ROYAL SWEDISH ACADEMY OF SCIENCE

AMBIO

A JOURNAL OF THE HUMAN ENVIRONMENT

Body Burden Reductions of PCBS, PBBs and Chlorinated Pesticides in Human Subjects

BY DAVID W SCHNARE, MAX BEN
AND MEGAN G SHIELDS

REPRINT

Report

By David W Schnare, Max Ben and Megan G Shields

With human exposure to environmental contaminants inevitable despite the best application of environmental laws and protection technologies, interest has grown in the potential to reduce the levels of contamination carried in the human host. This study demonstrates the promise of a comprehensive treatment for reduction of body burdens of polychlorinated and polybrominated biphenyis (PCB and PBB) and chlorinated pesticides. Adipose tissue concentrations were determined for seven individuals accidentally exposed to PBB. These patients underwent the detoxification treatment developed by Hubbard to eliminate fat-stored foreign compounds. Of the 16 organohalides examined, 13 were present in lower concentrations at post-treatment sampling. Seven of the 13 reductions were statistically significant; reductions ranged from 3.5 to 47.2 percent, with a mean reduction among the 16 chemicals of 21.3 percent (s.d. 17.1 percent). To determine whether reductions reflected movement to other body compartments or actual burden reduction, a post-treatment follow-up sample was taken four months later. Follow-up analysis showed a reduction in all 16 chemicals averaging 42.4 percent (s.d. 17.1 percent) and ranging from 10.1 to 65.9 percent. Ten of the 16 reductions were statistically significant. Future research stemming from this study should include further investigation of mobilization and excretion of xenobiotics in humans.

Between 1965 and 1978, over four million distinct chemical compounds were reported in the scientific literature-approximately 6000 per week. Of these, about *55* 000 are now in commercial production.

Managing the risk posed by this chemical panoply has come in three forms: exposure control, treatment of resultant diseases, and post-exposure pre-disease prophylactic treatment. As over 400 toxic chemicals, most of which bioaccumulate, have been identified in human tissues, increased research attention must be directed to reducing risks after human populations have been exposed but before disease processes begin (1). We report here on a pilot study of one such treatment.

The purpose of this study was to support decision-making on future work with regard to the value of studying this form of chemical body burden reduction and the difficulty of conducting this type of investigation, as well as to spur broader investigation into all aspects of body burden reduction.

The antecedents of body burden reduction research have narrowed active work to treatments which enhance excretion of chemicals through the bile and feces by ingestion of paraffin, activated carbon o saturated and unsaturated oils (2). The greatest successes have been with the unsaturated oils and have led to human participation in reduction studies, including other successes with the treatment reported on herein (3).

Reduction of fat-stored body burdens requires two basic steps: residue mobilization and enhanced excretion. The active fraction of the adipose tissue constitutes only five percent-the predominant chemical contaminant storage compartment -and does not appear to contain many contaminants found in the inactive fraction (4). However, it is clear that fat, and its associated contaminants, are regularly mobilized from deep stores (5). While knowledge in this area is relatively poor, mobilization of fat-stored chemicals in the absence of enhanced excretion pathways has been reported to cause latent exposure crises such as hallucinogenic "flashback" events which have kept occupationally drug-exposed police officers off the work force (6).

The key to enhanced excretion lies in overcoming enterohepatic recirculation. While cholestyramine, high-fiber diets vegetable diets, sucrose polyester and paraffin have all been used with varying degrees of success, only polyunsaturated oil has significantly enhanced excretion of extremely persistent chemicals and at the same time not increased fat deposition in the liver (7). There remains a question regarding the degree to which fecal excretion of persistent chemicals is due to biliary pathways or direct intestinal elimination (8).

Associated with overcoming enterohepatic recirculation is the potential for reduced adsorption of important nutrients and thus increased toxicity of persistent chemicals such as PBB. In such cases, increased administration of nutrients has been found to provide protection in the face of expected toxicity. For example, administration of lascorbic acid during PCB exposure eliminated liver enzyme activity degradation and negative histological changes otherwise normally observed (9). This was one of the compelling reasons for selecting the treatment used in this study.

EXPERIMENTAL

In 1973, a fire retardant consisting predominantly of polybrominated biphenyls (PBB) was substituted for a cattle feed supplement in the state of Michigan, USA. Milk and meat were consequently contaminated. In 1978, 97 percent of individuals tested in Michigan had detectable PBB in their adipose tissues (10). Because of the rich data base which has been developed on this large population (7 million individuals), seven healthy male volunteers age 20 to 30 were selected from this population for participation in the study. Each had been part of earlier studies on human PBB contamination, and they were expected to have total adipose tissue burdens of between 0.5-10.0 ppm PBB (lipid weight basis).

The treatment provided was that developed by Hubbard for the purpose of reducing body burdens of psychoactive chemicals (11). This treatment is currently in use in the United States for a variety of contamination incidents, including treatment of policemen exposed during arrests to illicit psychoactive drugs (e.g. phencyclidine) (6). It is also widely used in Sweden during drug rehabilitation. The treatment, described in detail elsewhere, is a relatively complex three-week regimen of polyunsaturated oil supplement, heat stress (sauna at 60°-82°C), and vitamin and mineral supplements (12). The regimen length is participant-specific and averaged 20 days in this study.

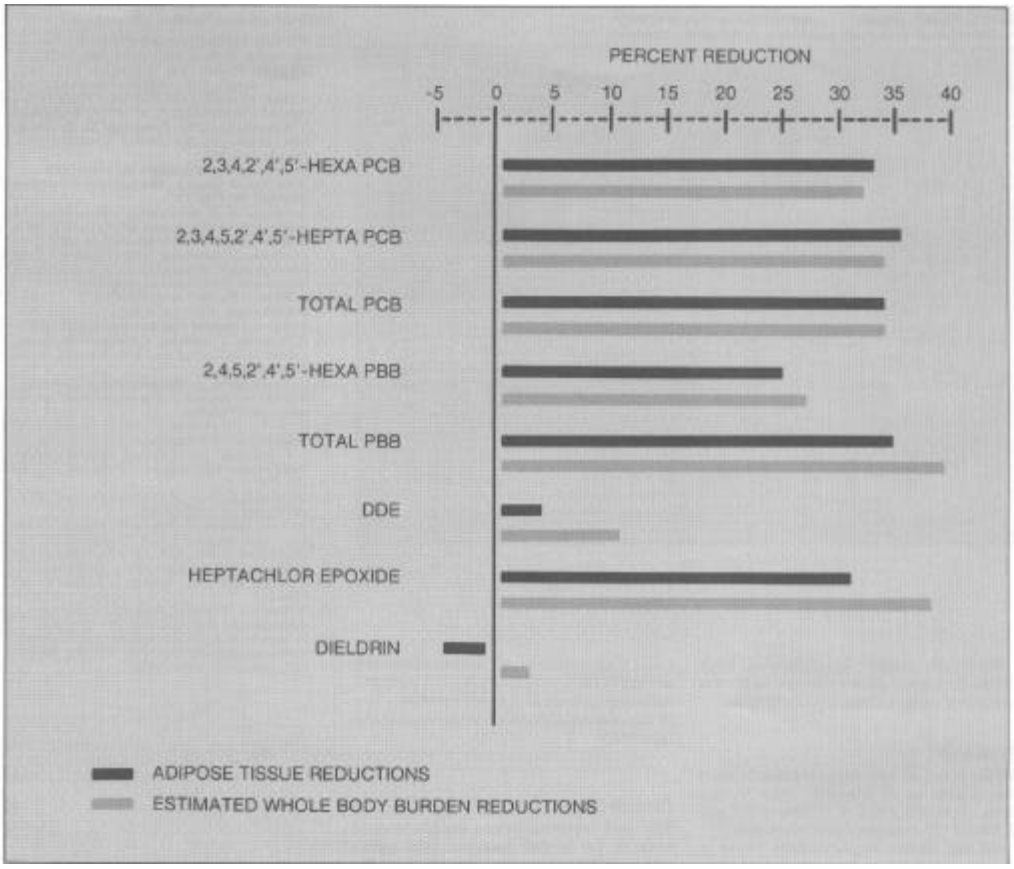

Figure1. Comparison of mean post-treatment reductions of adipose tisse concentrations and estimated body burdens (percent reductions).

Samples of adipose tissue were taken the day prior to initiation of the regimen, one day post-regimen and four months post-regimen (follow-up). These samples were obtained by subcutaneous needle aspiration, and were kept frozen until analyzed (13). Pre- and post-treatment samples were randomly coded to ensure unbiased chemical analysis and were forwarded for analysis as a group. Follow-up samples were analyzed later using a similar blind process. One patient was unavailable for follow-up sampling.

The chemicals targeted for analysis were the major congeners of PBB, polychlorinated biphenyls (PCB), and the residues of three commonly found chlorinated insecticides. Many PBB and PCB congeners are extremely persistent in mammalian organisms, with biological half-lives estimated to be from ten years to the lifetime of the organism (14). These chemicals, and therefore the test subjects, were especially chosen for this study due to their extremely persistent nature, thereby permitting the test subjects to serve as their own controls. We decided to use the patients as self-controls because of the extreme persistence of the target chemicals, the very small likelihood of re-exposure during treatment, the expected precision of the analytical procedure, and the pilot nature of this trial.

To determine chemical concentrations, tissue samples were mixed with sodium sulfate and extracted with petroleum ether. Lipid content was determined gravimetrically on a portion of the extract. Gel permeation chromatography followed by treatment on a Florisil column provided eluents which were concentrated and analyzed by gas chromatography with electron capture detection (15). Duplicate analyses were made on 20 percent of the samples for quality control purposes. The mean precision of the analytical procedure was ± 15.8 percent. Identification of specific chemicals and congeners was determined by gc-mass spectroscopy (16) (Table 1).

Table 1. Range of adipose tissue concentrations of targeted chlorinated pesticides, PCBs and PBBs (ppm on a lipid weight basis).

Contaminant	Range (ppm)	Level of Detection (ppm)
[PCB]		
2,3,4,2',4',5'-hexa	0.01-0.37	0.001
2,4,5,2',4',5'-hexa	0.09-0.73	0.001
2,4,5,2',3',6'-hexa	0.07-0.67	0.001
2,3,4,5,2',4',5'-hepta	0.02-0.20	0.001
2,3,4,6,2',3',4'-hepta	0.007-0.23	0.001
2,3,4,5,6,2',5'-hepta	0.08-0.59	0.005
2,3,5,6,3',4',5'-hepta	0.05-0.35	0.005
[PBB]		
2,4,5,3',4'-penta	nd-0.16	0.001
2,4,5,2',4',5'-hexa	0.01-2.72	0.001
2,3,4,2',4',5'-hexa	nd-0.22	0.001
2,4,5,3',4',5'-hexa	nd-0.09	0.001
2,3,4,5,2',3',4'-hepta	nd-0.26	0.001
2,3,4,5,2',4',5'-hepta	nd-0.01	0.001
DDE	0.30-1.58	0.05
Heptachlor Epoxide	0.20-0.82	0.005
Dieldrin	0.04-0.14	0.005

nd = not detectable

Table 2. Percent reductions In adipose tissue concentrations
In PBB-exposed Individuals receiving a detoxification treatment.

	Post-treatment			Four Month Follow-up		
Contaminant	percent	n	s.d.	percent	n	s.d.
[PCB]						
2,3,4,2',4',5'-hexa	32.8	6	17.9	60.6	6	24.9
2,4,5,2',4',5'-hexa	17.2	6	21.7	27.8	6	27.7
2,4,5,2',3',6'-hexa	20.4	5	21.6	45.3	5	25.1
2,3,4,5,2',4',5'-hepta	34.9	5	16.8	29.2	6	46.2
2,3,4,6,2',3',4'-hepta	26.2	6	30.8	56.5	6	32.0
2,3,4,5,6,2',5'-hepta	11.9	6	21.9	13.3	6	42.5
2,3,5,6,3',4',5'-hepta	37.0	6	28.0	59.0	6	21.6
Total PCB (sum of peaks)	34.2	6	24.4	38.4	6	27.7
[PBB]						
2,4,5,3',4'-penta	34.0	4	39.7	52.1	4	34.2
2,4,5,2',4',5'-hexa	25.0	6	21.4	65.9	6	37.3
2,3,4,2',4',5'-hexa	47.2	3	12.4	51.4	3	35.4
2,4,5,3',4',5'-hexa	4.2	4	84.3	30.3	5	50.5
2,3,4,5,2',3',4'-hepta	8.0	2	96.2	61.5	4	27.1
2,3,4,5,2',4',5'-hepta	36.3	5	34.0	37.5	5	95.4
Total PBB (sum of peaks)	34.5	6	20.	58.7	6	33.0
DDE	3.5	7	26.1	40.2	6	22.9
Heptachlor Epoxide	31.2	7	49.4	37.8	6	33.4
Dieldrin	3.9	7	19.9	10.1	6	21.9

* $p<.05$
** $p<.01$

To permit estimation of chemical body burdens, percent of body weight as fat was measured using submersion techniques.

RESULTS

While many of the target chemicals were measurable in all samples, some congeners, such as 2,3,4,2',4',5'-hexa PBB and 2,3,4,5,2',3',4'-hepta PBB, were not.

Of the sixteen organohalides found in the adipose tissue of the participants, thirteen were present in lower concentrations at post-treatment sampling (Table 2). Seven of the thirteen reductions were statistically significant. Reductions ranged from 3.5 to 47.2 percent, with a mean reduction among the sixteen chemicals of 21.3 percent (s.d. 17.0 percent).

The concentration of the chemicals in adipose tissue was generally at least 10 times the level of delectability. As the precision of the analytical method was approximately ± 16 percent, the measured reductions in adipose tissue concentrations do not appear to be due to analytical error.

In order to determine if changes in adipose tissue concentration after treatment were due to changes in fat mass, lean body mass measurements were made. There was a 0.45 percent decrease in body fat (nonsignificant), and reductions in adipose tissue concentrations were therefore not attributable to an increase in body fat. Reductions in adipose tissue concentrations were very similar to reductions in estimated body burdens (Figure 1).

To determine whether these post-treatment reductions reflected movement to other body compartments (eg liver) or an actual decrease in body burdens, follow-up samples were taken four months after treatment. Analysis of these samples indicated a reduction in all sixteen chemicals averaging 42.4 percent (s.d. 17.1 percent and ranging from 10. 1 to 65.9 percent. Ten of the sixteen reductions were statistically significant.

DISCUSSION

The data reported herein suggest several avenues for further research. The variation in percent reduction is a question d serving further study. It may be that those chemicals which showed relatively small post-treatment reductions but large follow-up reductions are also metabolites of other stored residues. For example, review of the chromatograms showed frank DDT in some of the tissue samples. Stored DDT residuals would be expected to metabolize to DDE rapidly once mobilized to the blood, with the potential for restorage of the metabolite.

Some of the variation in reduction ma also be due to low initial levels and/or the relative imprecision of the analytic method. The single case where we feel this may have been a problem in these target chemicals is Dieldrin, a well-known analytical challenge.

A second result which bears further examination is the continued reduction in body burdens after treatment was terminated. Others have observed this phenomenon as well, but explanation must await more extensive research on mobilization and excretion pathways (3).

With human exposure to environmental contaminants inevitable, research on reduction of body burdens is critical. The successful reductions, as indicated in this study, presage expansion in post-exposure pre-disease treatment.

References and Notes

1. US ERA**,** *Chemicals Identified In Human Biological Media, A Data Base,* EPA 560/13-80-036B, PB81-161-176 (US EPA, Washington, DC, 1980).

2. A brief sample of such studies would include: E Richter, J Lay, W Klein, F Korte, *Toxicology and Applied Pharmacology* **50,** 17 (1979); K Rozman, T Rozman, H Grein, *Toxicology* **22,** 33 (1981);

and J Street, *Canadian Medical Association Journal* 100, 16 (1969).

3. D Roehm, *Clinical Research* **31,** 243a (1983).

4. Y Oschry, B Shapiro, *Biochimica* **et** *Biophysica Acta* **664,** 201 (1981).

5. A discussion of mobilization and excretion of xenobiotics stored in human fat is available, along with the data produced in this study, in: *Reduction of Human Organohalide Body Burdens - Final Research Report* (Foundation for Advancements in Science and Education, Los Angeles, 1983).

6. R Warner, *The Backup* **3,** *5* (1983).

7. R Kimbrough, M Korver, V Burse, D Groce, *Toxicology and Applied Pharmacology* **52,** 442 (1980).

8. K Rozman, T Rozman, J Williams, H Greim, *Journal of Toxicology and Environmental Health* **9,** 611 (1982).

9. D Chakraborty, A Bhattacharyya, **J** Chatterjee, *International Journal of Vitamin and Nutrition Research* **48,** 22 (1978).

10. M Wolff, H Anderson, I Selikoff, *Journal of* the *American Medical Association* **247,** 2112 (1982).

11. L R Hubbard, *The Technical Bulletins* (Bridge Publications, Los Angeles, 1980) vol 12, pp 163-181.

12. D Schnare, G Denk, M Shields, S Brunton, *Medical Hypotheses* **9,** 265 (1982).

13. S Daum, J Knittle, K Rosenman, W Rom, E Holstein, *Environmental Health Perspectives* **23,** 183 (1978). This volume of the journal contains several articles on PBB contamination in Michigan.

14. **J** Miceli, **B** Marks, *Toxicology Letters* **9,** 315 (1981).

15. A Smrek, L Needham, *Bulletin of Environmental Contamination and Toxicology* **28,** 718 (1982).

16. R Moore, S **Aust,** *Biochemica and Biophysica Communications* **48,** 936 (1978); J Roboz, *Analytical Chemistry* **54,** 1104 1982 .