References and Notes

1. I. Yasuda, W. Noguchi, T. Sata, *J. Bone Joint Surg.* **37A**, 1292 (1955).
2. H. Iida, *J. Jap. Orthop. Surg. Soc.* **31**, 645 (1959).
3. C. A. L. Bassett, R. J. Pawluk, R. O. Becker, *Nature* **204**, 652 (1964).
4. L. S. Lavine, I. Lustrin, M. H. Shamos, *Nature* **224**, 1112 (1969).
5. L. S. Lavine, I. Lustrin, M. H. Shamos, M. L. Moss, *Acta Orthop. Scand.* **42**, 305 (1971).
6. J. H. McElhaney, R. Stalnaker, R. Bullard, *J. Biomech.* **1**, 47 (1968).
7. D. D. Levy, thesis, Polytechnic Institute of Brooklyn (1971).
8. E. S. Crelin and D. K. Dueker, *Yale J. Biol. Med.* **43**, 71 (1970).
9. B. T. O'Connor, H. M. Charlton, J. D. Currey, D. R. S. Kirby, C. Woods, *Nature* **222**, 162 (1969).
10. H. J. Hambury, J. Watson, A. Sivyer, D. J. B. Ashley, *ibid.* **231**, 190 (1971).
11. G. E. Palade and K. R. Porter, *J. Exp. Med.* **100**, 641 (1954).
12. K. R. Porter, *Biophys. J.* **4**, 167 (1964).
13. R. Ross, in *Treatise on Collagen*, B. Gould, Ed. (Academic Press, New York, 1968).
14. R. Ross and E. P. Benditt, *J. Cell Biol.* **22**, 365 (1964).

15. We thank Adam Narbut for technical help in assembling the power unit and Dr. John Bruno for clinical assistance. This work was supported by an NIH grant.

19 August 1971; revised 18 October 1971 ■

Mercury Concentrations in Museum Specimens of Tuna and Swordfish

Abstract. *The mercury levels of museum specimens of seven tuna caught 62 to 93 years ago and a swordfish caught 25 years ago have been determined by instrumental neutron activation analysis. These levels are in the same range as those found in specimens caught recently.*

Although there is considerable evidence that man-made mercury pollution of freshwater rivers and lakes has in some instances definitely increased the mercury levels in freshwater fish (*1*), there is relatively little information as to whether man-made sources of mercury pollution of the oceans have significantly increased the mercury levels in wide-ranging ocean fish. The large-scale confiscation of swordfish and the lesser-scale confiscation of tuna, as a consequence of the establishment by the U.S. Food and Drug Administration of a maximum permissible level of 0.5 part per million (ppm) for mercury in fish, have been interpreted by many persons as a consequence of the discovery of man-made pollution. Goldwater (*2*) and Hammond (*3*), however, have questioned the validity of this assumption. For example, Hammond estimates that the total amount of mercury processed by man since 1900 would, if put into the world's oceans and well mixed, increase the average mercury concentration of seawater (approximately 0.1 part per billion) by at most 1 percent—an essentially negligible increase (*4*).

To explore this subject further, we obtained samples of seven tuna and one swordfish that have been preserved in museums for varying lengths of time. The seven tuna (each slightly under 0.6 m in length) were caught between 1878 and 1909 and were preserved in toto, first in formaldehyde and then in alcohol, in the Smithsonian Institution. The single swordfish was caught in 1946, and its head was preserved in 40 percent isopropanol at the Museum of the California Academy of Sciences (*5*).

Samples of the flesh (*5, 6*) of these eight fish were analyzed for mercury by means of purely instrumental thermal-neutron activation analysis (NAA). In each case, a weighed sample (about 1 g) was sealed in a quartz ampule, activated for 3 hours in the nuclear reactor of the University of California at Irvine at a thermal-neutron flux of 0.7×10^{12} n cm^{-2} sec^{-1}, and then counted (still sealed in quartz) with a gamma-ray spectrometer [36-cm^3 Ge (Li) detector, 4096 channel], after 3 to 7 days decay. Several freshly prepared aqueous mercury standard solutions, also sealed in quartz (*7*), were activated along with the fish samples and counted with the same gamma-ray spectrometer, under the same conditions. The mercury contents of the samples and standards were measured unambiguously by means of the

Table 1. Mercury levels in museum and recent specimens of tuna and swordfish, determined by instrumental neutron activation analysis.

Description	Date	Samples	^{197}Hg measurements	Hg, mean ppm and S.D. of mean: Wet-weight basis	Hg, mean ppm and S.D. of mean: Dry-weight basis
Museum specimens					
Skipjack tuna, 21563*, Massachusetts	1878	2	4	0.27 ± 0.02	0.91 ± 0.06
Skipjack tuna, 21852*, Massachusetts	1878	1	2	0.64 ± 0.02	1.51 ± 0.04
Albacore tuna, 26873*, California	1880	2	8	0.27 ± 0.03	0.59 ± 0.06
Bluefin tuna, 37928*, Woods Hole	1886	1	4	0.38 ± 0.01	1.14 ± 0.04
Skipjack tuna, 41901*, San Diego	1890	2	4	0.45 ± 0.02	1.05 ± 0.04
Skipjack tuna, 52704*, Hawaii	1901	2	4	0.42 ± 0.02	0.92 ± 0.01
Skipjack tuna, 194901*, Philippines	1909	2	4	0.26 ± 0.01	0.53 ± 0.02
					0.95 ± 0.33‡
Swordfish, Baja California	1946	6	22	0.52 ± 0.10	1.36 ± 0.31
					1.36 ± 0.31‡
Recent specimens					
Albacore tuna, fresh, California		1	2	0.13 ± 0.01	0.44 ± 0.05
Skipjack tuna, fresh, Pacific		1	2	0.18 ± 0.03	0.62 ± 0.10
Albacore tuna, canned (A), in water†		1	2	0.48 ± 0.04	1.53 ± 0.12
Albacore tuna, canned (A), in oil†		1	2	0.30 ± 0.02	0.66 ± 0.04
Albacore tuna, canned (B), in water†		1	2	0.38 ± 0.03	1.29 ± 0.11
					0.91 ± 0.47‡
Six swordfish, fresh, California		14	40	0.23 to 1.27	0.94 to 5.08
					3.1 ± 1.5‡

* Smithsonian reference number. † The letters (A) and (B) are used to distinguish two brands of tuna. ‡ Mean mercury concentration.

gold K electron x-ray peak at 67 to 69 kev and the gold K electron x-ray and gamma-ray peak at 77 to 80 kev—both of which result from the electron-capture decay of 64-hour ^{197}Hg. Samples of "modern" tuna and swordfish, that is, fish caught during 1970–71, were activated and counted in the same fashion. The lower limit of the sensitivity of our instrumental NAA technique, as used in these experiments, was about 0.02 ppm for mercury in a 1-g sample.

A summary of the mercury levels observed in these fish samples is given in Table 1. After the activation analysis, the loss of weight on drying for 16 hours at 100°C was determined for each specimen, so that the mercury results could be expressed on a dry-weight basis (last column of Table 1). The volatile content (such as water or alcohol) varies appreciably among such samples. In fresh tuna and swordfish the mercury content (ppm) on a wet-weight basis is typically between 3.3 and 4.0 times lower than the mercury content (ppm) on a dry-weight basis; this corresponds to a moisture content of 70 to 75 percent.

The mean mercury concentration (dry-weight basis) in the seven museum tuna samples listed in Table 1 is 0.95 ± 0.33 ppm (the values range from 0.53 to 1.51 ppm). The corresponding mean mercury concentration for the five recent tuna samples is 0.91 ± 0.47 ppm (the values range from 0.44 to 1.53 ppm). It is evident that there is no significant difference in mercury concentration between the museum tuna samples, caught 62 to 93 years ago, and the samples of tuna caught recently. The average mercury value (1.36 ± 0.31 ppm on a dry-weight basis) for the single specimen of swordfish caught 25 years ago falls within the range of values (0.94 to 5.1 ppm) found for "modern" swordfish. The data for both tuna and swordfish lend support to the contention that the mercury levels now being found in wide-ranging ocean fish are not primarily the consequence of man-made pollution but are of natural origin.

There is no reason to believe that the seven museum specimens of tuna were contaminated with any additional mercury during the time they were preserved in formaldehyde, and then alcohol, in the Smithsonian Institution, although this possibility cannot be rigorously excluded. For the swordfish, samples of the original preservative solution were also available. In addition, "background" samples were obtained, consisting of a smaller fish—the pipefish (*Syngnathus griseolineatus leptorhyncus*)—caught in 1946 off Baja California, and its preservative solution, the same brand of 40 percent isopropanol. No mercury was detected in the isopropanol used for preserving the swordfish or the pipefish, and only a minute concentration of mercury, about 0.17 ± 0.15 ppm (dry-weight basis), was detected in the pipefish sample (whole fish). The absence of any significant mercury content in either the pipefish or its preservative solution is evidence that there was no mercury contamination from (i) the original preservative solution, (ii) subsequent external contamination during storage, or (iii) external contamination during our measurement procedures.

G. E. MILLER, P. M. GRANT
R. KISHORE, F. J. STEINKRUGER
F. S. ROWLAND, V. P. GUINN
Department of Chemistry,
University of California, Irvine 92664

References and Notes

1. R. A. Wallace, W. Fulkerson, W. D. Shults, W. S. Lyon, *Report ORNL-NSF-EP* (Oak Ridge National Laboratory, Oak Ridge, Tenn., 1971); K. Ljunggren, B. Sjostrand, A. G. Johnels, M. Olsson, G. Otterlind, T. Westermark, in *Nuclear Techniques in Environmental Pollution* (International Atomic Energy Agency, Vienna, 1971), p. 373.
2. L. J. Goldwater, *Sci. Amer.* **224**, No. 5, 15 (1971).
3. A. L. Hammond, *Science* **171**, 788 (1971).
4. The estimated mercury production is several times 10^5 metric tons, plus about 10^5 metric tons released by the burning of coal. In the world's oceans there are about 1.3×10^{18} metric tons of water, with an average concentration of mercury of about 0.1 ppb.
5. This broadbill swordfish (*Xiphias gladius*) specimen was not a typical specimen, although its abnormality presumably is not related to its mercury content. It was caught in August 1946 off the west coast of Baja California, and was found to have its bill bent around and embedded in its head. It weighed between 160 and 165 pounds (73 to 75 kg). The samples analyzed for mercury were flesh samples taken from regions near the head.
6. All tuna samples were homogenized portions of the light muscle taken from the center of the right dorsal side.
7. Loss of mercury from samples sealed in polyethylene vials has been reported by L. C. Bate [*Radiochem. Radioanal. Lett.* **6**, 139 (1971)] for much more intense neutron-irradiation conditions than ours. We have also observed a small loss of mercury from aqueous standard solutions in polyethylene vials, but not from fish samples, under our irradiation conditions.
8. We are indebted to M. E. Stansby, director of the Pioneer Research Laboratory, Seattle, and to the Smithsonian Institution for the old tuna samples. We are also indebted to C. Hubbs, of the Scripps Institution of Oceanography, and to W. N. Eschmeyer, of the California Academy of Sciences, for the swordfish samples, and to C. Hubbs for the samples of pipefish and isopropanol. This work was supported by the University of California at Irvine, with some assistance from AEC contract AT-(04-3)-34, agreement No. 126.

6 December 1971

Selenium: Relation to Decreased Toxicity of Methylmercury Added to Diets Containing Tuna

Abstract. *Japanese quail given 20 parts per million of mercury as methylmercury in diets containing 17 percent (by weight) tuna survived longer than quail given this concentration of methylmercury in a corn-soya diet. Tuna has a relatively high content of selenium and tends to accumulate additional selenium when mercury is present. A content of selenium in the diet comparable to that supplied by tuna decreased methylmercury toxicity in rats. Selenium in tuna, far from being a hazard in itself, may lessen the danger to man of mercury in tuna.*

Tuna and swordfish have a tendency to accumulate Hg in excess of 0.5 part per million (ppm), the content established by the U.S. Food and Drug Administration as the maximum allowable concentration. This fact raises the following important questions: (i) How toxic is a low concentration of Hg, as ingested in the form of tuna? and (ii) What factors present in tuna might cause Hg to accumulate or might modify its toxicity? We have been engaged in a study of the long-term effects of various concentrations of Hg in diets containing tuna. We here describe the surprising finding that tuna in the diet decreases the toxicity produced with high concentrations of methylmercury. Evidence is presented that Se present in tuna may be responsible for this effect.

Approximately 2000 pounds (906 kg) of canned tuna having an Hg content of 0.7 to 1.0 ppm (total contents of the can, fresh basis) was obtained from the processors. The cans were opened and drained, and the solids were lyophilized at ambient temperature to a moisture content of approximately 2 percent (by weight) and then packed in 5-gallon (18.9-liter) cans under nitrogen ("high-Hg tuna"). A smaller quantity of tuna with an Hg content of 0.09 to 0.14 ppm was obtained from the same sources and dried in the same way ("low-Hg tuna"). Preliminary experi-