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DIOXIN

What the U.S. Navy knew and didn't or wouldn't tell us.

An OP-ED Paper by Chuck Graham

I'm a U.S. Navy Vietnam Veteran and I have had a claim in place with the Dept of Veterans Affairs since 2003. Like so many of you I've been on the hamster wheel and suffered through the Haas appeal all to no avail. Over the years I've researched any available material that might help prove that the U.S. Navy had knowledge to support the findings of the Australian study, {ENTOX, also called NRCET from 2002}. This study involved the co- distillation of Dioxin through the fresh water evaporator systems commonly used aboard RAN, Royal Australian Naval Ships, that were present in Vietnam. The same evaporator systems were commonly used by U.S. Navy Ships, that were present in Vietnam, as the majority of the Australian Naval Ships were built at U.S. Naval Shipyards. It is my hope that the following information will shed some knowledge of what the U.S. Navy knew and had in their possession and if they knew then the DOD and more than likely the DVA also had to know.

{ My co-investigator on this paper is Ms Susie Belanger, known to many veterans across the country and without her help this would not have been possible.}

As far back as 1946 the U.S. Navy had knowledge of the dangers of distilling water for shipboard uses while in littoral waters or certain other locations. This was evidenced by the fact that while conducting atomic radiation testing at Bikini Atoll, they were warned not to utilize any seawater aboard ships in the area, for fear of contamination by the radiation which had contaminated the coastal waters. This was "Operation Crossroads" and 79 ships that were present during these tests, were salvaged and sent to Hunters Point Naval Shipyard in San Francisco for decontamination. An acid wash had to be used to decontaminate the evaporators and water purification systems.

In the U.S. Army Technical Manual TM 5-813-8 from September 1986 on water Desalination chapter 5-1 paragraph C where it "states" that dissolved organic materials will carry across a distillation / condensation process with the water. Pesticides and industrial organic chemicals may be difficult to remove by distillation/condensation.

Ok folks, lets look at and re-read that statement !! Someone in the Army had to have done some tests to make that statement. How else would they have known, without testing the condensate, that this was so. That proves that the Military knew that dioxin/ herbicides/pesticides would remain in distilled water.

The manual of Naval Preventive Medicine {NAVMED P -5010-6 rev 1990} chapter 6 Water Supply Afloat sec 6-3 "states" That water in harbors or off shore from habitations and when operating in fleet strength "must" be considered "polluted" and "unfit" for uses other than in fire and flushing systems and must not be used for other purposes.

In the U.S. Navy's Risk Analysis of Shipboard Drinking Water Chemical Contaminants, August 18, 2000 by Lieutenant Michael D. Cassady Medical Service Corps U.S. Navy

“states” An important aspect of the drinking water produced onboard ships and submarines is, its source. Ships and submarines routinely do not produce water unless they are at least 12 miles from the shoreline. However, the operational environment for ships and submarines is changing and more missions are requiring operations in littoral waters for extended lengths of time. Littoral waters are more likely to be at risk for primary and secondary contaminates.

Now while on the gun-line conducting NGS firing missions off the coast of Vietnam, we did not have time to pull off and run out 12 miles and make fresh water. We made water where we were at, 24/7

Now to get to the heart of the matter and the reason for this paper. We have discovered several Naval Documents that we feel should shed some light on the knowledge that the U.S. Navy had over the years starting in 1963 with “BUMED INSTRUCTION 6240.3B” from Chief, Bureau of Medicine and Surgery to ALL Ships and Stations. Subject : Standards for Potable Water. Purpose , to establish standards for water for drinking and culinary purposes throughout the Naval Establishment.

Pay special attention to page 3 where it lists “Chemical Characteristics Limits” Nowhere do you see “Herbicides” mentioned.

[Editor's Note: OK... here's a smoking gun. In 1963, water treatment specifications did not mention insecticides/herbicides in the water. In 1972, these same water treatment specifications mentioned insecticides/herbicides needing to be addressed. If they didn't know about the presence of herbicides in the water, why would that be specifically called out? Someone wearing brass and a lot of gold stripes lied to the American public. I wonder who that was?]

DEPARTMENT OF THE NAVY
Bureau of Medicine and Surgery
Washington 25, D.C.

BUMED 6240.3B
BUMED-7223-1ss
30 September 1963

BUMED INSTRUCTION 6240.3B

From: Chief, Bureau of Medicine and Surgery
To: All Ships and Stations

Subj: Standards for potable water

Ref: (a) ONMINST 5711.9 dated 16 May 1958 (NOTAL)
(b) BUMEDINST 5711.2 dated 30 January 1959 (NOTAL)

1. **Purpose.** To establish standards for water for drinking and culinary purposes throughout the Naval Establishment.

2. **Cancellation.** BUMED Instruction 6240.3A is canceled.

3. **Background**

a. **Policy.** The Department of Defense has established the policy of compliance by the Military Departments with United States Public Health Service Drinking Water Standards, as may be modified by the Medical Services of the Departments, or as may be modified by competent authority for purposes of international agreement.

b. **International agreement.** Naval Tripartite Standardization Agreement ABC-NAVY-STD-23 was promulgated by references (a) and (b). The object of the agreement is to provide the United States Navy, the Royal Navy, and the Royal Canadian Navy assurance that drinking and culinary water delivered to each other's ships from installations under their cognizance meets certain minimum standards of quality.

4. **Quality Standards.** The standards for bacteriological quality, physical and chemical characteristics, and radioactivity shall be those in "Public Health Service Drinking Water Standards, 1962." Department of Health, Education, and Welfare. The Standards, as modified, may be found in NAVMED P-5010-5, "Water Supply Ashore," available through the Navy Supply System.

5. **Definition of Terms.** The following terms are defined for clarification in interpretation of standards:

a. **Adequate protection by natural means** involves one or more of the following processes of nature that produce water consistently meeting the requirements of these Standards: dilution, storage, sedimentation, sunlight, aeration, and the associated physical and biological processes which tend to accomplish natural purification in surface waters and, in the case of ground waters, the natural purification of water by infiltration through soil and percolation through underlying material and storage below the ground water table.

b. **Adequate protection by treatment** means any one or any combination of the controlled processes of coagulation, sedimentation, absorption, filtration, disinfection, or other processes which produce a water consistently meeting the requirements of these standards. This protection also includes processes which are appropriate to the source of supply; works which are of adequat capacity to meet maximum demands without creating health hazards, and which are located, designed, and constructed to eliminate or prevent pollution; and conscientious operation by well-trained and competent personnel whose qualifications are commensurate with the responsibilities of the position.

c. **The coliform group** includes all organisms considered in the coliform group as set forth in Standard Methods for the Examination of Water and Wastewater, current edition, prepared and published jointly by the American Public Health Association, American Water Works Association, and Water Pollution Control Federation.

d. **Health hazards** mean any conditions, devices, or practices in the water supply system and its operation which create, or may create, a danger to the health and well-being of the water consumer. An example of a health hazard is a structural defect in the water supply system, whether of location, design, or construction, which may regularly or occasionally prevent satisfactory purification of the water supply or cause it to be polluted from extraneous sources.

e. **Pollution,** as used in these Standards, means the presence of any foreign substance (organic, inorganic, radiological, or biological) in water which tends to degrade its quality so as to constitute a hazard or impair the usefulness of the water.

f. **The standard sample** for the bacteriological test shall consist of:

(1) For the bacteriological fermentation tube test, five standard portions of either:

(a) 10 milliliters
(b) 100 milliliters

(2) For the membrane filter technique, not less than 50 milliliters.

g. **Water supply system** includes the works and auxiliaries for collection, treatment, storage, and distribution of the water from the sources of supply to the freeflowing outlet of the ultimate consumer.

6. **Source and Protection**

a. The water supply should be obtained from the most desirable source which is feasible, and effort should be made to prevent or control pollution of the source. If the source is not adequately protected by natural means, the supply shall be adequately protected by treatment.

b. Frequent sanitary surveys shall be made of the water supply system to locate and identify health hazards which might exist in the system.

c. Approval of water supplies shall be dependent in part upon:

(1) Enforcement of rules and regulations to prevent development of health hazards:

(2) Adequate protection of the water quality throughout all parts of the system, as demonstrated by frequent surveys;

(3) Proper operation of the water supply system under the responsible charge of personnel whose qualifications are acceptable to the Bureau of Yards and Docks or the Bureau of Ships, as appropriate;

(4) Adequate capacity to meet peak demands without development of low pressures or other health hazards; and

(5) Record of laboratory examinations showing consistent compliance with the water quality requirements of these Standards.

7. **Standards.** The limits listed below are generally those contained in "Public Health Service Drinking Water Standards, 1962." For sampling procedures and techniques, refer to NAVMED P-5010-5.

a. **Bacteriological quality: limits.** The presence of organisms of the coliform group as indicated by samples examined shall not exceed the following limits:

(1) When 10 ml. standard portions are examined, not more than 10 percent in any month shall show the presence of the coliform group. The presence of the coliform group in three or more 10 ml. portions of a standard sample shall not be allowable if this occurs:

(a) In two consecutive samples;
(b) In more than one sample per month when less than 20 are examined per month; or
(c) In more than five percent of the samples when 20 or more are examined per month.

When organisms of the coliform group occur in three or more of the 10 ml. portions of a single standard sample, daily samples from the same sampling point shall be collected promptly and examined until the results obtained from at least two consecutive samples show the water to be of satisfactory quality.

(2) When 100 ml. standard portions are examined, not more than 60 percent in any month shall show the presence of the coliform group. The presence of the coliform group in all five of the 100 ml. portions of a standard sample shall not be allowable if this occurs:

(a) In two consecutive samples;
(b) In more than one sample per month when less than five are examined per month; or
(c) In more than 20 percent of the samples when five or more are examined per month.

When organisms of the coliform group occur in all five of the 100 ml. portions of a single standard sample, daily samples from the same sampling point shall be collected promptly and examined until the results obtained from at least two consecutive samples show the water to be of satisfactory quality.

(3) When the membrane filter technique is used, the arithmetic mean coliform density of all standard samples examined per month shall not exceed one per 100 ml. Coliform colonies per standard sample shall not exceed 3/50 ml., 4/100 ml., 7/200 ml., or 13/500 ml. in:

(a) Two consecutive samples;
(b) More than one standard sample when less than 20 are examined per month; or
(c) More than five percent of the standard samples when 20 or more are examined per month.

When coliform colonies in a single standard sample exceed the above values, daily samples from the same sampling point shall be collected promptly and examined until the results obtained

from at least two consecutive samples show the water to be of satisfactory quality.

b. Physical characteristics: limits. Drinking water should contain no impurity which would cause offense to the sense of sight, taste, or smell. Under general use, the following limits should not be exceeded:

Turbidity	5 units
Color	15 units
Threshold Odor Number	3

c. Chemical characteristics: limits. Drinking water shall not contain impurities in concentrations which may be hazardous to the health of the consumers. It should not be excessively corrosive to the water supply system. Substances used in its treatment shall not remain in the water in concentrations greater than required by good practice. Substances which may have deleterious physiological effect, or for which physiological effects are not known, shall not be introduced into the system in a manner which would permit them to reach the consumer.

(1) The following chemical substances should not be present in a water supply in excess of the listed concentrations where, in the judgment of the Bureau of Yards and Docks and the Bureau of Medicine and Surgery, other more suitable supplies are or can be made available.

Substance	Concentration in mg/l (ppm)
Alkyl Benzene Sulfonate (ABS)	0.5
*Antimony (Sb)	0.01
Arsenic (As)	0.01
Chloride (Cl)	250.
Copper (Cu)	1.
Carbon Chloroform Extract (CCE)	0.2
Cyanide (CN)	0.01
Fluoride (F)	(See (3))
Iron (Fe)	0.3
Manganese (Mn)	0.05
Nitrate [1] (NO_3)	45.
Phenols	0.001
Sulfate (SO_4)	250.
Total Dissolved Solids	500.
Zinc (Zn)	5.

[1] In areas in which the nitrate content of water is known to be in excess of the listed concentration, the public should be warned of the potential dangers of using the water for infant feeding.

* Not contained in Drinking Water Standards but this limit was determined by the Public Health Service and the Bureau of Medicine and Surgery.

(2) The presence of the following substances in excess of the concentrations listed shall constitute grounds for rejection of the supply:

Substance	Concentration in mg/l (pp
*Antimony (Sb)	0.05
Arsenic (As)	0.05
Barium (Ba)	1.0
Cadmium (Cd)	0.01
Chromium (Hexavalent) (Cr^{+6})	0.05
Cyanide (CN)	0.2
Fluoride (F)	(See (3))
Lead (Pb)	0.05
Selenium (Se)	0.01
Silver (Ag)	0.05

* Not contained in Drinking Water Standards this limit was determined by the Public Health Service and the Bureau of Medicine and Surgery

(3) Fluoride. When fluoride is naturally present in drinking water, the concentration should not average more than the appropriate upper limit in the following Table I. Presence fluoride in average concentrations greater than two times the optimum values in Table I shall constitute grounds for rejection of the supply. When fluoridation (supplementation of fluoride drinking water) is practiced, the average fluoride concentration shall be kept within the upper and lower control limits in Table I.

TABLE I

Annual average of maximum daily air temperatures [2]	Recommended control limits--Fluoride concentrations in mg/l (ppm)		
	Lower	Optimum	Upper
50.0 - 53.7	0.9	1.2	1.7
53.8 - 58.3	0.8	1.1	1.5
58.4 - 63.8	0.8	1.0	1.3
63.9 - 70.6	0.7	0.9	1.2
70.7 - 79.2	0.7	0.8	1.0
79.3 - 90.5	0.6	0.7	0.8

[2] Based on temperature data obtained for a minimum of five years.

d. Radioactivity: limits.

(1) The effects of human radiation exposure are viewed as harmful and any unnecessary exposure to ionizing radiation should be avoide

Approval of water supplies containing radioactive materials shall be based upon the judgment that the radioactivity intake from such water supplies when added to that from all other sources is not likely to result in an intake greater than the radiation protection guidance [3] recommended by the Federal Radiation Council and approved by the President. Water supplies shall be approved without further consideration of other sources of radioactivity intake of Radium-226 and Strontium-90 when the water contains these substances in amounts not exceeding 3 and 10 μμc/liter, respectively. When these concentrations are exceeded, a water supply shall be approved by the certifying authority if surveillance of total intakes of radioactivity from all sources indicates that such intakes are within the limits recommended by the Federal Radiation Council for control action

(2) In the known absence [4] of Strontium-90 and alpha emitters, the water supply is acceptable when the gross beta concentrations do not exceed 1,000μμc/liter. Gross beta concentrations in excess of 1,000 μμc/liter shall be grounds for rejection of supply except when more complete analyses indicate that concentrations of nuclides are not likely to cause exposures greater than the Radiation Protection Guides as approved by the President on recommendation of the Federal Radiation Council.

[3] The Federal Radiation Council, in its Memorandum for the President, Sept. 13, 1961, recommended that "Routine control of useful applications of radiation and atomic energy should be such that expected average exposures of suitable samples of an exposed population group will not exceed the upper value of Range II (20μμc/day of Radium-226 and 200μμc/day of Strontium-90)."

[4] Absence is taken here to mean a negligibly small fraction of the above specific limits, where the limit for unidentified alpha emitters is taken as the listed limit for Radium-226.

8. **Technical Assistance.** Assistance with potable water problems may be requested from the following:

a. Preventive Medicine Units, in accordance with BUMED Instruction 6200.3A of 2 July 1957, Subj: U.S. Navy Preventive Medicine Units.

b. Bureau of Yards and Docks' Field Engineering Offices, in accordance with BUDOCKS Instruction 5450.19A of 21 September 1962, Subj: Sanitary Engineering Responsibilities of the Bureau of Yards and Docks Field Engineering Offices.

A. S. CHRISMAN
Deputy and Assistant Chief

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Then in 1972 we see "BUMED INSTRUCTION 6240.3C" From: Chief, Bureau of Medicine and Surgery To : ALL Ships and Stations Subj: Standards for Potable Water.

Pay special attention to page 6 on Chemical Concentrations where it now includes Pesticides, Herbicides, Fungicides and see footnote {2} This is just a short period of 9 years ,1963 through 1972 that "something brought to their attention" that it would be desirable to remove Pesticides and Herbicides from our drinking water. In my humble opinion scientific tests of some sort had to be conducted to verify this concern over Herbicides.

Maint

DEPARTMENT OF THE NAVY
Bureau of Medicine and Surgery
Washington, D.C. 20390

BUMEDINST 6240.3C CH-1
722-PAT:cb
13 December 1972

BUMED INSTRUCTION 6240.3C
CHANGE TRANSMITTAL 1

From: Chief, Bureau of Medicine and Surgery
To: All Ships and Stations

Subj: Standards for potable water

1. *Purpose.* To promulgate change 1 to the basic instruction to eliminate possible confusion concerning how nitrate and nitrite levels are to be determined. These levels are to be expressed as nitrate nitrogen or nitrite nitrogen which is in consonance with current testing procedures.

2. Action. On page 4, table, line 12, opposite entry for Nitrate and Nitrite, in the Concentration column, to present "10." add "(as N)" so that it will read:

10. (as N)

G. M. DAVIS

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DEPARTMENT OF THE NAVY
Bureau of Medicine and Surgery
Washington, D.C. 20390

BUMEDINST 6240.3C
722-PAT:cb
25 August 1972

BUMED INSTRUCTION 6240.3C

From: Chief, Bureau of Medicine and Surgery
To: All Ships and Stations

Subj: Standards for potable water

* Ref: (a) NAVMATINST 5711.9A of 17 June
* 1965 (NOTAL)
* (b) BUMEDINST 5711.2A of 3 December
* 1965

1. Purpose. To establish standards for water for
* drinking and culinary purposes throughout the Naval
* Establishment and prescribe the use of the DD Form
* 686, Bacteriological Examination of Water, and DD
* Form 710, Physical and Chemical Analysis of Water.

* 2. Cancellation. BUMED Instructions 6240.3B and
* 6240.5 are canceled.

3. Background

a. Policy. The Department of Defense has established the policy of compliance by the Military Departments with United States Public Health Service Drinking Water Standards, as may be modified by the Medical Services of the Departments, or as may be modified by competent authority for purposes of international agreement.

b. International Agreement. Naval Tripartite Standardization Agreement ABC-NAVY-STD-23A was promulgated by references (a) and (b). The object of the agreement is to provide the United States Navy, the Royal Navy, and the Royal Canadian Navy assurance that drinking and culinary water delivered to each other's ships from installations under their cognizance meets certain minimum standards of quality.

4. Quality Standards. The standards for bacteriological quality, physical and chemical characteristics, and radioactivity shall be those in "Public Health Service Drinking Water Standards, 1962" Department of Health, Education, and Welfare. The Standards, as modified, may be found in NAVMED P-5010-5, Water Supply Ashore, available through the Navy Supply System.

5. Definition of Terms. The following terms are defined for clarification in interpretation of standards:

a. Adequate protection by natural means involves one or more of the following processes of nature that produce water consistently meeting the requirements of these standards: dilution, storage, sedimentation, sunlight, aeration, and the associated physical and biological processes which tend to accomplish natural purification in surface waters and, in the case of ground waters, the natural purification of water by infiltration through soil and percolation through underlying material and storage below the ground water table.

b. Adequate protection by treatment means any one or any combination of the controlled processes of coagulation, sedimentation, absorption, filtration, disinfection, or other processes which produce a water consistently meeting the requirements of these standards. This protection also includes processes which are appropriate to the source of supply; works which are of adequate capacity to meet maximum demands without creating health hazards, and which are located, designed, and constructed to eliminate or prevent pollution; and conscientious operation by well trained and competent personnel whose qualifications are commensurate with the responsibilities of the position.

c. The coliform group includes all organisms considered in the coliform group as set forth in Standard Methods for the Examination of Water and Wastewater, current edition, prepared and published jointly by the American Public Health Association, American Water Works Association, and Water Pollution Control Federation.

d. Health hazards mean any conditions, devices, or practices in the water supply system and its operation which create, or may create, a danger to the health and well-being of the water consumer. An example of a health hazard is a structural defect in the water supply system, whether of location, design, or construction, which may regularly or occasionally prevent satisfactory purification of the water supply or cause it to be polluted from extraneous sources.

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(2)

e. Pollution, as used in these standards, means the presence of any foreign substance (organic, inorganic, radiological, or biological) in water which tends to degrade its quality so as to constitute a hazard or impair the usefulness of the water.

f. The standard sample for the bacteriological test shall consist of:

(1) For the bacteriological fermentation tube test, five standard portions of either:

(a) 10 milliliters
(b) 100 milliliters

(2) For the membrane filter technique, not less than 50 milliliters.

g. Water supply system includes the works and auxiliaries for collection, treatment, storage, and distribution of the water from the sources of supply to the freeflowing outlet of the ultimate consumer.

6. Source and Protection

a. The water supply should be obtained from the most desirable source which is feasible, and effort should be made to prevent or control pollution of the source. If the source is not adequately protected by natural means, the supply shall be adequately protected by treatment.

b. Frequent sanitary surveys shall be made of the water supply system to locate and identify health hazards which might exist in the system.

c. Approval of water supplies shall be dependent in part upon:

(1) Enforcement of rules and regulations to prevent development of health hazards;

(2) Adequate protection of the water quality throughout all parts of the system, as demonstrated by frequent surveys;

(3) Proper operation of the water supply system under the responsible charge of personnel whose qualifications are acceptable to the Navy Facilities Engineering Command or Navy Ship Systems Command.

(4) Adequate capacity to meet peak demands without development of low pressures or other health hazards; and

(5) Record of laboratory examinations showing consistent compliance with the water quality requirements of these standards.

7. Standards. The limits listed below are generally those contained in Public Health Service Drinking Water Standards, 1962. For sampling procedures and techniques, refer to NAVMED P-5010-5.

a. Bacteriological Quality (Limits). The presence of organisms of the coliform group as indicated by samples examined shall not exceed the following limits:

(1) When 10 ml. standard portions are examined, not more than 10 percent in any month shall show the presence of the coliform group. The presence of the coliform group in three or more 10 ml. portions of a standard sample shall not be allowable if this occurs:

(a) In two consecutive samples;
(b) In more than one sample per month when less than 20 are examined per month; or
(c) In more than five percent of the samples when 20 or more are examined per month.

When organisms of the coliform group occur in three or more of the 10 ml. portions of a single standard sample, daily samples from the same sampling point shall be collected promptly and examined until the results obtained from at least two consecutive samples show the water to be of satisfactory quality.

(2) When 100 ml. standard portions are examined, not more than 60 percent in any month shall show the presence of the coliform group. The presence

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of the coliform group in all five of the 100 ml. portions of a standard sample shall not be allowable if this occurs:

(a) In two consecutive samples;
(b) In more than one sample per month when less than five are examined per month; or
(c) In more than 20 percent of the samples when five or more are examined per month.

When organisms of the coliform group occur in all five of the 100 ml. portions of a single standard sample, daily samples from the same sampling point shall be collected promptly and examined until the results obtained from at least two consecutive samples show the water to be of satisfactory quality.

(3) When the membrane filter technique is used, the arithmetic mean coliform density of all standard samples examined per month shall not exceed one per 100 ml. Coliform colonies per standard sample shall not exceed 3/50 ml., 4/100 ml., 7/200 ml., or 13/500 ml. in:

(a) Two consecutive samples;
(b) More than one standard sample when less than 20 are examined per month; or
(c) More than five percent of the standard samples when 20 or more are examined per month.

When coliform colonies in a single standard sample exceed the above values, daily samples from the same sampling point shall be collected promptly and examined until the results obtained from at least two consecutive samples show the water to be of satisfactory quality.

b. Bacteriological Examination of Water. Bacteriological Examination of Water, DD Form 686, shall * be used by all naval facilities, both ashore and afloat, to conduct bacteriological examination of water.

c. Physical Characteristics (Limits). Drinking water should contain no impurity which would cause offense to the sense of sight, taste, or smell. Under general use, the following limits should not be exceeded:

Turbidity	5 units
Color	15 units
Threshold Odor Number	3

d. Chemical Characteristics (Limits). Drinking water shall not contain impurities in concentrations which may be hazardous to the health of the consumers. It should not be excessively corrosive to the water supply system. Substances used in its treatment

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shall not remain in the water in concentrations greater than required by good practice. Substances which may have deleterious physiological effect, or for which physiological effects are not known, shall not be introduced into the system in a manner which would permit them to reach the consumer.

(1) The following chemical substances should not be present in a water supply in excess of the listed concentrations where, in the judgement of the Navy Facilities Engineering Command and the Bureau of Medicine and Surgery, other more suitable supplies are or can be made available.

Substance	Concentration in mg/l (ppm)	
Antimony (Sb) (See footnote 1.)	0.01	
Arsenic (As)	0.01	
Chloride (Cl)	250.	
Carbon Chloroform Extract (CCE)	0.15	*
Copper (Cu)	1.	
Cyanide (CN)	0.01	
Fluoride (F)	See 7d(3)	
Iron (Fe)	0.3	
Manganese (Mn)	0.05	
Mercury (Hg) (See footnote 2.)	0.005	
Methylene Blue-Active Substance (Including ABS)	0.5	*
Nitrate (NO_3), Nitrite (NO_2) (See footnote 3.)	10.	*
pH (Range)	6.0 - 9.0	*
Phenols	0.001	
Sulfate (SO_4)	250.	
Total Dissolved Solids	500.	
ZINC (Zn)	5.	

Footnotes:

1. Not contained in Drinking Water Standards but this limit set by PHS and BUMED.

2. Not contained in Drinking Water Standards but this limit set by BUMED upon recommendation of EPA. *

3. In areas in which the nitrate or nitrite content of water is known to be in excess of the listed concentration, * the public should be warned of the potential dangers of using the water for infant feeding.

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(2) The presence of the following substances in excess of the concentrations listed shall constitute grounds for rejection of the supply:

Substance	Concentration in mg/l (ppm)
Antimony (Sb) (See footnote 1.)	0.05
Arsenic (As)	0.05
Barium (Ba)	1.0
Cadmium (Cd)	0.01
Chromium (Hexavalent) ($Cr^{+}6$)	0.05
Cyanide (CN)	0.2
Fluoride (F)	See 7d(3)
Lead (Pb)	0.05
Pesticides, Herbacides, Fungicides (See footnote 2.)	
Chlorinated hydrocarbons	0.003 - 0.1
Organo-phosphates	0.1
Chlorophenoxy herbacides	0.005 - 1.00
Selenium (Se)	0.01
Silver (Ag)	0.05

Footnotes:

1. Not contained in Drinking Water Standards but this limit set by PHS and BUMED.

2. Concentrations represent range of levels for each group of chemicals. Individual pesticides have specific concentrations - queries should be directed to BUMED (Code 72).

(3) **Fluoride.** When fluoride is naturally present in drinking water, the concentration should not average more than the appropriate upper limit in the following table. Presence of fluoride in average concentrations greater than two times the optimum values in the table shall constitute grounds for rejection of the supply. When fluoridation (supplementation of fluoride in drinking water) is practiced, the average fluoride concentration shall be kept within the upper and lower control limits in the table.

Annual average of maximum daily air temperatures, based on data obtained for a minimum of 5 years	Recommended control limits-Fluoride concentrations in mg/l (ppm)		
	Lower	Optimum	Upper
50.0 - 53.7	0.9	1.2	1.7
53.8 - 58.3	0.8	1.1	1.5
58.4 - 63.8	0.8	1.0	1.3
63.9 - 70.6	0.7	0.9	1.2
70.7 - 79.2	0.7	0.8	1.0
79.3 - 90.5	0.6	0.7	0.8

e. Physical and Chemical Analysis of Water. Physical and Chemical Analysis of Water, DD Form 710, shall be used by all naval facilities, both ashore and afloat, to conduct physical and chemical analysis of water.

CLW

f. Radioactivity (Limits).

(1) The effects of human radiation exposure are viewed as harmful and any unnecessary exposure to ionizing radiation should be avoided. Approval of water supplies containing radioactive materials shall be based upon the judgement that the radioactivity intake from such water supplies when added to that from all other sources is not likely to result in an intake greater than the radiation protection guidance recommended by the Federal Radiation Council and approved by the President. (The Federal Radiation Council, in its 13 September 1961, Memorandum for the President, recommended that "Routine control of useful applications of radiation and atomic energy should be such that expected average exposures of suitable samples of an exposed population group will not exceed the upper value of Range II (20 μμc/day of Radium-226 and 200 μμc/day of Strontium-90).") Water supplies shall be approved without further consideration of other sources of radioactivity intake of Radium-226 and Strontium-90 when the water contains these substances in amounts not exceeding 3 and 10 μμc/liter, respectively. When these concentrations are exceeded, a water supply shall be approved by the certifying authority if surveillance of total intakes of radioactivity from all sources indicates that such intakes are within the limits recommended by the Federal Radiation Council for control action.

(2) In the known absence (taken here to mean a negligibly small fraction of the above specific limits, where the limit for unidentified alpha emitters is taken as the listed limit for Radium-226) of Strontium-90 and alpha emitters, the water supply is acceptable when the gross beta concentrations do not exceed 1,000 μμc/liter. Gross beta concentrations in excess of 1,000 μμc/liter shall be grounds for rejection of supply except when more complete analyses indicate that concentrations of nuclides are not likely to cause exposures greater than the Radiation Protection Guides as approved by the President on recommendation of the Federal Radiation Council.

8. Technical Assistance. Assistance with potable water problems may be requested from the following:

a. Environmental and Preventive Medicine Units, in accordance with BUMED Instruction 6200.3C series, Subj: Environmental and Preventive Medicine Units.

b. Navy Facilities Engineering Command's Field Engineering Offices in accordance with current NAVFAC Instruction 5450.19 series, Subj: Sanitary Engineering Responsibilities of the Naval Facilities Engineering Command Field Division.

9. Procurement of DD Form 686 and DD Form 710. DD Form 686, Bacteriological Examination of Water, and DD Form 710, Physical and Chemical Analysis of Water, may be obtained from Cognizance I stock points of the Navy Supply System.

G. M. DAVIS

Distribution:
SNDL Parts 1 and 2
MARCORPS Code CC (less MarBks)

Stocked:
COMNAVDIST WASH DC
(Supply & Fiscal Dept.—Code 514.3)
Wash. Navy Yard
Wash., D.C. 20390

CLW

0000000150

(7)

Then in February 1987 we have the document from Naval Facilities Engineering Command Guide Performance Work Statement {GPWS } For Water Plants and System Operation and Maintenance. Prepared by Southern Division Naval Facilities Engineering Command in Charleston, South Carolina.

It "states" The contractor shall produce and store treated water free of taste and or odor and that meets the minimum water quality standards described below: see page 44 {PDF DOC } Where we see Herbicides are a concern again.

NAVAL FACILITIES ENGINEERING COMMAND

GUIDE PERFORMANCE WORK STATEMENT (GPWS)

FOR

WATER PLANTS AND SYSTEMS OPERATION AND MAINTENANCE

PREPARED BY

SOUTHERN DIVISION

NAVAL FACILITIES ENGINEERING COMMAND

2155 EAGLE DRIVE, P. O. BOX 190010

CHARLESTON, SOUTH CAROLINA 29419-9010

FEBRUARY l987

shall produce sufficient potable water to meet demand up to a maximum of ! INSERT NUMBER OF GALLONS! gallons per !INSERT TIMEFRAME!. The Contractor shall maintain the system so that in the event of a draw down below the system's minimum storage limit, storage volumes can be replaced at the rate of INSERT NUMBER OF GALLONS! gallons per !INSERT TIMEFRAME!. The Contractor shall produce and store treated water free of taste and/or odor, and that meets the minimum water quality standards described below:

MEASURABLE OUTPUT	UNIT OF MEASURE	PERFORMANCE LEVEL (# OF UNITS)
INORGANIC CHEMICALS		MAX. ALLOWABLE
Arsenic	mg/l	0.05
Barium	mg/l	1.0
Cadmium	mg/l	0.010
Chromium	mg/l	0.05
Lead	mg/l	0.05
Mercury	mg/l	0.002
Nitrate (as N)	mg/l	10.0
Selenium	mg/l	0.01
Silver	mg/l	0.05
ORGANIC CHEMICALS		MAX. ALLOWABLE
Endrin	mg/l	0.0002
Lindane	mg/l	0.004
Methoxychlor	mg/l	0.10
Toxaphene	mg/l	0.005
2, 4-D	mg/l	0.10
2, 4, 5-TP Silvex	mg/l	0.01
TTHM	mg/l	0.10
TURBIDITY	NTU	1.0
MICROBIOLOGICAL (coliform)	NOTE (1)	NOTE (1)
(a) Membrane filter technique		
or		
(b) Fermentation tubes with 10 ml. standard portions; 5 - tube MPN.		
RADIOACTIVITY		
Radium 226 + Radium 228	p Ci/l	5.0
Gross Alpha (NOTE 3)	p Ci/l	15.0
Beta particle/photon	mrem/yr	4.0
CHLORINE RESIDUAL		
Sample point (a)	mg/l	0.2(min. allowable)
Sample point (b)	mg/l	0.2
Sample point (c)	mg/l	0.2
Sample point (d)	mg/l	0.2
Sample point (etc.)	mg/l	0.2
FLUORIDE	mg/l NOTE (2)	0.7-1.2
HARDNESS	mg/l as $CaCO_3$	180

NOTE (1): Obtain values from 40 CFR 141.14 and 141.21 for: (a) membrane filter technique and (b) fermentation tubes with 10 ml standard portions; 5-tube MPN.

Finally, see the following study where Researchers in Vietnam in 1970 tested fish and crustaceans For the presence of TCDD {Dioxin}. These are the same researchers that were mentioned in the Australian ENTOX study and the fish tested were caught by local fishermen in Vietnam, both in fresh water as well as saltwater. This shows that dioxin's were present in local fish in 1970 and If dioxin "stopped" at lands-end, as DVA would have us believe, how did it pollute saltwater fish and crustaceans.

An Analytical Method for Detecting TCDD (Dioxin): Levels of TCDD in Samples from Vietnam

by Robert Baughman* and Matthew Meselson*

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is an extraordinarily toxic substance that is produced as an unwanted side product in the industrial synthesis of 2,4,5-trichlorophenol, an intermediate in the manufacture of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) (*1*, *2*). Because of its chemical stability and its lipophilic nature, the possibility exists that TCDD released into the environment could accumulate in food chains. A direct test of the possibility of biologically significant accumulation in animal tissues requires an analytical method able to detect TCDD at levels well below those known to be toxic. The lowest value known for the lethal dose of TCDD is that observed in the guinea pig, for which the single oral dose LD_{50} is 600 parts per trillion (ppt) body weight (*3*). Allowing for sublethal toxic effects and providing for a conservative margin of safety, it seems desirable to have an analytical sensitivity of at least 1 ppt. For a 1-g sample this means the method must have a sensitivity of about 10^{-12}g or 1 picogram (pg).

The most common method for analyzing chlorinated organic compounds in tissue samples is gas–liquid chromatography (GLC) with an electron capture detector. Its limit of detection for TCDD, about 10^{-10}g, is inadequate. This method is also susceptible to interference from other compounds and so is not very specific.

Mass spectrometry offers better possibilities. It is high sensitive and in the high resolution mode of operation it is highly specific. We have previously described a time averaged mass spectroscopic method with an adequate limit of detection (*4*). However, full sensitivity could not be realized in most sample types because of interference from DDE (a major degradation product of DDT) and polychlorinated biphenyls (PCBs). In this paper we describe a cleanup procedure that overcomes this difficulty.

Homogenized samples are saponified in alcoholic potassium hydroxide and extracted with hexane. The extract is shaken with sulfuric acid and chromatographed on alumina. Elution with carbon tetrachloride–hexane removes most of the DDE and PCBs. Chlorinated dioxins are then eluted with dichloromethane–hexane. The TCDD containing fraction is further purified by preparative gas–liquid chromatography and analyzed by mass spectroscopy by use of a multichannel analyzer to average successive scans.

We also report the levels of TCDD found in a limited number of samples of fish and crustaceans from locations in South Vietnam near areas heavily exposed to 2,4,5-T.

*Harvard University, Cambridge, Massachusetts 02138.

Experimental

Reagents and Apparatus

Hexane (pesticide grade, Fisher Scientific), dichloromethane (reagent grade, Eastman), carbon tetrachloride (reagent grade, Merck), 95–97% sulfuric acid (reagent grade, Dupont), sodium carbonate (powdered) (reagent grade, Mallinckrodt), and ethanol (pesticide grade, Matheson, Coleman and Bell) were used.

Activated alumina was Fisher A–540, activated at 130° C for 24 hr.

The gas chromatograph was a Bendix Model 2200 equipped with a thermal conductivity detector. The column was 5% SE–30 on 60/80 Chromosorb W, 2 m × 2 mm (id) stainless steel. The trap for preparative gas chromatography was a 150 mm × 1.5 mm (id) glass tube packed with 30 mm of glass wool.

An Associated Electrical Industries MS–9 double focusing mass spectrometer and a Varian 1024 time-averaging computer interfaced with the MS–9 as described earlier (*4*), were used.

Cleanup Procedure for the Analysis of TCDD in Tissue Samples

(1) The sample was weighed and homogenized with 1.0–1.2 parts EtOH.

(2) This homogenate was transferred to a round-bottomed flask equipped with a reflux condenser (Teflon tape used on the ground glass joint). The sample was spiked with approximately 1000 ppt ^{37}Cl TCDD; 2 parts 40% aqueous KOH were added, and this mixture was refluxed for 2 hr. One part always refers to the original samples.

(3) The solution was partially cooled and 1 part hexane added.

(4) The solution was transferred to a separatory funnel, and the phases were separated. The aqueous phase was extracted with three more identical portions of hexane; the hexane extracts were combined and collected in the original round-bottomed flask.

(5) The hexane phase was transferred to the separatory funnel, the round-bottomed flask was rinsed twice with a few milliliters of EtOH and then twice with a few milliliters of hexane; the solvent was refluxed each time; and the hexane was extracted with 1 part 1.0*N* NaOH.

(6) The hexane was extracted four times (or until acid phase was colorless) with 2 parts 95–97% H_2SO_4. Emulsions were broken with a few drops of saturated Na_2CO_3 solution.

(7) The hexane was extracted with 1 part water, and several grams of Na_2CO_3 were added to the hexane.

(8) The hexane was filtered through a column of Na_2CO_3 (100 mm × 10 mm id for 300 ml hexane), the Na_2CO_3 first being prewashed with several milliliters of hexane.

(9) The hexane was concentrated to 3–4 ml (Snyder column).

(10) The hexane residue was chromatographed on a column of activated Al_2O_3 (50 mm in a 5 mm disposable pipet). The column should not be prewashed. Elution was with 12 ml of 20% CCl_4 in hexane, then 1 ml of hexaane, and finally 4 ml of 20% CH_2Cl_2 in hexane.

(11) The 20% CH_2Cl_2 fraction was concentrated carefully to about 50 μl, 100–200 μl benzene added, and concentration repeated to 20 μl.

(12) A few micrograms of m-terphenyl in benzene were added to the residue and the mixture subjected to preparative chromatography. The retention time of *m*-terphenyl relative to that of TCDD was determined beforehand and used to make certain that the TCDD collection was carried out at the right retention time.

(13) The GLC trap containing TCDD was eluted with 60μl followed by 10μl of benzene. The total amount of eluant collected was measured, and the fraction size for the planned number of fractions (typically ten) calculated.

(14) The fractions for TCDD analysis were prepared in the sample tubes described previously (*4*). A known amount of TCDD was added to three or more fractions for quantitation of any TCDD observed. The amount of TCDD added per fraction for

quantitation should be approximately three or four times the amount expected to be present.

(15) The fractions were analyzed with the MS–9 instrument. Typical conditions were: source 220°C, resolution 10,000 (based on a 10% valley between peaks), trap current 1.0 mA (rhenium filament), electron multiplier 700, ionizing voltage 70 eV, time averaging at four scans per second.

(16) Peak heights were measured at *m/e* 321.894. The quantity of TCDD (picagrams), present in the fractions to which TCDD has not been added was computed from the ratio of their mean peak heights to the mean peak heights found with added TCDD.

(17) Steps (14)–(16) were repeated, but ^{37}Cl TCDD was added and peak heights were measured at *m/e* 327.885 in order to compute the amount of ^{37}Cl TCDD recovered. The recovery through the complete cleanup procedure was then calculated based on the amount of ^{37}Cl TCDD added to the sample at the beginning of the cleanup.

(18) The quantity of TCDD computed in step (15) was corrected by the recovery factor obtained in step (16) to give the final result.

Sample Collection

Freshly caught fish and crustaceans were collected in South Vietnam in August and September 1970 from local fishermen. The samples were homogenized with a meat grinder, placed in acetone-rinsed glass bottles with aluminum foil-lined caps, and immediately frozen in solid CO_2. Later on the same day, samples were placed in a Linde LR–35 liquid nitrogen refrigerator where they remained until analysis. Water blanks were present in the liquid nitrogen refrigerator throughout the storage period and were analyzed with the samples. Fresh Cape Cod butterfish (*Poronotus tricanthus*, family *Stromateidae*) were obtained from a local market, homogenized, and kept at −20° C until analysis. Domestic beef livers were obtained and treated similarly.

Results

Methodology

The mass spectra of natural and ^{37}Cl TCDD are shown in Figure 1. The most intense signal for natural TCDD occurs at *m/e* 321.894 (nominal *m/e* 322), corresponding to the isotopic isomer with one atom of ^{37}Cl and three atoms of ^{35}Cl. The natural abundances of the Cl isotopes are 75.53 and 24.47%, respectively. The observed spectrum for the synthetic ^{37}Cl TCDD corresponds to an isotopic purity of 95.5% ^{37}Cl, the same as the value claimed by the manufacturer (Oak Ridge National Laboratory) of the NaCl used in the synthesis of the labeled TCDD. The synthetic ^{37}Cl TCDD contributes only 0.042% as much to the peak at *m/e* 322 as it contributes to its most intense signal at *m/e* 328. The contribution at *m/e* 320 is even lower, by a factor of nearly 100. This allows an excess of ^{37}Cl TCDD to be added to each sample before cleanup without interfering

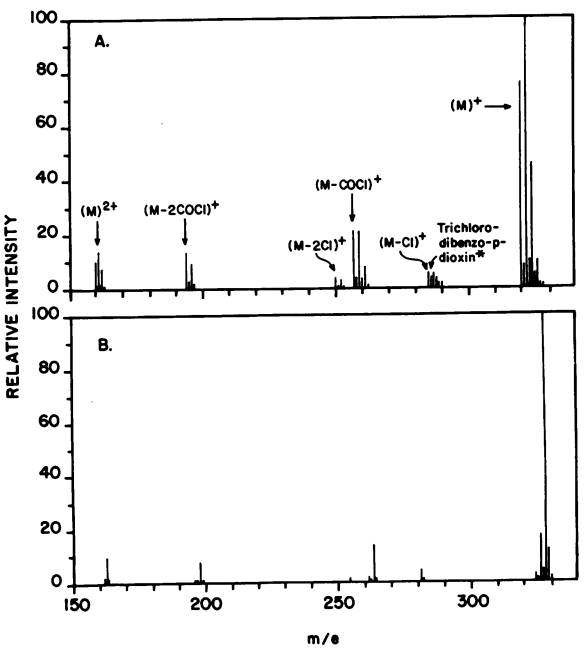

FIGURE 1. Mass spectra of (A) TCDD and (B) ^{37}Cl-labeled TCDD. The isotopic purity of the ^{37}Cl is 95.5%. The asterisk denotes an impurity. The multiplicity of lines associated with each major molecular species results from the presence of various isotopes of Cl and C.

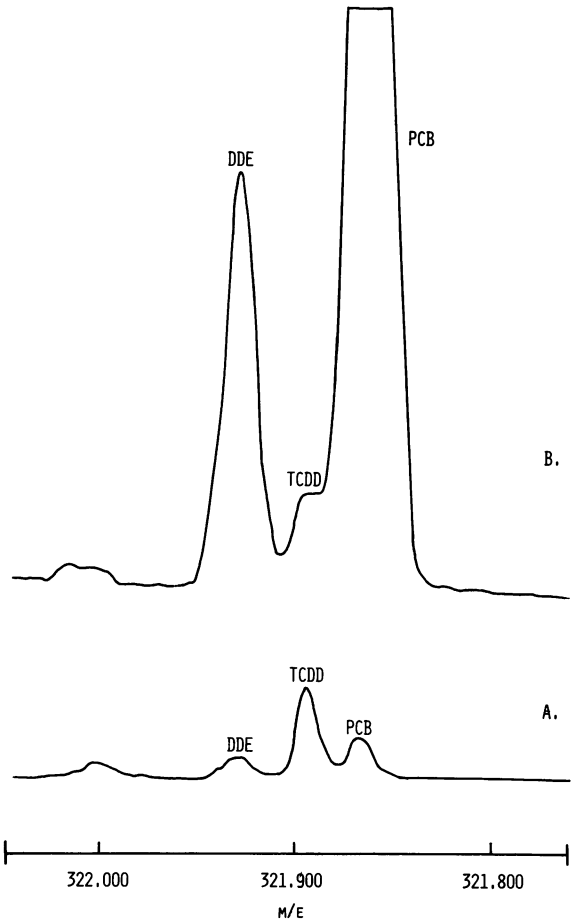

FIGURE 2. Mass spectra showing reduction of DDE and PCB levels in fish residue by means of alumina chromatography. Following the sulfuric acid cleanup step, the residue in hexane is added to a column of activated alumina: (*A*) Trace from the material eluted by 20% CH_2Cl_2 in hexane after the column was first eluted with 20% CCl_4 in hexane; (*B*) trace obtained from a similar 20% CH_2Cl_2-in-hexane elution after the column was first eluted with 1% CH_2Cl_2 in hexane. Elution with 1% CH_2Cl_2 in hexane was reported to be effective in reducing the amount of PCB residues (*5*). Elution with 20% CCl_4 is clearly even more effective and was routinely used in obtaining the results reported here.

with analysis of natural TCDD at *m/e* 322 and 320. The addition of ^{37}Cl TCDD provides a carrier and makes possible the calculation of absolute recoveries.

An alumina chromatography step has been developed which, when combined with the cleanup steps described previously, (*4*) makes possible the measurement of picogram quantities of TCDD in samples initially containing more than a millionfold excess of DDE and PCBs. Figure 2 shows the effectiveness of this procedure.

The calculation of TCDD levels described in steps (14)–(16) of the experimental section assumes a linear relationship between peak height and amount of TCDD present in any given sample. Figure 3 demonstrates that the response is indeed linear over the full range of TCDD amounts introduced into the MS–9 in the course of the analyses reported here.

The reproducibility and overall recovery of the complete analytical procedure is illustrated in Table 1. A sample of beef liver was homogenized and divided into three portions each of which was then spiked with 20 ppt TCDD and 1000 ppt ^{37}Cl TCDD. The three samples were independently put through the cleanup procedure up to the GLC step. Each sample was then split into three portions before preparative GLC and mass spectrometric analysis, giving rise to a total of nine separate values for the recovery of both TCDD and ^{37}Cl TCDD. The average recovery was 34 ± 7% for TCDD and 27 ± 5% for ^{37}Cl TCDD. When the slight background signal at *m/e* 322 in an unspiked

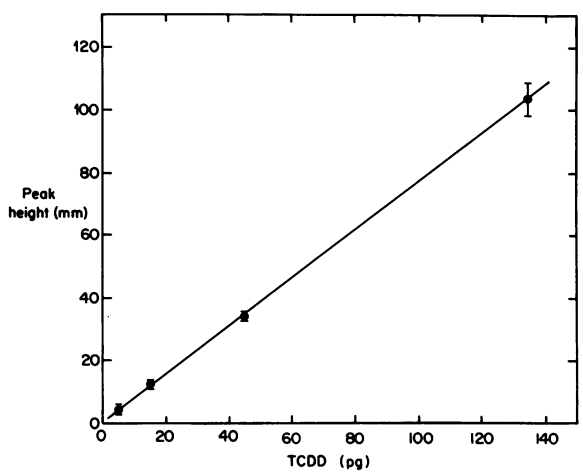

FIGURE 3. Linearity of response for TCDD in the presence of beef liver residue. The TCDD values are the amounts introduced into individual runs on the MS-9.

Table 1. Recoveries of TCDD (added at 20 ppt) and ^{37}Cl TCDD (added at 1000 ppt) from beef liver.

Sample	Recovery, %	
	TCDD	^{37}Cl TCDD
Sample A		
GLC 1	47	24
GLC 2	36	30
GLC 3	36	25
Sample B		
GLC 1	28	35
GLC 2	31	29
GLC 3	24	20
Sample C		
GLC 1	29	27
GLC 2	40	32
GLC 3	37	21
Mean recovery for A, B, and C	34 ± 7.2	27 ± 5.0

sample of the same liver is taken into account, the calculated recoveries from the spiked samples become even more nearly equal. Experiments performed separately with each individual cleanup step established that the step with lowest recovery is preparative gas–liquid chromatography.

We conclude from these and other controls that the present analytical method provides the sensitivity and reproducibility required for biologically meaningful analyses of animal tissue samples. The method makes possible investigations of such samples at levels approximately 10^{-4} times those reported heretofore (*6*).

Observed TCDD Levels

Signals at *m/e* 320 and 322 were conspicuously present in each of the fish and crustacean samples from Vietnam. The calculated levels of TCDD, summarized in Table 2, range from 18 ppt to 814 ppt, based on total wet body weight.

No peak was observed at *m/e* 320 or 322 with Cape Cod butterfish. The background signal corresponded to a level of 3 ppt of TCDD. No peaks were observed in water blank samples present in the liquid nitrogen refrigerator throughout the sample collection and storage period.

Confirmation that peaks observed at *m/e* 320 and 322 are in fact produced by TCDD is routinely provided by the criteria outlined in part A of Table 3. All three of these criteria are met by the mass spectra from each of the Vietnamese samples.

The additional confirmatory procedures listed in part B of Table 3 were carried out on a sample of Vietnamese fish. This sample, carp from the Dong Nai River, exhibited a mean TCDD level of 540 ppt. The mass spectrum in the region *m/e* 322 is shown in Figure 4. The compound observed in this fish behaved identically to TCDD in each of the three additional confirmatory tests. We consider it extraordinarily unlikely that this compound is anything other than a tetrachlorodibenzo-*p*-dioxin. In contrast to the significant amounts of 2,3,7,8-tetrachlorodi-

Table 2. TCDD levels in fish and crustaceans.

Map site [a]			Level, ppt total wet body weight [b]			
			I	II	III	Mean
A	Dong Nai River (interior)	Carp (Cyprininae)	690	320	610	540
B	Dong Nai River (interior)	Catfish (Siluridae)	610	1020		810
B	Dong Nai River (interior)	Catfish (Tachysuridae)	510	530		520
C	Sai Gon River (interior)	Catfish (Schilbeidae)	52	89		70
C	Sai Gon River (interior)	River Prawn (Palaemonidae)	34	49		42
D	Can Gio Village (seacoast)	Croaker (Sciaenidae)	110	49		79
D	Cape Gio Village (seacoast)	Prawn (Peneidae)	23	14		18
	Cape Cod, Massachusetts	Butterfish (Stromateidae)				≤3

[a] Letters refer to sites on map in Figure 5.

[b] Roman numerals refer to independent cleanups of different portions of the same sample. All values are corrected for recovery.

Table 3. Confirmation Procedures

A.	Routine
	1. Follows ^{37}Cl TCDD through highly specific cleanup
	2. Has expected mass (±2-3 mmu) at *m/e* 320 and 322
	3. Has expected ratio of isotopic isomers at *m/e* 320 and 322
B.	Additional [a]
	1. M^+-COCl fragmentation peak has expected mass and isotopic isomer ratio
	2. Percent recovery after partial photolytic decomposition equals that of ^{37}Cl TCDD (*7*, *8*)
	3. Partition coefficient between dichloromethane-hexane and acetonitrile equals that of ^{37}Cl TCDD (*7*).

[a] Steps 2 and 3 of the additional procedures were carried out on the dichloromethane–hexane eluant from the alumina chromatography prior to preparative GLC.

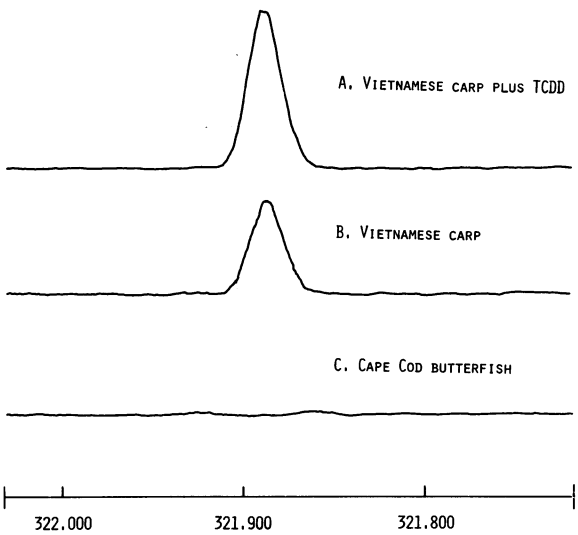

FIGURE 4. TCDD signals observed in fish samples: (*A*) Vietnamese carp plus 60 pg TCDD, (wet weight of fish 0.18 g); (*B*) Vietnamese carp, (wet weight of fish 0.18 g); (*C*) Cape Cod butterfish (wet weight of fish 0.16 g).

benzo-*p*-dioxin known to have been disseminated as a contaminant of 2,4,5-T (*1*), we know of no likely route by which other isomers of TCDD might have been introduced into the Vietnamese environment.

The locations from which the Vietnamese samples were obtained are designated in Figure 5. The letters correspond to those in Table 2. Areas heavily treated with 2,4,5-T before its use was ordered discontinued in

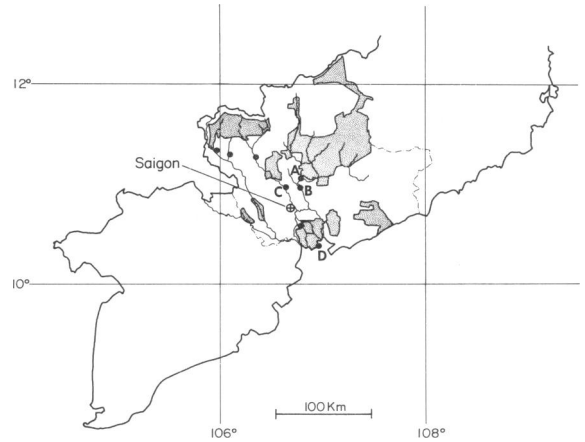

FIGURE 5. Map showing sampling sites in relation to rivers and principal sprayed areas. Sites A and B are located on the Dong Nai River, site C is on the Sai Gon River, and site D is on the coast at Can Gio. Sprayed areas are depicted only within the region bounded by the dashed lines (– · –).

April 1970 are shown as stipled. The number of samples is not adequate to permit reliable conclusions concerning the differences between various locations and species, although this certainly should be a subject of future studies.

Discussion

Considering the limited number of samples we have analyzed and the fact that they were collected 2½ yr ago, it does not seem appropriate to attempt any detailed evaluation of the possible toxicological significance of our results. Such discussion is made even more difficult by the complexity and incompleteness of the existing toxicological data. However, in order to provide perspective for such discussion, a tabulation of some of the principal toxicity data on TCDD is presented in Table 4. It may be noted that guinea pigs consuming their weight of food contaminated with TCDD at a level of 600 ppt would have ingested a quantity corresponding to the lethal dose. In contrast, a far greater quantity of TCDD is required to reach the LD_{50} cited for rats. The table shows that teratogenesis in the rat occurs at doses substantially lower than those required to kill.

Table 4. Levels of TCDD giving various biological effects.

Effect	TCDD to obtain effect, ppt body weight	Reference
Lethality		
Female rat, single oral dose LD_{50} (observations terminated at 44 days)	45,000	(3)
Male rat, single oral dose LD_{50} (observations terminated at 44 days)	23,000	(3)
Male guinea pig, single oral dose LD_{50} (observations terminated at 50 days)	600	(3)
Teratogenicity		
Cleft palate in 50% NMRI mice, daily oral dose, days 6–15	5,000	(9)
Intestinal hemorrhage and subcutaneous edema in 50% Sprague-Dawley rats, daily oral dose, days 6–15	125-500	(3)
Edema and death in chicken embryo, single injection	20	(10)
Enzyme induction		
Doubling of δ-aminolevulinic acid synthetase in chicken embryo, single injection	30	(11)
Mitotic arrest		
Lily endosperm, ambient concentration	<200	(12)

Feeding studies in monkeys show that dioxin poisoning is cumulative (13). Various levels of a toxic fat known to contain chlorodioxins were incorporated into the daily diet of *Macaca mulatta* monkeys. As pointed out by the investigators, the mean survival time depended inversely on the daily dose. A plot of their data (Fig. 6) conforms rather well to the relation $T = K/D + K'$, where T is mean survival time, D is daily dose, and K and K' are constants corresponding respectively, to the accumulated lethal dose and to the lag time between the accumulation of this dose and the time of death. No departure from this relation is seen even at the lowest level of toxic fat tested, where the mean survival time was 445 days. The importance of this result is that repeated intake of quantities of TCDD individually equal to only a small percentage of the single oral dose LD_{50} may over time cause serious poisoning. Unfortunately, the LD_{50} for TCDD in these primates cannot be computed since all the animals died (5/5), even at the lowest dose level, and the concentration of TCDD in the toxic fat has not been established.

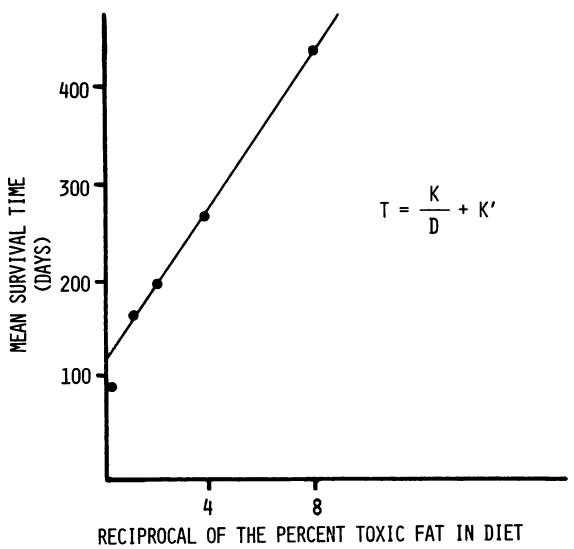

FIGURE 6. Mean survival time of monkeys fed toxic fat plotted against the reciprocal of the per cent of toxic fat present in the diet (13).

In South Vietnam itself we have little information regarding the possible occurrence of toxic effects of TCDD in humans. Certainly, it should be pointed out that while we were in South Vietnam in 1970, the medical member of our group, Dr. John Constable, Professor of Surgery at Harvard Medical School, did not encounter evidence of any severe and widespread unusual illness in visiting Can Gio and several other villages or in discussions with officials of the South Vietnamese Ministry of Health. However, it was felt that certain indications in birth statistics ought to be investigated further

for possible connections with herbicide exposure (*14*). It is of obvious interest to survey appropriately chosen populations in South Vietnam more closely, especially if TCDD residues should be found in human tissue samples.

Finally, turning from questions of environmental toxicology to the biological mechanisms of action, we note that TCDD seems to be particularly toxic to proliferating tissues, as suggested by its effects on spermatogenesis and hematopoiesis and its apparent toxicity to the intestinal epithelium (*13*) and the thymus (*15*). These indications are consistent with the effects of a mitotic poison, such as TCDD is known to be in the African blood lily (*12*) and possibly in *Drosophila melanogaster* (*16*). We are led by these observations to speculate that TCDD may be able catalytically to disrupt microtubules, the subcellular elements of which spindle fibers are constructed and which are ubiquitous in their structural roles in cell extension and cell movement.

Summary

A procedure has been developed for the reliable detection of TCDD in animal tissues down to levels approaching 1 ppt. It makes use of chemical cleanup, preparative gas-liquid chromatography, and analysis by time-averaged high resolution mass spectroscopy.

A limited number of fish and crustacean samples was collected in South Vietnam in 1970 near areas heavily exposed to the herbicide 2,4,5-T. TCDD was detected in these samples at levels ranging from 18 to 810 ppt. TCDD was not detected in a sample of Cape Cod butterfish used as a control.

These results suggest that TCDD may have accumulated to biologically significant levels in food chains in some areas of South Vietnam exposed to herbicide spraying.

Note added in proof: Overall recoveries have been increased to 60–80% by replacing the GLC step with an additional Al_2O_3 column step. Details of this procedure will be described in a future publication.

Acknowledgement

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REFERENCES

1. Report on 2,4,5-T. A Report of the Panel on Herbicides of the President's Science Advisory Committee, Executive Office of the President, Office of Science and Technology, March 1971.
2. Effects of 2,4,5-T on Man and the Environment. Hearings before the Subcommittee on Energy, Natural Resources, and the Environment of the Committee on Commerce, United States Senate, 91st Congress, April 7 and 15, 1970.
3. Sparschu, G. L., Dunn, F. L., and Rowe, V. K. Study of the Teratogenicity of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in the Rat. Food Cosmet. Toxicol. 9: 405 (1971).
4. Baughman, R., and Meselson, M. An improved analysis for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. In: Advances in Chemistry Ser. 120, E. Blair, Ed., American Chemical Society, Washington, DC., 1973, p. 92.
5. Porter, M. L., and Burke, J. A. Separation of three chlorodibenzo-*p*-dioxins from some polychlorinated biphenyls by chromatography on an aluminum oxide column, 1971. J. Assoc. Offic. Anal. Chem. 54: 1426 (1971).
6. Woolson, E. A., Reichel, W. I., and Young, A. L. Dioxin residues in lakeland sand and eagle samples. In Advances in Chemistry Ser. 120, E. Blair, Ed., American Chemical Society, Washington, D.C., in press.
7. Woolson, E. A., Thomas, R. F., and Ensor, P. D. J. Survey of polychlorodibenzo-*p*-dioxin content in selected pesticides. J. Agr. Food Chem. 20: 351 (1972).
8. Crosby, D. G., Wong, A. S., Plimmer, J. R., and

Woolson, E. A. Photodecomposition of chlorinated dibenzo-*p*-dioxins. Science 173: 748 (1971).

9. Neubert, D., and Dillman, I. Embryotoxic effects in mice treated with 2,4,5-T and tetrachlorodibenzo-*p*-dioxin. Naunyn-Schmiedbergs Arch. Pharmacol. 272: 243 (1972).
10. Verret, J. In: Effects of 2,4,5-T on Man and the Environment. Hearings before the Subcommittee on Energy, Natural Resources, and the Environment of the Committee on Commerce, United States Senate, 91st Congress, April 7 and 15, 1970.
11. Poland, A., and Glover, E. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin: a potent inducer of δ-aminolevulinic acid synthetase. Science 173: 243 (1973).
12. Jackson, W. T. Regulation of mitosis: III. Cytological effects of 2,4,5-T and of dioxin contaminants in 2,4,5-T formulations, J. Cell Sci. 10: 15 (1972).
13. Allen, J. R., and Carstens, L. A. Light and electron microscopic observations in *Macaca mulatta* monkeys fed toxic fat. Am. J. Vet. Res. 28: 1513 (1967).
14. Herbicide Assessment Commission. Background Material Relevant to Presentations at the 1970 Annual Meeting of the AAAS and Preliminary Report of the Herbicide Assessment Commission; both reprinted in Congressional Record 118(32): S3226-3233 March 3, 1972.
15. Buu-Hoi, N. P., et al. Organs as targets of dioxin intoxication, Naturwiss. 59: 174 (1972).
16. Davring, L., and Sunner, M. Cytogenetic effects of 2,4,5-trichlorophenoxyacetic acid on oogenesis and early embryogenesis in *Drosophila melanogaster*. Hereditas 68: 115 (1971).