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Waterborne Methylene *bis*(2-chloroaniline) and 2-Chloroaniline Contamination around Adrian, Michigan

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In September 1978, the National Institute of Occupational Safety and Health published a "Special Hazard Review with Control Recommendations for 4,4'-Methylene Bis(2-Chloroaniline)" (USDHEW 1978) which was subsequently reviewed by the Office of Toxic Materials Control of the Michigan Department of Natural Resources (MDNR). A review of industrial surveillance reports for the State of Michigan compiled as part of the Critical Materials Registry program of MDNR revealed a small specialty chemical company located in Adrian, Michigan (Lenawee County) which produced in excess of one million pounds of 4,4'-methylene bis(2-chloroaniline) (M2CA) annually. A further search of agency files on this company documented a number of environmental problems associated with wastewater discharges. In response to these concerns, the Michigan Department of Natural Resources began an intensive investigation of the potential environmental contamination of the area around this facility by M2CA and other chlorinated anilines in October 1978.

In February 1979, MDNR called upon the Food and Drug Administration's Division of Chemical Technology (FDA-DCT) to confirm their findings and investigate the potential for food chain contamination. M2CA has been manufactured under at least two tradenames: MOCA and Curene 442. The commercial preparations frequently contain 2-chloroaniline (2CA) and a compound containing three 2CA moities joined by methylene groups as well as M2CA. M2CA is an animal carcinogen (STULA et al. 1977) and has been banned in food and food-contact surfaces by the FDA. The principal use of M2CA is in curing urethane and epoxy resins, and cross-linking urethane foams (ANONYMOUS 1978).

In this paper, we report results which trace waterborne M2CA and 2CA contamination from the company's waste treatment lagoon, through the municipal wastewater treatment plant and surface runoff, to the South Branch of the Raisin River at Adrian, Michigan. In addition we report residues of 2CA in fish taken from the river near the treatment plant discharge. Sampling sites are shown in Figure 1.

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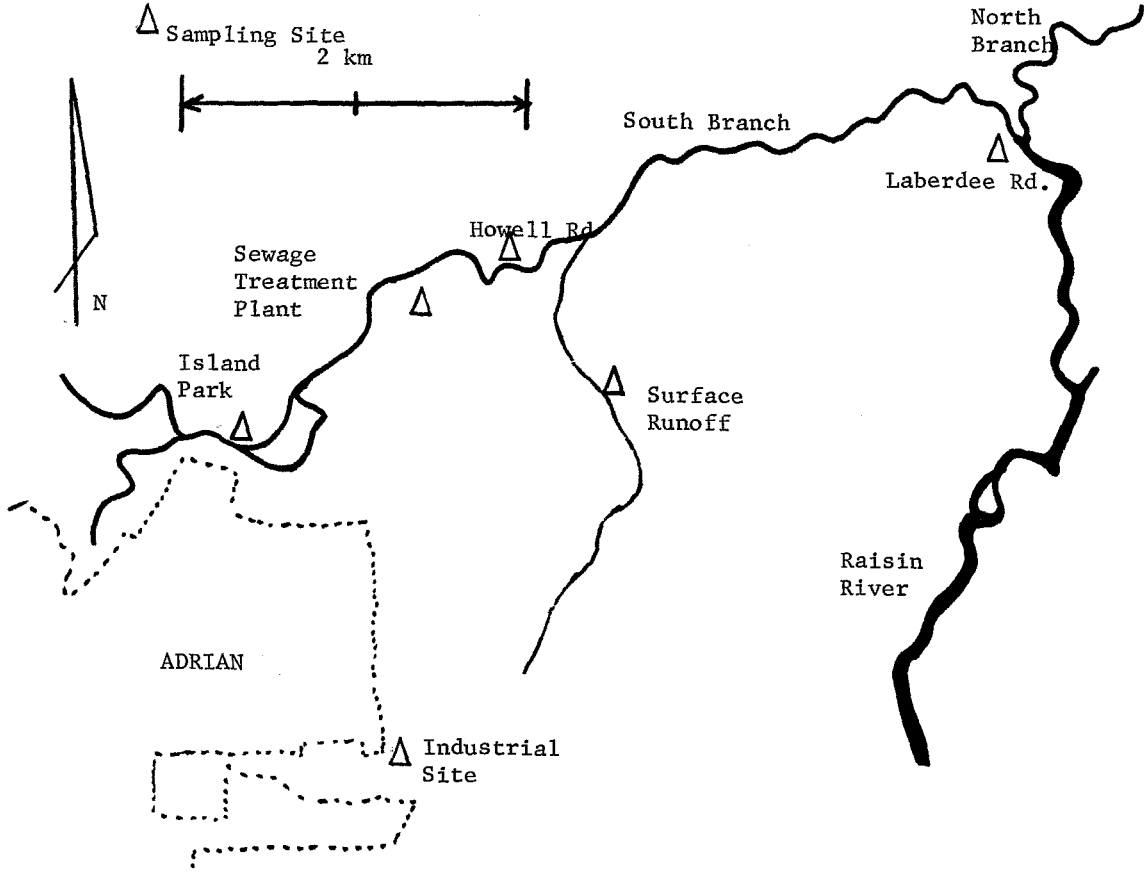
Sampling Site
2 km
North
Branch
South Branch
Laberdee Rd.
Howell Rd.
Sewage
Treatment
Plant
N
Island
Park
Surface
Runoff
Raisin
River
ADRIAN
Industrial
Site

MATERIALS AND METHODS

Samples. All samples were collected by MDNR. Sediment and water samples were shipped and stored on ice. Fish were shipped and stored frozen.

Water and Sediment Survey for M2CA. Water samples were made basic (pH 10-11) and extracted with diethyl ether. The extracts were dried and concentrated for gas chromatography. Recovery studies indicated that M2CA could be recovered in better than 90% yield from water spiked at 6 to 1000 ppb. Sediment and sludge samples were diluted with water and extracted successively with petroleum ether and diethyl ether. The diluted samples were then made basic and extracted with diethyl ether. No recovery experiments were conducted on the sediment or sludge samples. The samples were analyzed on two gas chromatographic columns (4' glass, 2 mm i.d., 6% OV-17 on Chromosorb WHP and 4' glass, 2 mm i.d., 6% Apiezon L on 2% KOH-coated Chromosorb WHP) at 235°C. The retention time of M2CA was 9-10 min on each column using a nitrogen carrier flow of 30 mL/min. A nitrogen/phosphorus (N/P) selective detector was used and quantitation was by peak height comparison with standards. Gas chromatography-mass spectrometry was used in the full scan and selective ion modes to confirm M2CA.

Procedure for M2CA and 2CA. Sludge and sediment samples were extracted successively with ethyl acetate and methanol. The ethyl acetate extract was dried and concentrated, hexane was added, and the organic bases were extracted into acidic aqueous methanol. The aqueous methanol was then diluted with water and made basic. The organic bases were extracted successively with hexane and ethyl acetate to yield two fractions. These two fractions and the original methanol extract were dried, concentrated and analyzed separately. M2CA and 2CA could be determined in the same chromatographic run using a temperature program of 4°C/min starting at 140°C and finishing at 300°C (6' glass, 4 mm i.d., 10% OV-101 on Chromosorb WHP). With a helium flow of 30 mL/min, the retention time of 2CA was about 3 min while M2CA eluted in about 31 min.

Analysis of Fish. Analysis of fish tissue after digestion with NaOH (DIACHENKO 1979) was very similar to the method for 2CA and M2CA in sediments noted above. It was found that 2CA, unlike most other anilines (e.g., those without substituents ortho to the amino group), can be partially recovered from Florisil columns (120 mm x 22 mm i.d.) with 6% diethyl ether in petroleum ether (MILLS 1959). This additional step was applied to achieve very selective cleanup for 2CA. It was also found that 2-chloronitrobenzene (2CNB) was reduced to 2CA in the digestion process. To analyze fish without digestion, ground tissue was extracted with ethyl acetate in a blender. The ethyl acetate was washed with dilute acid to remove basic amines (e.g., aliphatic amines). After drying and concentrating, the sample could be chromatographed, but much cleaner samples were obtained for 2CA analysis by displacing the ethyl acetate with hexane, then using the

Florisil cleanup step described above. After 2CA elutes with 6% diethyl ether in petroleum ether, 2CNB can be eluted with 15% diethyl ether in petroleum ether. M2CA unfortunately could not be recovered from Florisil.

RESULTS AND DISCUSSION

The study was pursued by FDA-DCT in three phases: (1) Water, sediment and sludge were surveyed for M2CA to estimate extent of waterborne contamination. (2) M2CA and 2CA were tracked from the plant to the Raisin River. (3) Fish from the Raisin River were analyzed for residues of M2CA, 2CA and 2CNB.

Waterborne M2CA Survey. Table 1 lists the results for M2CA in water, sediment and sewage sludge leading away from the

TABLE 1

Waterborne 4,4'-Methylene Bis(2-chloroaniline) Survey Around Adrian, Michigan

Sample Description	Concentration of M2CA
Industrial lagoon sediment	minimum 1600 ppm (dry weight)
Industrial lagoon effluent water	250 ppb
Industrial site deep well water	1.5 ppb
Surface runoff water from industrial site	1 ppb
Sewage treatment plant (STP) Adrian, MI, influent water	possible trace <0.5 ppb
STP, effluent water	possible trace <0.5 ppb
STP, activated sludge	estimated 18 ppm (dry weight)
Raisin River water @ Howell Rd.	not detected ≤0.1 ppb
@ Laberdee Rd.	not detected ≤0.1 ppb
@ Island Park	not detected ≤0.1 ppb

manufacturing plant. As expected, high levels of M2CA were found in water and sediment of the industrial waste lagoon. The identification of M2CA was readily confirmed by full scan gas chromatography-mass spectrometry. Contamination was also found in a deep well on the manufacturing site and in surface runoff from the site. No more than a trace of M2CA was present in the influent or effluent water from the sewage treatment plant which at one time received large amounts of manufacturing waste. Also, no M2CA was detected in water from the Raisin River near the sewage treatment plant outfall. However, appreciable quantities of M2CA were found in the activated sludge from the sewage treatment plant. Some of this sludge has in the past been given to local residents for fertilizing gardens. While it was not possible to obtain confirmation of M2CA in these samples by full scan mass spectrometry, the m/z 266 and 231 ions of M2CA were observed in the deep well and sewage treatment plant samples. These results suggested a pattern of waterborne contamination which warranted an evaluation not only of M2CA but also 2CA from which M2CA is prepared.

Tracking M2CA and 2CA. In the sediment from the industrial lagoon, a total of 3800 ppm M2CA and 600 ppm 2CA were found (dry weight basis). Over 79% of each compound was recovered in the "ethyl acetate extract/hexane fraction" (see experimental) and less than 6% of either compound was found in the "methanol extract". Thus, ethyl acetate efficiently extracts 2CA and M2CA and the quantities found in the "ethyl acetate extract/hexane fraction" are indicative of the relative concentrations of M2CA and 2CA. In the sludge from the sewage treatment plant, 10 ppm M2CA and 30 ppm 2CA were found in the "ethyl acetate extract/hexane fraction". Thus, M2CA is the dominant compound in the waste lagoon, while 2CA is the dominant compound at the sewage treatment plant. These results suggest that 2CA may be more readily mobilized through the wastewater systems because of its water solubility or lesser tendency to sorb to sediments. The predominance of 2CA over M2CA at the sewage treatment plant suggested that 2CA (or a metabolite such as 2-chloronitrobenzene) might be a major contaminant of the Raisin River food chain.

Contamination of Raisin River Fish. Table 2 reports the recovery of 2CA, 2CNB and M2CA from spiked portions of edible tissue of a carp collected from the Raisin River upstream from Adrian, Michigan. Without base digestion, 2CNB and 2CA are extracted and at least partially recovered through the analytical procedure. M2CA might be extracted without base digestion, but since it was found not to be eluted from Florisil, M2CA could not be determined by this procedure. With base digestion, 2CA and M2CA are recovered, but the sample cannot be cleaned up by acid-base partitioning alone to allow detection limits below 1 ppm for M2CA. 2CNB is completely reduced to 2CA during the digestion step and cannot be directly determined by this procedure.

TABLE 2

Recovery of 2-Chloroaniline, 2-Chloronitrobenzene and 4,4'-Methylene Bis(2-chloroaniline) Spikes from Edible Tissue of Carp[a]

Compound[b]	Spiking Level (PPM)	Fraction	% Recovered
		Without Base Digestion	
[2CA	0.512	6% EE/PE	39
2CNB	0.832	6% and 15% EE/PE total	84
M2CA]	1.05	c	c
		With Base Digestion	
[2CNB]	3.00	6% and 15% EE/PE total	0[d]
[2CA	0.103	EtOAc extract/ hexane fraction	78[e,f]
2CNB	0.168	6% and 15% EE/PE total	0
M2CA]	0.211	EtOAc extract/ hexane fraction	g
[2CA	1.84	EtOAc extract/ hexane fraction	139[e]
2CNB	3.00	6% and 15% EE/PE total	0
M2CA]	3.87	EtOAc extract/ hexane fraction	78

a) Abbreviations: EE = diethyl ether, PE = petroleum ether, EtOAc = ethyl acetate; 6% and 15% refer to the percentage diethyl ether in petroleum ether used to elute the compound from Florisil.
b) Compounds in brackets were spiked simultaneously.
c) Not eluted from Florisil.
d) 2CA representing 32% of the 2CNB spike was found in EtOAc extract/hexane fraction.
e) These numbers reflect contribution from 2CNB.
f) After Florisil cleanup, 37% recovery.
g) Not distinguishable from interference.

Two white suckers collected near the Adrian sewage treatment plant outfall were filleted and composited. One portion of edible fish tissue was extracted without digestion. The extract was cleaned up by chromatography using Florisil. No 2CA or 2CNB was detected (M2CA would not have been recovered through the procedure). The solid material remaining after extraction was digested with 1N NaOH, extracted and worked up as previously described. This extract contained 13 ppb 2CA (not corrected for recovery) based on the original weight of tissue.

Another portion of the fish tissue was digested without prior extraction and analyzed. This portion contained 49 ppb 2CA (uncorrected). After Florisil chromatography, the sample was clean enough to allow full scan mass spectrometric confirmation of 2CA. No M2CA was detected in the samples, but the detection limit for this compound was rather poor (e.g., 1000 ppb).

These results suggest that 2CA residues are chemically bound to macromolecules of the tissue, and do not exist free in the tissues. It is tempting to try to account for the difference in 2CA found in the two experiments by attributing a portion of the bound 2CA to conjugates which are extractable with ethyl acetate (before digestion), but which do not pass through the analytical procedure. However, the low recoveries of spiked 2CA virtually guarantee a poor overall precision in the analysis. Thus, little significance can be attributed to the difference between 13 ppb and 49 ppb. More work is planned to determine the roles of free, altered, conjugated and bound residues of 2CA in fish.

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