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Analyses of Human Milk Samples Collected in Hawaii for Residues of Organochlorine Pesticides and Polychlorobiphenyls

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Analyses of human milk samples collected in Hawaii for residues of chlorinated hydrocarbons were carried out by the Hawaii Epidemiologic Studies Program (Hawaii ESP). This work is believed to be the first extensive study of its kind on residents of the State of Hawaii. Surveys on human milk samples for organochlorine pesticide and/or polychlorobiphenyl residues have been carried out in other states or on samples from other states, but apart from the analyses of 6 samples for a national survey (SAVAGE 1977), no other characterizing study specifically on residents of Hawaii is believed to have been conducted prior to this work.

The State of Hawaii is composed of 8 major islands grouped closely together and geographically separated from the continental states by over 2,000 miles of Pacific Ocean. Hawaii's isolation from the mainland U.S.A., its limited industry, and the ethnic diversity of its resident population all contribute to the unique nature of this state. Therefore, the question naturally arises as to whether Hawaiian milk samples are similarly exposed to chlorinated hydrocarbon contaminants as human milk samples of other states.

At the time of the Hawaiian milk analyses, the Hawaii ESP laboratory was also participating in a national human milk study (EPA directive, VANDERMER 1979). The Colorado Epidemiologic Studies Center, in conjunction with the Washington State ESP, coordinated the study and provided intra- and interlaboratory quality control assurance programs. In the national survey, milk samples from various states throughout the mainland were shipped to the Hawaii laboratory from the Colorado Center. A total of 102 samples were completely analyzed according to the requirements outlined in the EPA directive (VANDERMER 1979). The collection and analytical schemes were predetermined by the center coordinating the survey and were not known to the participating laboratories providing analytical support.

Human milk samples collected from residents of Hawaii were handled and analyzed concurrently in the same manner as the national samples. The plan of collection was outlined by the Hawaii ESP.

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[1] Presently, Pesticide Hazard Assessment Project

SAMPLE COLLECTION AND ANALYSES

Milk samples were collected by manual expression from lactating mothers, up to 1 month, post partum, who were willing donors and residents in the State of Hawaii. Fifty-four samples were collected in sterilized specimen jars, 40 OD x 85 mm, with teflon-lined screw-caps and frozen for storage. Most (42) of the donors resided on the island of Oahu where Honolulu and the majority of the state's population is located, 10 were residents of the island of Hawaii, and 2 were residents of Kauai. The durations of donor residency in the state were, 1-37 years, up to life-time. Five donors had lived in Hawaii for less than 5 years and the average years of residency among 43 immigrants was 18 years. Eleven were life-time residents. Collections were made during 1979 and 1980 from apparently healthy donors, and the residue analyses were carried out concurrently from August 24, 1979, through July 18, 1980.

The method of chemical analyses as outlined in the EPA directive, VANDERMER (1979), was the multiresidue analyses by TESSARI & SAVAGE (1980), and included the target chlorinated hydrocarbon residues, p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD, α-HCH, β-HCH, γ-HCH (lindane), dieldrin, heptachlor epoxide, oxychlordane, HCB, mirex, aldrin, trans-nonachlor (TNC), and polychlorobiphenyls (PCBs). Heptachlor and chlordane were originally on the list of detectable residues but were not found in any of the in vivo samples. The analytical method, a gas-liquid chromatography (GLC) determination, electron capture (EC) detection, involved extracting the residues from the lipids of milk which were first isolated, and processing the extracts (cleanup and separation of residues) for GLC. The lipids were drawn from milk with acetone and hexane. An aliquot of the extract was used for the determination of milk lipid content, and the remainder was used for the analyses of the chlorinated hydrocarbons. The residues were extracted from the lipids with acetonitrile and partitioned into hexane with large volumes of the 2 solvents and 2% sodium sulfate solution. Further cleanup and separation of the residues in the hexane fraction were then carried out by Florisil column chromatography followed by silicic acid column chromatography. Four eluates, 1 off the Florisil column and 3 off the silicic acid column were analyzed by GLC, EC detection, on two columns having different resolving characteristics for chlorinated hydrocarbons. The residues detected in the milk samples were quantified on both whole milk and lipid bases. The GLC peaks for PCB detection in the milk samples corresponded to those for Aroclor® 1254.

For GLC a Tracor Micro-Tek 220 instrument operating with 2-^{63}Ni detectors and 2 borosilicate glass columns, 1.8m x 6.25 OD x 4.0 ID mm, was used. One column was packed with 4% SE-30/6% OV-210 on 80-100 mesh Gas Chrom Q, and the 2d column was packed with 1.50% OV-17/1.95% QF-1 on 80-100 mesh Gas Chrom Q. Other operating parameters were, temperatures: column, 203°C; detectors, 280°C; inlet, 233°C; transfer, 250°C; carrier gas, nitrogen, 60-80 mL/min. Chromatographically reproducible Florisil and silicic acid for

TABLE 1. Results of the analyses of human milk samples, Hawaii, for organochlorine residues (1979-1980)

Residues in ppm - Lipid Basis

Sample No.	Lipid %	p,p'-DDT	p,p'-DDE	α-HCH	β-HCH	Dieldrin	Heptachlor epoxide	PCBs	Oxychlor-dane	HCB	TNC
1	2.7	0.13	3.8	--	0.15	0.050	0.053	1.9	0.12	0.057	0.15
2	5.2	0.097	1.1	--	0.099	0.062	0.036	0.86	0.051	0.034	0.061
3	2.6	0.23	1.6	--	0.082	--	0.019	0.43	0.043	0.020	0.047
4	3.1	0.085	2.3	--	0.076	0.027	0.037	0.56	0.055	0.032	0.070
5	2.1	0.087	2.7	--	0.13	0.079	0.042	0.50	0.065	0.025	0.067
6	2.9	0.17	2.5	--	0.052	0.040	0.029	1.5	0.061	0.031	0.11
7	4.1	0.069	1.4	--	0.0083	0.036	0.018	0.78	0.045	0.037	0.044
8	1.5	0.13	1.5	--	0.028	0.053	0.041	0.85	0.043	0.043	0.081
9	4.9	0.13	1.5	--	0.058	0.040	0.032	0.65	0.050	0.041	0.092
10	4.1	0.058	0.92	--	0.030	0.024	0.042	0.29	0.020	0.023	0.047
11	2.0	0.15	5.7	--	0.12	0.017	0.068	2.2	0.10	0.083	0.13
12	4.2	0.26	1.3	--	--	0.024	0.016	0.94	0.036	0.027	0.018
13	2.7	0.041	1.1	--	0.064	0.037	0.026	0.74	0.057	0.040	0.066
14	3.9	0.14	2.1	--	0.054	0.066	0.017	0.53	0.025	0.028	0.079
15	3.9	0.20	4.7	--	0.15	0.028	0.039	0.24	0.55	0.021	0.082
16	1.9	0.18	2.3	--	0.48	0.042	0.042	1.2	0.16	0.081	0.22
17	3.7	0.22	1.7	0.017	0.10	0.057	0.046	0.65	0.087	0.043	0.12
18	2.6	0.074	0.39	0.037	0.074	0.040	0.046	0.83	0.048	0.063	0.053
19	1.5	0.21	3.8	--	--	0.029	0.044	1.3	0.076	0.052	0.092
20	0.7	0.28	1.7	--	0.063	--	0.046	0.62	0.079	0.050	0.15
21	5.7	0.11	1.3	--	0.031	0.029	0.036	0.85	0.050	0.037	0.052
22	3.0	0.24	3.6	--	0.059	0.056	0.035	0.91	0.064	0.044	0.066
23	3.4	0.11	0.54	0.073	0.016	0.072	0.015	0.13	0.014	0.031	0.037
24	3.5	0.52	3.3	0.025	0.042	0.057	0.021	0.18	0.011	0.018	0.027
25	2.1	0.12	1.7	0.40	0.036	0.071	0.014	0.51	0.020	0.031	0.038
26	3.4	0.11	0.74	--	0.026	0.054	0.029	0.57	0.042	0.026	0.059
27	3.4	0.19	1.8	--	0.045	0.052	0.022	1.2	0.069	0.034	0.086

TABLE 1. Continued

Residues in ppm - Lipid Basis

Sample No.	Lipid %	p,p'-DDT	p,p'-DDE	α-HCH	β-HCH	Dieldrin	Heptachlor epoxide	PCBs	Oxychlor-dane	HCB	TNC
28	3.6	0.22	2.4	0.78	0.066	0.076	0.057	0.58	0.079	0.044	0.099
29	3.4	0.46	3.5	0.003	2.58	0.053	0.049	1.2	0.030	0.11	0.061
30	3.2	0.18	1.2	0.030	0.049	0.048	0.043	0.67	0.058	0.046	0.079
31	2.1	0.16	1.5	--	--	0.0029	0.022	0.87	0.044	0.031	0.076
32	2.2	0.13	1.6	--	--	0.014	0.042	1.9	0.034	0.38	0.064
33	3.7	0.31	1.7	--	--	0.015	0.056	0.82	0.088	0.038	0.12
34	5.4	0.12	0.86	0.010	0.026	0.043	0.026	0.38	0.029	0.031	0.052
35	3.6	0.17	2.3	--	0.076	0.071	0.038	0.53	0.092	0.049	0.10
36	3.4	0.17	3.9	0.007	0.14	0.034	0.041	0.64	0.064	0.037	0.069
37	3.8	0.11	1.0	--	0.043	0.027	0.044	1.0	0.062	0.031	0.087
38	3.5	0.083	1.2	--	0.11	0.022	0.051	0.52	0.036	0.025	0.061
39	2.7	0.032	0.25	--	--	0.020	0.042	0.63	0.049	0.029	0.063
40	1.6	0.032	0.94	--	--	0.008	0.027	0.93	0.020	0.023	--
41	2.7	0.15	0.93	--	--	0.022	0.033	0.69	0.055	0.036	0.080
42	2.9	0.059	0.42	--	--	0.024	0.029	0.62	0.046	0.034	0.057
43	2.3	0.16	3.2	--	--	0.034	0.027	1.1	0.062	0.025	0.089
44	3.1	0.079	1.9	--	0.086	0.049	0.045	0.17	0.098	0.047	0.13
45	3.2	0.17	4.3	--	0.033	0.042	0.028	0.12	0.074	0.026	0.087
46	2.5	0.27	1.8	--	0.049	0.095	0.057	0.76	0.070	0.057	0.064
47	3.3	0.35	2.3	--	0.075	0.064	0.016	0.79	0.042	0.041	0.060
48	2.4	0.063	1.5	--	0.066	0.085	0.045	0.82	0.052	0.033	0.051
49	2.9	0.13	1.2	--	0.083	--	0.051	0.77	0.11	0.052	0.10
50	2.7	0.27	3.5	--	0.21	0.052	0.026	0.87	0.065	0.035	0.13
51	5.5	0.12	1.2	--	0.069	0.048	0.032	0.78	0.053	0.039	0.063
52	3.2	0.12	2.1	--	0.078	0.034	0.043	1.3	0.10	0.041	0.12
53	2.2	0.087	1.4	--	0.027	--	0.023	1.4	0.040	0.031	0.10
54	2.8	0.22	2.2	--	0.075	0.020	0.046	0.53	0.084	0.048	0.096

Note: All whole milk sample masses about 7.000g (according to EPA directive) except sample N. 48 which was 5.6865g.
-- = not detected

TABLE 2. Comparison of results for the analyses of organochlorine residues in Hawaiian and Mainland human milk samples

Residues in ppm - Lipid Basis

Statistics	Sample Class	p,p'-DDT	p,p'-DDE	Heptachlor epoxide	Oxychlordane	HCB	PCBs	TNC	Dieldrin	β-HCH
Incidence, % Positives	Hawaii (n=54)	100	100	100	100	100	100	98.2	94.4	81.5
	Nat'l (n=102)	100	100	97.1	99.0	98.0	100	98.0	91.2	83.3
Mean ± SD ($\bar{x}$ of Positives ± Standard Deviations)	Hawaii	0.16 ±0.098	2.0 ±1.2	0.036 ±0.013	0.068 ±0.43	0.046 ±0.049	0.80 ±0.43	0.81 ±0.035	0.042 ±0.021	0.080 ±0.075
	Nat'l	0.19 ±0.20	1.9 ±1.8	0.055 ±0.039	0.054 ±0.049	0.031 ±0.025	0.97 ±0.59	0.079 ±0.071	0.062 ±0.036	0.096 ±0.080
Range (Minimum - Maximum)	Hawaii	0.032-0.52	0.25-5.7	0.014 0.068	0.011-0.55	0.018-0.38	0.13-2.2	ND-0.22	ND-0.095	ND-2.6
	Nat'l	ND-1.7	0.24-11.0	ND-0.20	ND-0.44	ND-0.23	0.076-4.4	ND-0.47	ND-0.17	ND-0.47

TABLE 2. Continued

Statistics	Sample Class	Residues in ppm - Lipid Basis							
		∝-HCH	ϒ-HCH	o,p'-DDT	o,p'-DDE	p,p'-DDD	Chlordane	Mirex	Aldrin
Incidence, % Positives	Hawaii	18.5	ND	ND	ND	ND	ND	ND	ND
	Nat'l	22.6	3.92	5.88	ND	ND	ND	0.98	1.96
Mean ± SD ($\bar{x}$ of Positives ± Standard Deviations)	Hawaii	0.10 ±0.23	ND	ND	ND	ND	ND	ND	ND
	Nat'l	0.10 ±0.18	0.023 ±0.017	0.032 ±0.009	ND	ND	ND	--	0.082 ±0.019
Range (Minimum-Maximum)	Hawaii	ND-0.78	--	--	--	--	--	--	--
	Nat'l	ND-0.85	ND-0.044	ND-0.046	--	--	--	ND-0.25	ND-0.10

ND = Not Detected

column chromatography were supplied by the Quality Assurance Section, Environmental Toxicology Division, EPA, Research Triangle Park, North Carolina and by the Washington State ESP, respectively, to ensure uniformity in analyses between participating laboratories.

RESULTS AND DISCUSSION

The results for the analyses of Hawaiian milk samples are shown in Table 1. The quantities of chlorinated hydrocarbon residues detected are shown on the basis of lipid content. The residues in the analytical protocol which were not detected (ND) in the milk samples besides heptachlor and chlordane were o,p'-DDT, o,p'-DDE, p,p'-DDD, γ-HCH (lindane), mirex, and aldrin, and are also not included in the Table. A comparison of these results with those for the mainland samples in the national survey analyzed in this laboratory revealed that there were generally little or no differences between the two. Apart from isolated low and high levels of organochlorine residues, the results for the two groups of samples were basically similar. Table 2 compares the mean levels of residues $\pm$ standard deviations (SD), the incidences, and the ranges of residue levels for the Hawaiian and mainland milk samples. The means are for the positive residue detections and do not include the ND's and traces (positives but below analytical detection limits). Among the mainland samples analyzed, only one was found to contain mirex and two others contained aldrin. For the organochlorine pesticide, β-HCH, one Hawaiian sample (No. 29) had a residue level of 2.58 ppm, which was much higher than the other samples of the group. Without this high value, the mean $\pm$ SD for β-HCH is 0.080 $\pm$ 0.075, as shown in the Table, which is about the same as the mean $\pm$ SD for the mainland samples. Further statistical treatments to determine significant differences between Hawaiian and mainland samples were not necessary.

This work has revealed that the chlorinated hydrocarbon residues found in human milk samples collected from residents in the State of Hawaii were statistically the same residues found in mainland human milk samples. Moreover, the levels at which these residues were detected in Hawaiian samples were comparable to those detected in mainland samples, and differences between the two groups of samples were not apparent. The close correlation between residue analyses was unexpected considering Hawaii's geographic isolation and the distinct ethnic diets of its population. They do indicate, however, that the uptake of chlorinated hydrocarbon contaminants by the population of this state is not specific and is most likely due to some physiological means of entry which is common to the populations of the mainland states. Inasmuch as contaminated foods have been considered the main source of chlorinated hydrocarbon residues which accumulate in human milk , the most probable means of entry is through the diet of lactating mothers. The ingestion of chlorinated hydrocarbon residues from contaminated foods by lactating mothers in Hawaii must then be equivalent to that of lactating mothers on the mainland, and this is apparently the situation in spite of the varied ethnic foods which are not usually found on the mainland and which are available for consumption by

Hawaiian women throughout the course of their pre- and post-natal care.

The residue analyses for chlorinated hydrocarbons in Hawaiian and mainland milk samples are believed to be base line levels for each of the U.S.A. regions compared. The samples were randomly collected without bias and were believed to be representatives for Hawaii and for the mainland. The mainland samples were from 33 different states covering all of the five regions of the National Human Milk Study, SAVAGE (1977).

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