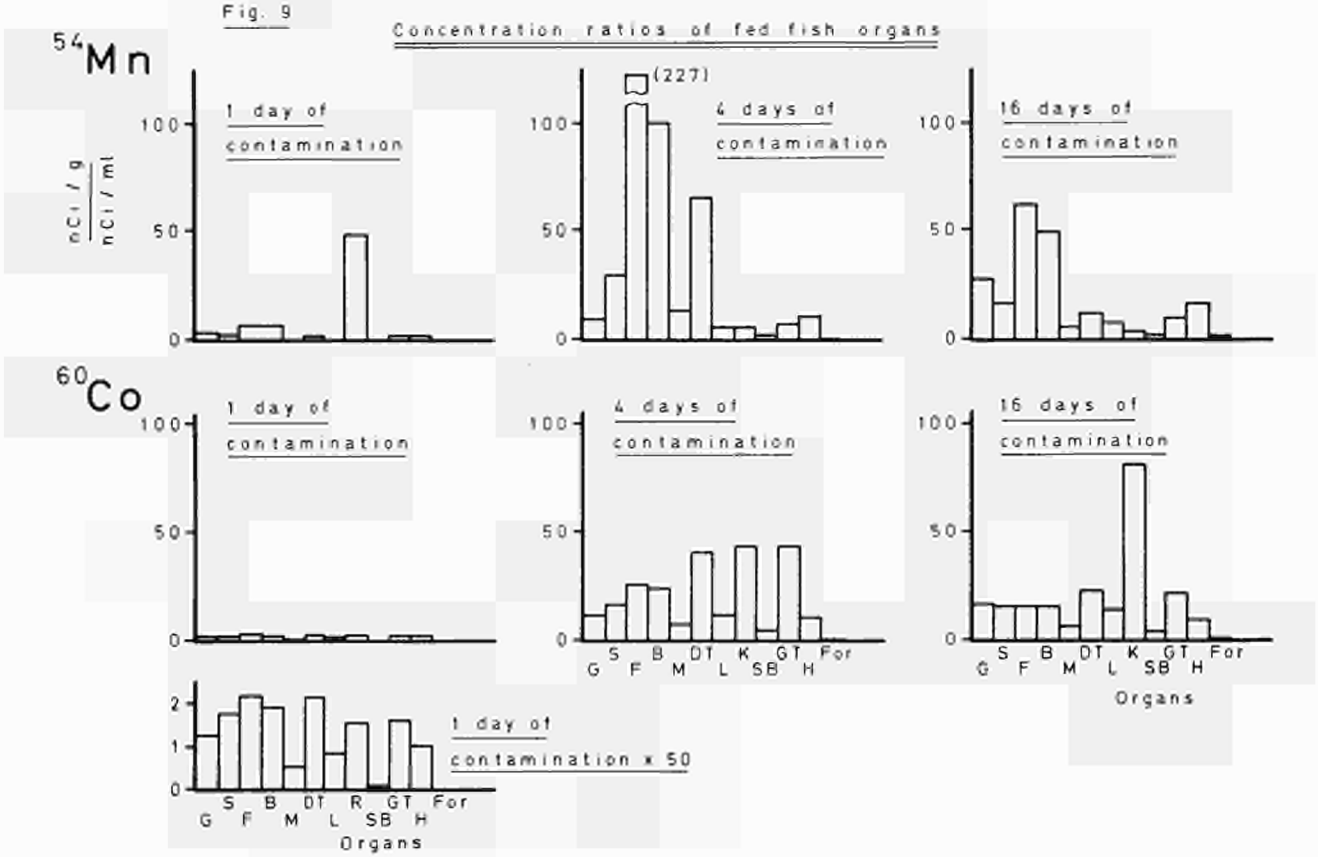


Fig. 10

60Co: Radioactivity of starving fish organs measured in nCi / organ

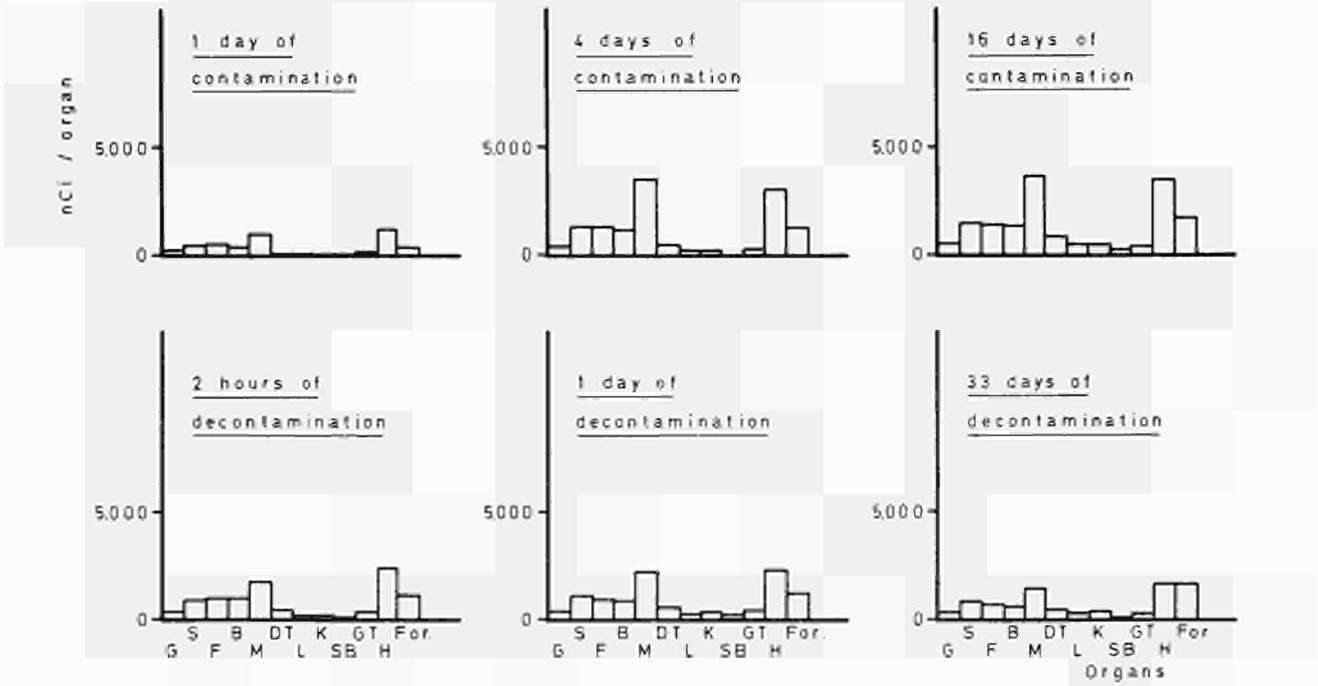


Fig. 11

^{54}Mn: Activity of starving fish organs measured in nCi / g organ

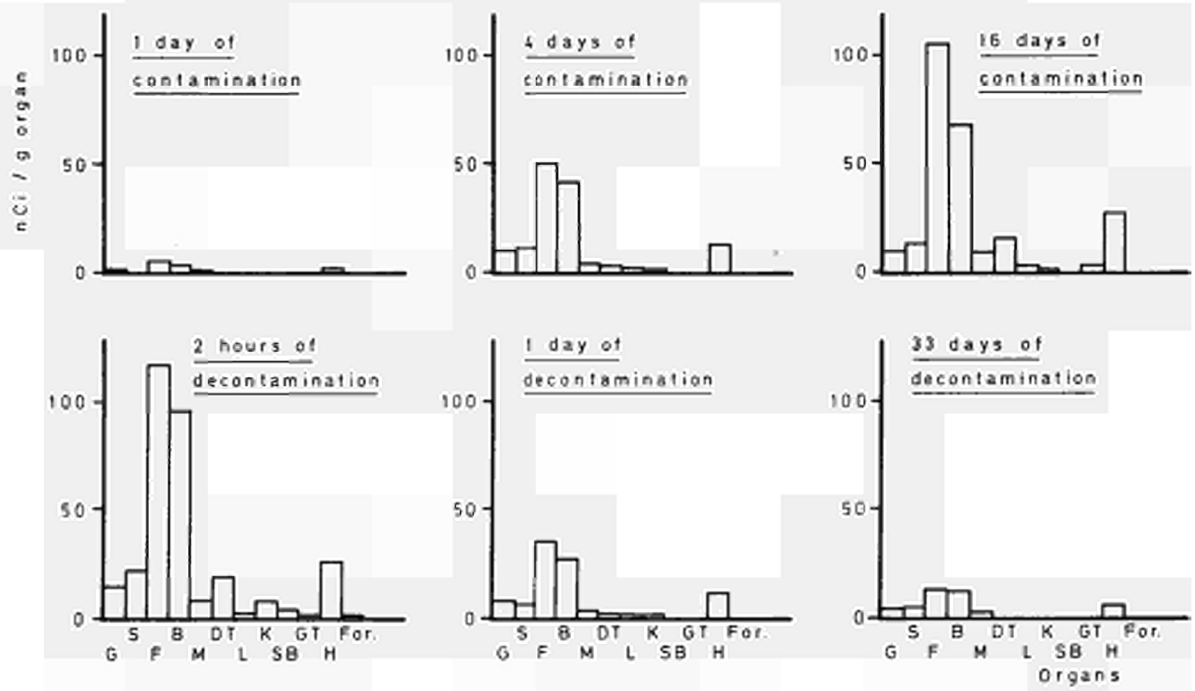


Fig. 12

Concentration ratios in starving fish organs

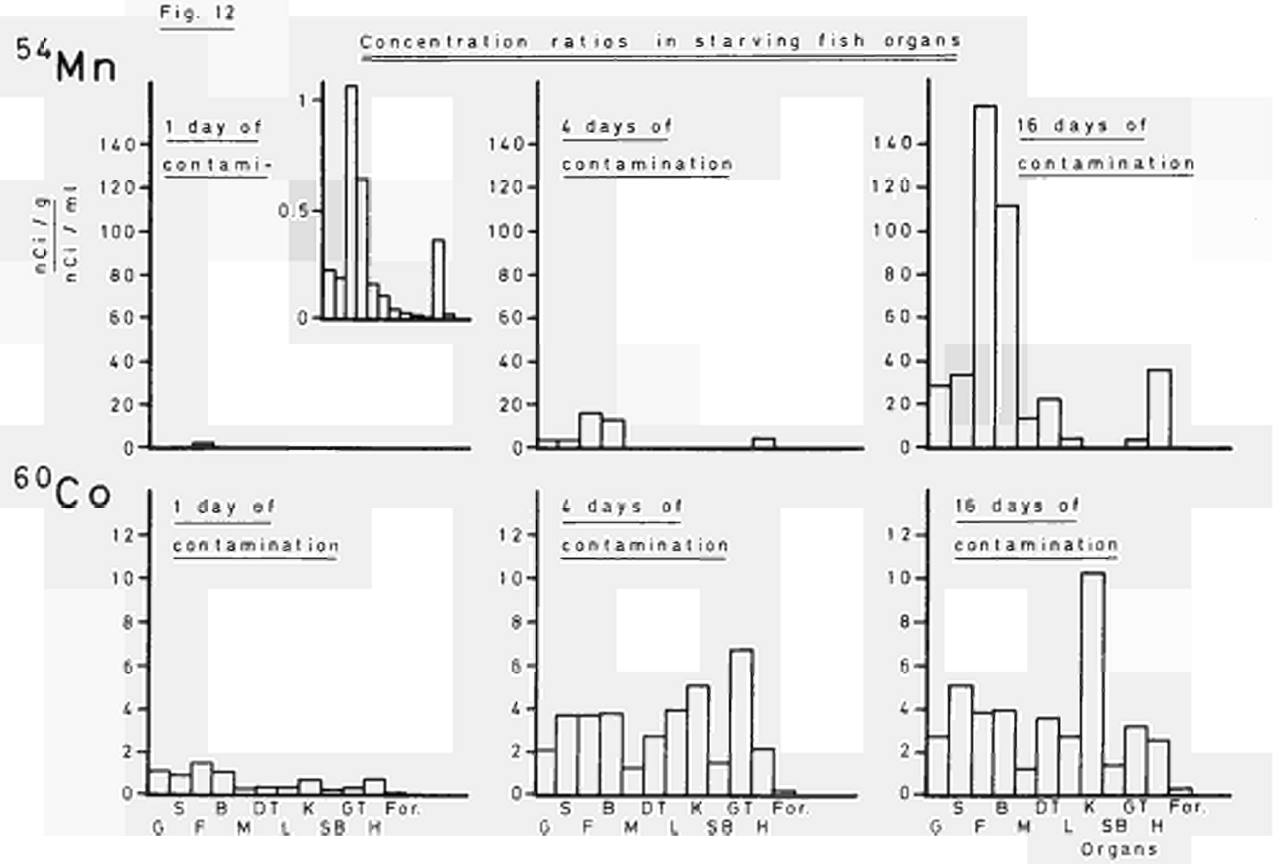


Fig. 13

Radioactivity of dead fish organs after 8 days of contamination

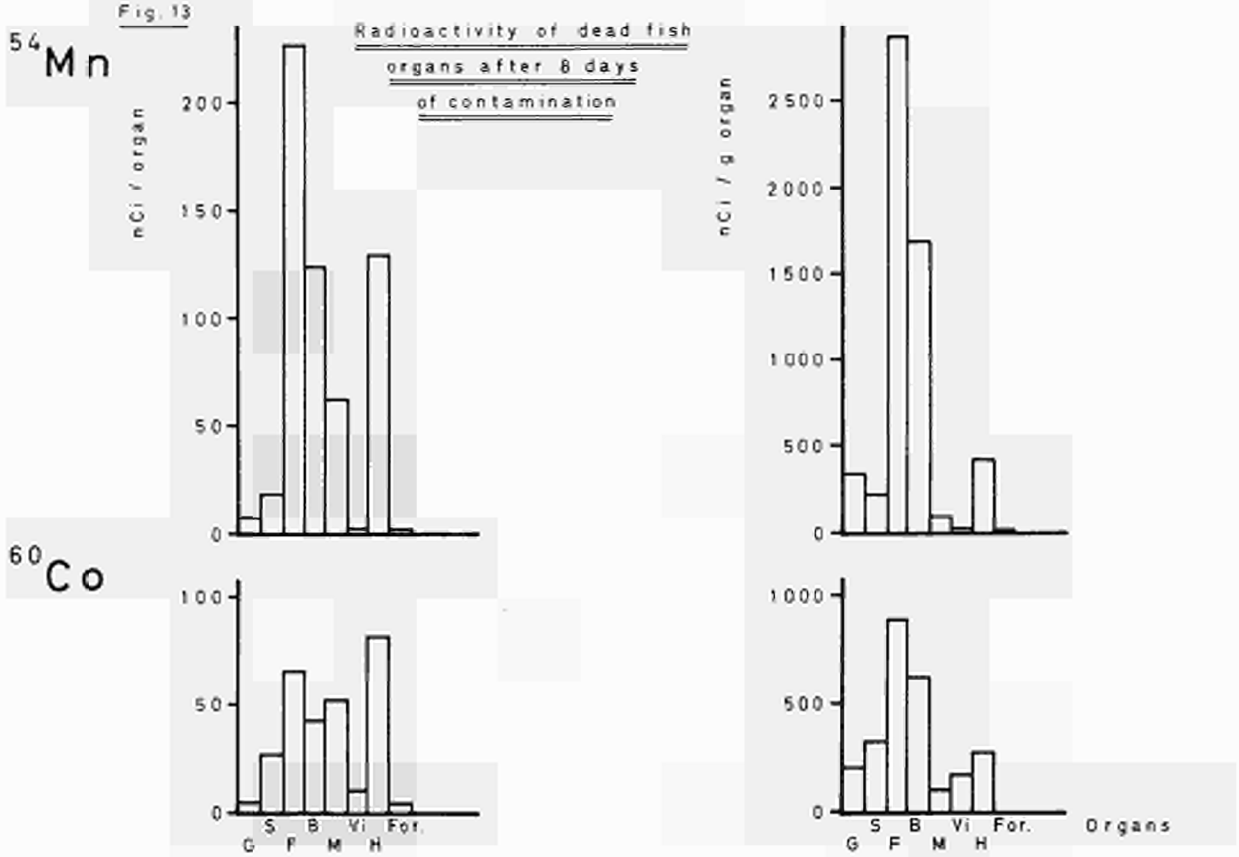


Fig. 14

Concentration ratios of dead fish organs after 8 days of contamination

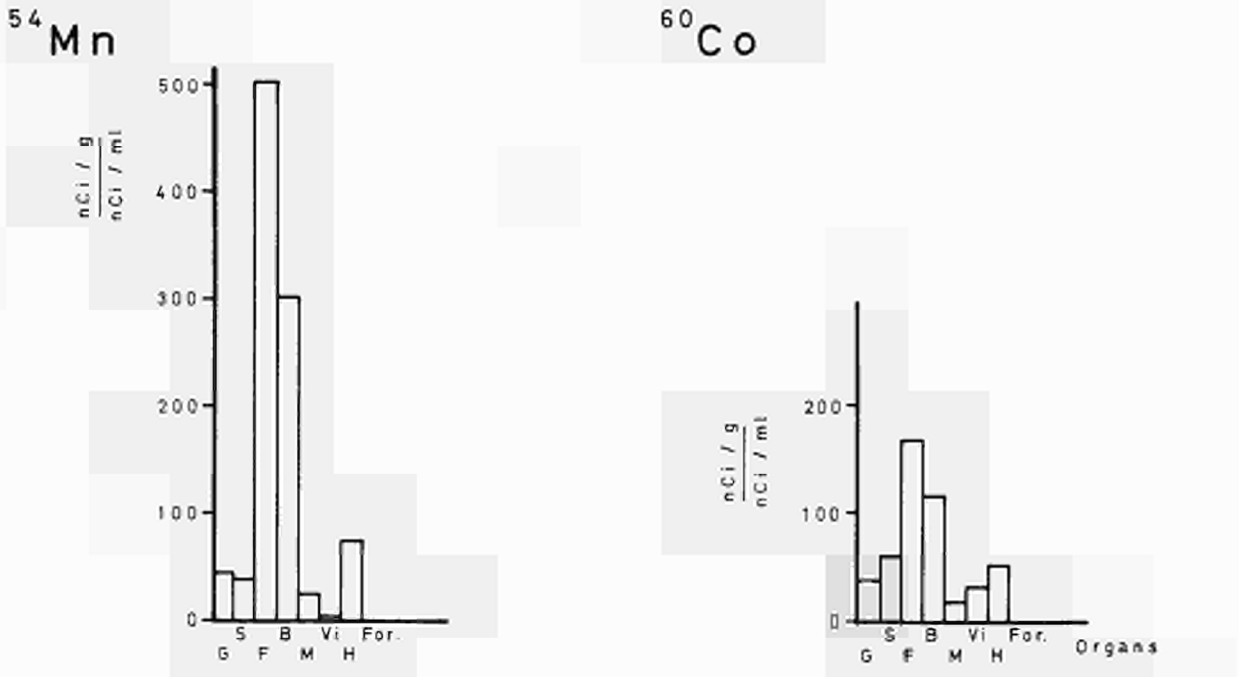


Fig. 15 ^{60}Co: Radioactivity of fed fish organs measured in nCi / organ

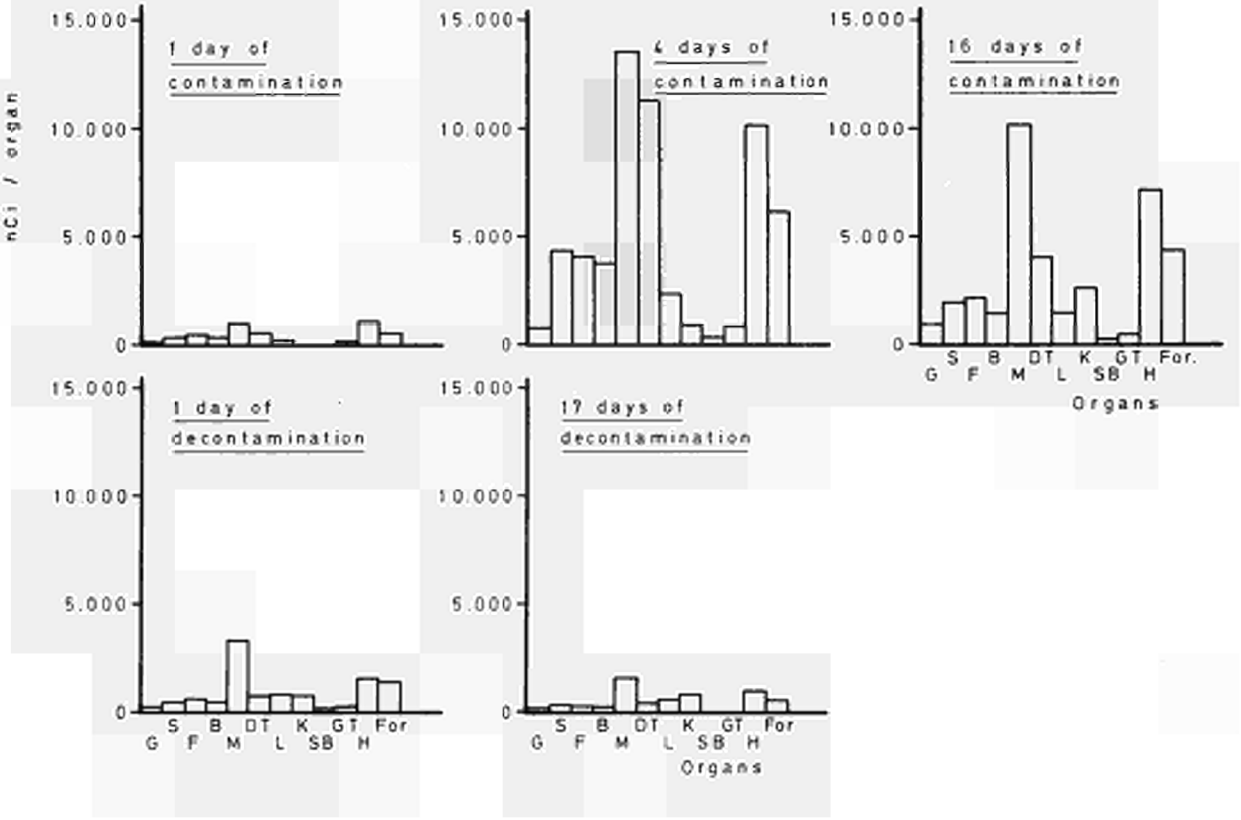


Fig. 16 ^{60}Co: Activity of fed fish organs measured in nCi / g organ

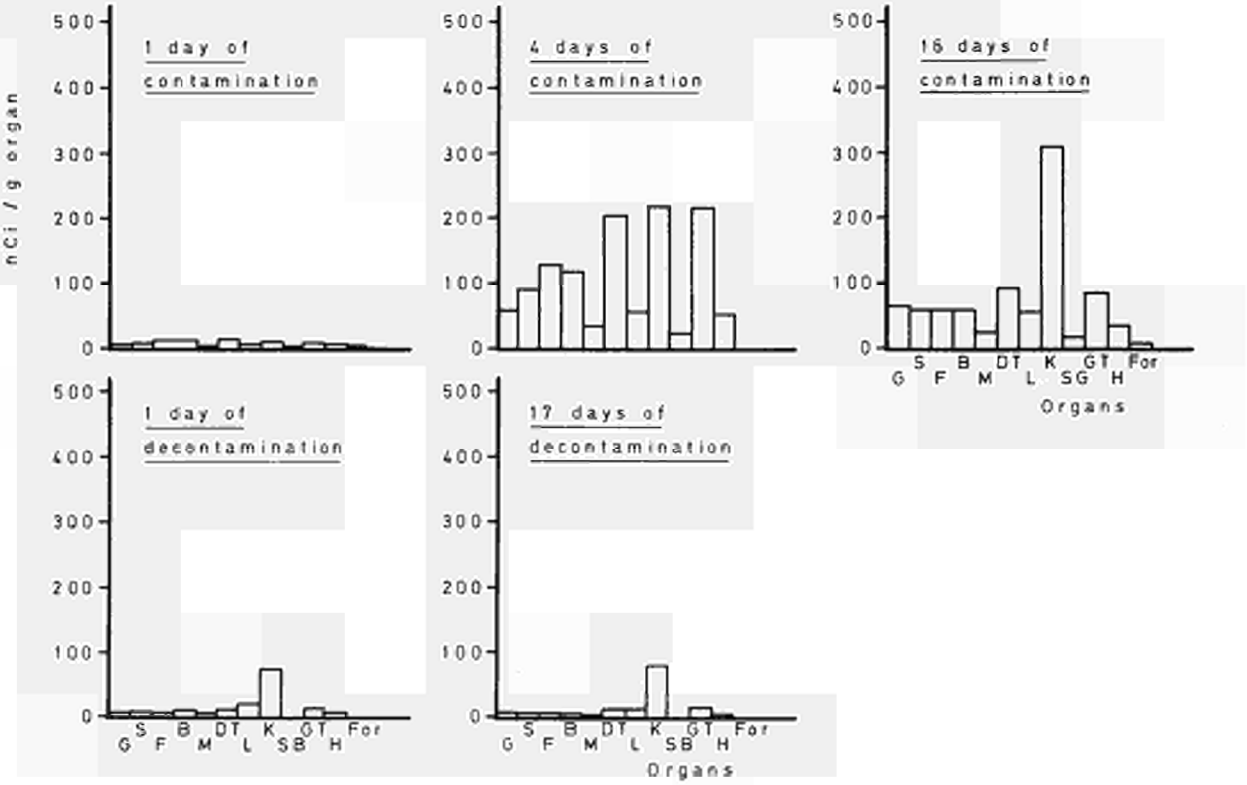


Fig. 17

^{60}Co: Radioactivity of starving fish organs measured in nCi / organ

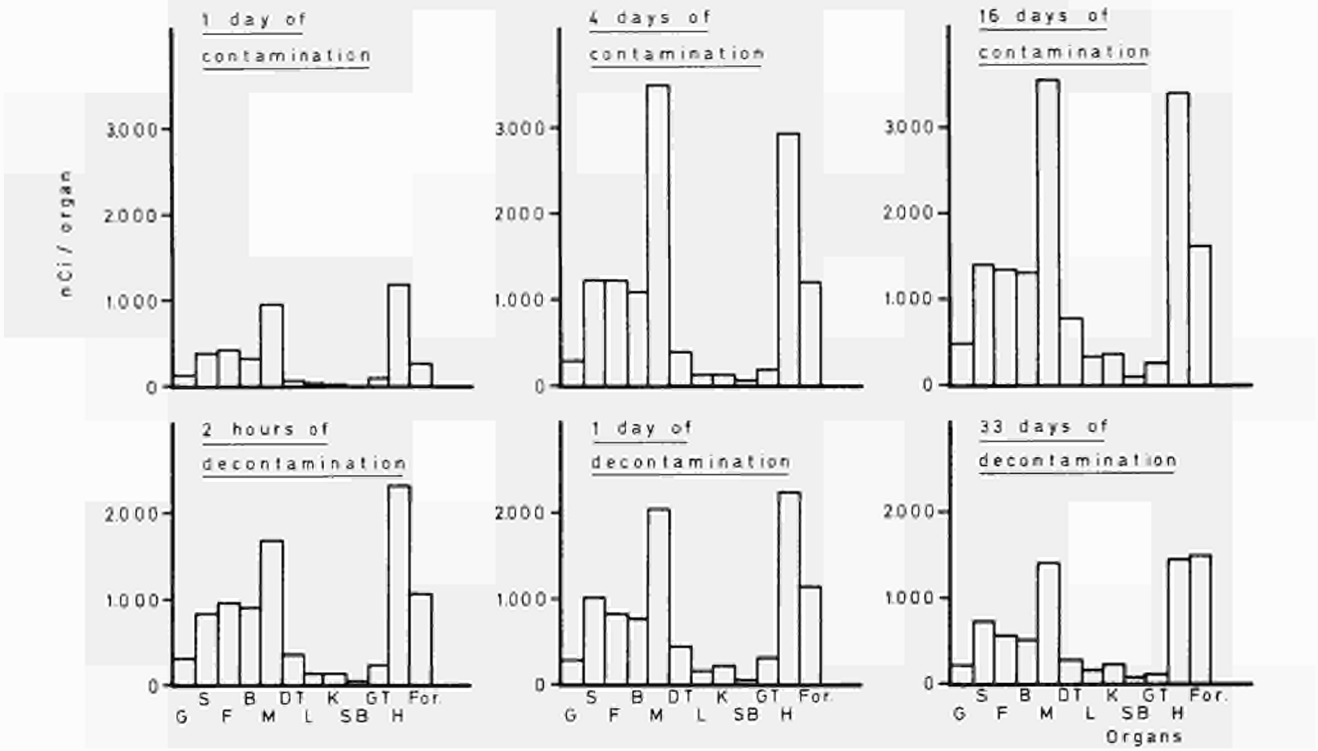
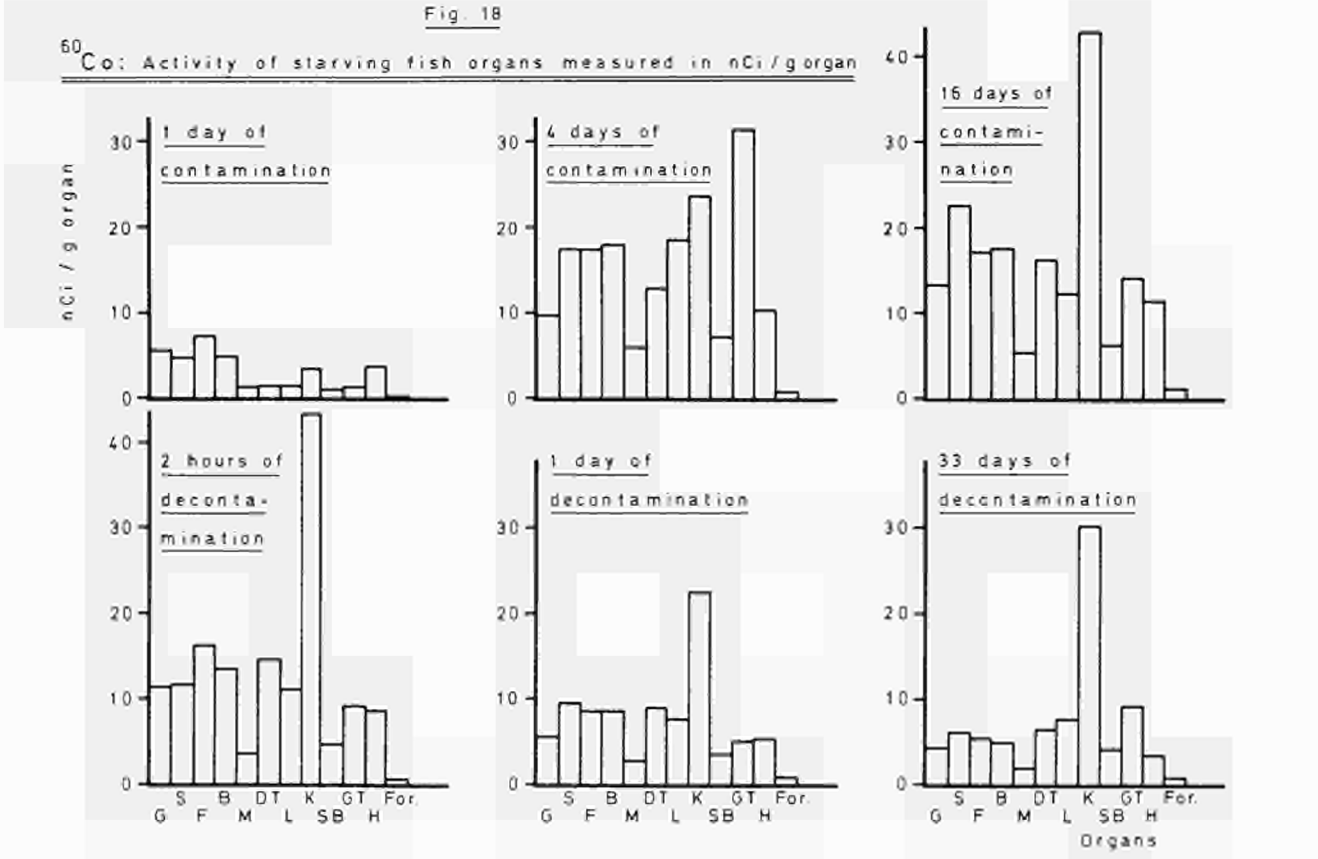


Fig. 18

^{60}Co: Activity of starving fish organs measured in nCi / g organ



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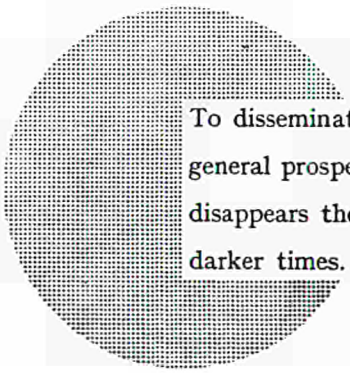

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Alfred Nobel

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