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FISHING INDUSTRY

RESEARCH TRUST ACCOUNT

TITLE OF PROPOSAL Preparation of fish flour of known mercury and selenium content for animal feeding trials

APPLICANTS NAME CSIRO

ORGANISATION Tasmanian Food Research Unit

FINANCES SOUGHT BY APPLICANT:

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FISHING INDUSTRY RESEARCH COMMITTEE

CSIRO DIVISION OF FOOD RESEARCH

Tasmanian Food Research Unit, Hobart

Final Report to the Fishing Industry Research Committee

Preparation of fish flour of known mercury and selenium content for animal feeding trials.

Supervisor:-

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Experimental Officer:-

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Technical Assistant - Mr Russell Joiner employed at CSIRO Division of Applied Organic Chemistry, Melbourne.

Date Commenced: - 1/7/77 Date finished - 30/11/78

The report of this work takes the form of the first unrefereed draft of a paper for Australian Fisheries. The purpose of this paper is to highlight the importance of selenium in fish at a time when the Australian report on mercury in fish is about to be released. It is hoped that publication of the paper will draw from the NH & MRC the kind of experiments which they would like to see done next and also suggestions from laboratories in Australia or overseas.

Design of the first feeding trial

An initial feeding trial using rats was to test the biological availability of selenium in the presence of the mercury in the shark flour. To do this eight diets were prepared which used either Torula yeast, a low mercury low selenium protein supplement, or fish flour as the protein component of the diet. The flour used was prepared from school shark.

Sodium selenite was added to the torula yeast diet at three levels to enable a dose response curve for the effect of selenite on blood glutathione peroxidase levels to be constructed. Mercury concentrations in the diet were varied by adjusting the relative proportions of torula yeast and fish flour. The availability of the selenium in the fish flour and of added selenite in the presence of mercury are being measured from the standard glutathione peroxidase response curve.

The difficulty of measuring selenium is illustrated by the results of the analyses of these diets (Table 2). While mercury presented no real problems the selenium results are, at this stage, inconsistent and unreliable.

Statement of expenditure as at 30 September 1978

	Provision	Total Committed	Balance Not Committed
Salary	4,000	2,478	1,522
Equipment	300	138	162
Maintenance	800	47	753

The financial balance will be spent on selenium estimations.

Table 1.

Outline of Initial Feeding Trial to Examine Mercury/Selenium Interactions

Diet Number	Protein Source (%)		Expected Concentrations (ppm)	
	Torula Yeast	Fish Flour	Mercury	Selenium
1	20	-	Nil	0.02*
2	20	-	Nil	0.16
3	20	-	Nil	0.32
4	10	10	1.0	0.08*
5	10	10	1.0	0.16
6	10	10	1.0	0.32
7	-	20	2.0	0.16*
8	-	20	2.0	0.32

*These diets have no selenium added as selenite.

Table 2.

Comparison of measured concentrations of mercury and selenium with those expected in the diet

Diet Number	Mercury Concentration (ppm)		Selenium Concentration (ppm)	
	Measured	Expected	Measured	Expected
1	nd	Nil	0.11	0.02
2	nd	Nil	0.18	0.16
3	nd	Nil	0.20	0.32
4	1.21, 1.02	1.0	0.13	0.08
5	1.13, 1.21	1.0	0.16	0.16
6	1.17, 1.15	1.0	0.12	0.32
7	2.00, 2.14	2.0	0.22	0.16
8	2.24, 2.15	2.0	0.33	0.32

First preliminary draft of a tentative paper for Australian Fisheries. It would be appreciated if it were not circulated outside the FIRTA Committee. J.O.

The moonstone, a glimmer of light in the mercury tunnel

by S. J. Thrower and June Olley

Mercury (Hg), a constraint on the marketing of fish

In August 1972 the Victorian Government banned the landing and sale for human consumption of school shark more than 28 inches in length, because of mercury contents above the permitted level of 0.5 ppm.

The Tasmanian Food Research Unit of the CSIRO, Division of Food Research is close to the harbour, just five minutes walk up the hill on Hobart's historical Battery Point. The unit specializes in research into fish technology; it was not surprising therefore that when the Victorian ban was announced a delegation of shark fishermen appeared spontaneously at the laboratory seeking advice and banging the library table. A few months later an irate fisherman informed us by telephone that because we were in comfortable government service, we did not care. Recently, in addition to shark the mercury content of large gemfish has also come under scrutiny. This article describes the approach we are taking to these problems.

Does selenium (Se) detoxify mercury?

Selenium, Greek selene, is a trace element named after the moon. Its stable water soluble salt, sodium selenite has been called "the moonstone" (Robinson 1975). This author reports a dietary deficiency of selenium in New Zealand and West Indian babies fed on New Zealand dried milk. Fish products constitute an important source of selenium

Stephen Thrower is an experimental Officer and June Olley a Senior Principal Research Scientist with the CSIRO, Tasmanian Food Research Unit, Hobart. Their article describes the background to a FIRTA Grant application and the subsequent developments.

to attain a good dietary level (Egaas and Braekkan, 1977). As early as 1972, Ganther in the United States had produced some evidence that the selenium naturally present in tuna protected Japanese quail against the toxic effects of mercury in the fish and against added mercury. Quail chicks are particularly sensitive to toxicants in foods and are increasingly being used to screen for them. As a result of his work Ganther presented a petition to the U.S. Food and Drug Administration asking them to reconsider and distinguish between their position on the dangers of the mercury naturally present in fish, and that caused by industrial discharge as at Minimata. In the last few years considerable evidence has accrued to show that selenium does indeed protect against the toxic effects of mercury in many subtle ways. This data has come from laboratories world wide and concerns both selenium naturally present in fish and "the moonstone" sodium selenite which has been added to experimental diets; to list a few references from the U.S.A. (Stoewsand *et al.* 1974, Ganther and Sunde 1974); Czechoslovakia (Parizek *et al.* 1974); Yugoslavia (Kosta *et al.* 1975) and Japan (Seki *et al.* 1975, Tamura *et al.* 1976, Konno *et al.* 1976). Selenium would thus seem to be central to the arguments as to the safety of fish containing mercury.

An approach to Dr. R. Fleming of the Australian National Health and Medical Research Council showed that the HN & MRC took the attitude that the protective effect of selenium against potential mercury toxicity would have to be proven with Australian species of fish caught in Australian waters.

The Tasmanian Food Research Unit therefore applied for a grant from the Fishing Industry Research Trust Account to prepare stable

samples of fish flour containing known amounts of mercury and selenium which would store indefinitely and which could be packaged and sent anywhere in Australia, or in the world for that matter, for feeding trials to evaluate its safety.

Selenium as an essential element

The essential role of selenium in animal nutrition was discovered in 1957 and selenium supplements are now routinely distributed to animals in selenium deficient areas (Underwood 1971). Recently selenium has been found to be an essential component of the enzyme glutathione peroxidase; some forms of this enzyme may contain four molecules of selenium to a molecule of protein. The amount of this enzyme in blood and tissues is an index of the selenium status of an animal. This is fortunate as glutathione peroxidase is easily estimated enzymically, while elemental selenium is extremely difficult to estimate. Hoekstra (1974) has postulated an hypothesis which interrelates the role of Vitamin E, unsaturated fatty acids and the selenium containing enzyme. Glutathione peroxidase in animal tissues catalyses the destruction of hydroperoxides from oxidised fats. The formation of hydroperoxides in the body is deleterious and may lead indirectly to such well known diseases as muscular dystrophy in sheep. Fish are rich sources of Vitamin E (Nobile *et al.* 1976) and this vitamin can spare selenium by relieving it of some of its antioxidant function. Conversely fish oils are rich in fats with five and six double bonds which are readily oxidised to hydroperoxides thus creating a demand for antioxidants.

Welsh and Soares (1976) found that diets containing 30 mg/100 g of Vitamin E strikingly decreased the mortality rate of Japanese quail fed diets containing 30 ppm mercury and 0.1 ppm selenium. Even large fish do not approach these levels of mercury and we are more concerned

with levels in the 0.5 ppm to 2 ppm range. The Vitamin E requirements in the diet would presumably be at least ten times less. In order to study the effects of dietary selenium on potential mercury toxicity, it was therefore necessary to remove other sources of interaction with selenium from the fish such as polyunsaturated fatty acids and Vitamin E. Vitamin E can be subsequently added to diets in measured amounts.

Fish flour for feeding trials

Comminuted gemfish flesh contains approximately 5% fat (Bremner 1978) while comminuted school shark and bronze whaler were found to contain 1.8% and 0.55% fat respectively. Preliminary attempts to dry the shark flesh at low temperature resulted in the flesh becoming strongly ammoniacal. It was realised that a solvent extraction method which could sterilise the flesh and at the same time remove water, fats, water-and-fat soluble vitamins, and substrates for ammonia production such as urea would be the method of choice.

The process of fish flour production developed by the Fisheries Research Board of Canada was therefore chosen. This process of treating fish flesh with isopropanol:water mixtures (Idler 1968) leaves an odourless, tasteless, stable fine white powder. The step of adding polyphosphoric acid to soften connective tissue was omitted; since acidification is known to release heavy metals from proteins. To determine whether the mercury and selenium would stay with the fish flour or whether the metals would be partially extracted into the isopropanol:water solvent, a preliminary experiment was carried out on a seven-gill shark netted by the Hobart Marine Board in the Derwent Estuary. Extraction of the flesh with isopropanol according to the Canadian procedure showed that the mercury and selenium indeed stayed

with the shark flour (Table 1).

The next problem was to obtain large sharks and large gemfish in sufficient quantities to make a stockpile of fish flour of high mercury content. A number of State Government Departments co-operated in supplying material as did several Tasmanian fishermen. Bronze whaler sharks which comprise 30% of the shark catch in Western Australia (Hancock *et al.* 1977) were shipped by Fridgmobile by arrangement with John Edmonds, Research Officer at the Western Australian Marine Research Laboratories, while large gemfish were donated by Terry Gorman (New South Wales State Fisheries) from catches aboard the "F.R.V. Kapala". The cost incurred in the purchase and transport of much of this material was considerable.

The shark and gemfish flesh was comminuted in a Bibun meat separator purchased for the laboratory with funds from an earlier FIRTA grant. The mince was frozen in 1-kilo blocks and stored at $-18^{o}C$ for transport to a solvent extraction plant. The solvent extraction plant which needed slight modification was located in the CSIRO, Division of Applied Organic Chemistry, Melbourne, and Dr. Stan Johns took charge of the production whilst Mr. Russell Joiner was employed under the FIRTA grant as the technical assistant. The solvent extracted flour was freeze dried to 5% moisture.

Analyses of the first batches of school shark flour showed that two solvent extractions reduced the fat and total volatile base (ammonia) content of the flour to acceptable levels (Table 2). A third extraction was omitted in the large scale production due to the high costs of isopropanol ($77 a litre). The isopropanol content of the flour was, however, unacceptably high.

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Isopropanol is held tenaciously by fish flour (Ackman and Odense 1968). Stachowski (1966) pointed out that solvent residues in fish flour can only be replaced by a compound of higher polarity. Isopropanol can conveniently be removed by water in a steaming process. Steaming was done at the CSIRO Division of Food Research, North Ryde.

The flour was placed in a vacuum tumbler drier, a vacuum drawn, and steam injected. The material was tumble dried below 40°C under vacuum. The flours were successively steamed and dried through several cycles and were then ready for feeding trials.

The amounts of these materials available for feeding trials are shown in Table 3. Some of this material could be made available to outside laboratories should the experiments proposed be considered of relevance to the problem.

Feeding trials currently organised and in progress

The first consideration is whether the selenium in the shark and gemfish flours is as available to an animal as sodium selenite. The activity of the enzyme glutathione peroxidase is as already mentioned an index of the selenium status of an animal. By chance we learnt that the Victorian Veterinary Research Laboratories at West Meadows had an automated analysis for glutathione peroxidase and furthermore Dr. Ivan Caple and Mrs. Kathy Andrewartha at that station were willing to conduct feeding trials.

The CSIRO Division of Human Nutrition in Adelaide has considerable experience in selenium investigation and Dr. Ken Godwin of that Division prepared diets for an initial feeding trial which is now in progress at West Meadows.

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The aspect of the toxicity or otherwise of the fish flours will be tested by Dr. Marie Coates using Japanese quail at the National Institute for Research in Dairying, Shinfield, Reading, England as arranged by one of us (S.J.T.) on a visit to the U.K. in 1976.

Logistics

Some of the school shark were transported by Fridgmobile from Stanley, North West Tasmania and comminuted in Hobart. The frozen material was air freighted to Melbourne, solvent extracted there and steamed in Sydney. The diets were prepared in Adelaide and sent back to Melbourne for feeding trials.

The logistics of this exercise have been immense and may explain to fishermen why there is no easy answer to their questions.

Acknowledgements

The authors would like to thank the fishermen who generously donated shark; Mr. Gerry Stanley and Dr. D. Casimir, of the CSIRO Division of Food Research, North Ryde for isopropanol determinations and steaming the flours respectively, also the Department of Public Health, Tasmania and the Tasmanian Fisheries Development Authority for meeting additional costs for isopropanol and shark.

References

Ackman, R.G., Hingley, H.J. and Power, H.E. (1967). Determination of isopropyl alcohol in fish protein concentrate by solvent extraction and gas-liquid chromatography. *J. Fish. Res. Bd Can.* 24: 1521.

Ackman, R.G. and Odense, P.H. (1968). Reinterpretation of isopropyl alcohol retention in fish protein concentrate. *J. Fish. Res. Bd Can.* 25: 805.

Bremner, H.A. (1978). Mechanically separated fish flesh from Australian species - a summary of results of storage trials. *Food Technol. Aust.* 30: 393.

Egaas, E. and Braekkan, O.R. (1977). The selenium content in some Norwegian fish products. *Fisk. Dir. Skr. Ser. Ernering* 1(3): 87.

Ganther, H.E. and Sunde, M.L. (1974). Effect of tuna fish and selenium on the toxicity of methylmercury : a progress report. *J. Food Sci.* 39: 1.

Hancock, D.A., Edmonds, J.S. and Edinger, J.R. (1977). *Fish Res. Bull.* No.18.

Hanson, S.W.F. and Olley, J. (1963). Application of the Bligh and Dyer method of lipid extraction to tissue homogenates. *Biochem. J.* 89: 101P.

Hoekstra, W.G. (1974). Biochemical role of selenium. *In* Trace element metabolism in animals - 2. Ed. Hoekotra, W.G., Suttie, J.W., Ganther, H.E. and Mertz, W. University Park Press, Baltimore p. 61.

Idler, D.R. (1968). The development and scope of the Halifax process. Canadian Fisheries Report 10. Proceedings Conference on fish protein concentrate, p. 45.

Konno, H. *et al.* (1976). Evaluation of selenite as a therapeutic agent for methylmercury poisoning in rats. *Ann. Rep. Tokyo Metr. Res. Lab.* P.H. 27: 297. (Translation available).

Kosta, L., Byrne, A.R. and Zelenko, V. (1975). Correlation between selenium and mercury in man following exposure to inorganic mercury. *Nature* (Lond.) 254: 238.

Nobile, S., Woodhill, H.M. and Rosevear, P.E. (1976). A survey of the vitamin content of foods as they are consumed by selected groups of the Australian population. *Food Technol. Aust.* 28: 427.

Opstvedt, J. (1973). Broiler growth, carcass quality and Vitamin E. *Acta Agric. Scand. Suppl.* 19; 64.

Parizek, J. *et al.* (1974). Interaction of selenium with mercury, cadmium and other toxic metals. *In* Trace element metabolism in animals - 2. Ed. Hoekstra, W.G., Suttie, J.E., Ganther, H.E. and Mertz, W. University Park Press, Baltimore p. 119.

Robinson, M.F. (1975). The moonstone, more about selenium. The Nutrition Society of New Zealand. First Muriel Bell Memorial Lecture, Ed. E.L. Hove.

Seki, H. *et al.* (1975). Effectiveness of selenium in tuna meat in modifying methylmercury toxicity. *Ann. Rep. Tokyo Metr. Res. Lab.* P.H. 26: 294. (Translation available).

Stachowski, J. (1966). Solvent residues in fish protein concentrates. Abstracts of papers 2nd International Congress of Food Science and Technology, Warsaw, Poland p. 37.

Stoewsand, G.S., Bache, C.A. and Lisk, D.J. (1974). Dietary selenium protection of methylmercury intoxication of Japanese quail. *Bull. Env. Cont. Toxic.* 11: 152.

Tamura, Y. *et al.* (1976). Influence of selenite on the transplacental transition of methylmercury (changes noted in the distributional patterns in rat organs). *Ann. Rep. Tokyo Metr. Res. Lab.* P.H. 27: 179. (Translation available).

Underwood, E.J. (1971). The trace elements in human and animal nutrition, 3rd Edit. Academic Press, New York and London, p. 350.

Welsh, S.O. and Soares, J.H. (1976). The protective effect of Vitamin E and selenium against methylmercury toxicity in the Japanese quail. *Nutr. Rep. Int.* 13: 43.

Table 1. Effect of isopropanol:water extraction on the Hg and Se content of seven-gill shark

	Hg ppm dry weight*	Se ppm dry weight+
Shark flesh	0.88	4.1
Shark flour	0.87	4.4

* Analysis by courtesy of Australian Government Analytical Laboratory

\+ Analysis by courtesy of CSIRO, Division of Human Nutrition, Adelaide

Table 2. Analysis of school shark flour prepared by solvent extraction

Component before steaming	Number of isopropanol extraction 2nd	3rd
Fat %*	0.20	0.08
Total volatile base N mg/100g+	20.2	15.1
Isopropanol %≠	4.6	4.7

* Chloroform methanol extraction (Hanson & Olley, 1963)

\+ Conway method

≠ Method of Ackman *et al.* (1967) but using 4% of methylated carbowax 1450 on chromasorb G as the stationary phase for gas chromatography

Table 3. Inventory of fish flours prepared for feeding trials

Fish species	Kilos of flour prepared		Hg content*	Se content*
	Committed for trials	Available for further work	mg/kg	mg/kg
School shark *Galeorhinus Australis*	20	30	10.0	
Bronze whaler *Carcerhinus obscurus*		25		
Gemfish *Rexea solandri*		15		

* The bronze whaler flours and gemfish flours are awaiting the final steaming before Hg and Se analysis.

+ There have been considerable difficulties in the analysis for selenium. See FIRTA Report.