Bull. Environ. Contam. Toxicol. (2002) 69:667–673

DOI: 10.1007/s00128-002-0113-7

Bulletin of Environmental Contamination and Toxicology

Pesticide Residue Analysis of Infant Formula in India

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Received: 21 July 2001/Accepted: 2 July 2002

In the first year of life, an infant gets four categories of food. They may be distinguished as breast milk, milk formula, commercial formula, and formula made by the mother using normal food (*Manz,1993)*(like mashed khichari, pulses and rice combination and mashed fruits etc.).Consumption of breast milk and formula of normal food results in an obligatory and occasionally high intake of pesticide residues.The concentration of pesticide residues in milk formula and commercial infant formula can be actively influenced, however, by the infant food manufacturing industry, as infants show a high intake of these products during the first year of life (Wiles,1993).

Infants and children are generally more vulnerable to toxic substances, the EPA, in contrast, allows infants and children to eat adult approved doses of pesticides that have not been evaluated in terms of their safety for infants and young children. Infants and children react differently than adults to many drugs and toxic substances. In many cases they suffer far more serious damage as a result (Reis,1993). Aspirin can cause Reyes syndrome (a condition that kills 80 percent of its victims) in children and teenagers, but it does not cause this condition in adults (Wiles,1993). Lead causes permanent loss of mental capacity when infants and children are exposed at levels of little consequence to adults (Wiles,1993).

In spite of this evidence, the EPA evaluates the safety of most pesticides using protocols that require testing only sexually mature animals (EPA,1994). These tests provide little information relevant to infant or fetal exposure to pesticides. A faster and different metabolism, and rapid growth and development are the basic reasons for an infant's increased vulnerability to any toxic substance, including pesticides (EPA,1994). After birth the most pronounced period of growth is the first year of life, during which the human infant triples in weight. Different organs grow at different rates as the infant matures, creating a roulette of infant organ susceptibility. The brain of a newborn child grows rapidly and is particularly sensitive to toxic substances. At birth, the human brain weighs about one third an adult brain, compared to the infant body which weighs about 4 percent of an adult body. This relatively large brain grows rapidly in the newborn child, achieving 50 percent of its adult weight by 6 months of age, with 75 percent of all brain cells present by age 2. In contrast, 50 percent of adult weight in the liver, heart and kidneys is not reached until age nine. In infants the sensitivity towards pesticides is as high as 10 times in comparison to adults, still there are no standards to protect infants, children or anyone else from multiple pesticides in food or from other sources (Guzelian,1992).The EPA has begun to consider the additive effects of certain groups

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of pesticides. The additive adverse effects of these combined exposures on the infants has not been studied so far and needs special attention (Yess,1993). In general, but not always, rapid development will increase the risk of cancer from toxic exposure (NRC,1993) While the issue is complex, the NAS Committee on Pesticides in the Diets of Infants and Children concluded that in the absence of other factors, "direct carcinogens are more potent in rapidly growing animals", adding that, "Infants and children are subject to rapid tissue growth and development, which will have an impact on cancer risk". The incidence of childhood brain cancer and childhood leukemia has increased 33 percent since 1973. Cancer now kills more children under age 14 than any other disease.

A 1992 review of data on 22 chemicals from separate studies that began dosing animals in utero, in infancy and in adulthood concluded that exposure early in life increases the rate of cancer in the exposed population and that these cancers generally occur earlier in life. But cancer is only one cause for concern. Several organ systems, including the nervous, immune, reproductive, and endocrine systems, which are not fully developed at birth may "demonstrate particular sensitivity during the postnatal period". The nervous system of the infant and young child is extraordinarily sensitive to some toxins. Infants and toddlers, for example, are far more sensitive than adults to the immediate and long term effects of toxins. Many pesticides found in baby food are toxic to the nervous system, but the EPA has not evaluated their ultimate toxic effect on children (NRC,1993).

The most common neurotoxic pesticides are the organophosphate and organochlorine insecticides which inhibit the normal function of the nervous system enzyme, acetylcholinesterase. According to the NAS, "... emerging data suggest that neurologic and behavioral effects may result from low-level chronic exposure to some organophosphate and organochlorine pesticides".Current safety standards, the NAS observed, there are no guarantee of protection for children: "The data strongly suggest that exposure to neurotoxic compounds at levels believed to be safe for adults could result in permanent loss of brain function if it occurred during the prenatal or early childhood period of brain development. This information is particularly relevant to dietary exposure to pesticides, since policies that established safe levels of exposure to neurotoxic pesticides for adults could not be assumed to adequately protect a child less than four years of age,"(NRC,1993).

The reproductive system is also vulnerable to the toxic effects of pesticides. Evidence is mounting that exposure to chemical pollutants and pesticides in the womb or in early childhood can interfere with normal sexual development and may be contributing to declining male reproductive health in the industrialized world. Every day, people are exposed to chemicals that mimic the female hormone estrogen and or otherwise may disrupt the human hormone system. The severity and consequences of this exposure are not known nor are they being systematically studied. We do know, however, that several currently used pesticides commonly found in food mimic the female hormone estrogen, and others clearly interfere with normal hormone function. In addition, recent work shows that the effect of estrogenic pesticides is additive, and that exposure to estrogenic mixtures at low levels can cause an effect produced by a single chemical, only when administered at a higher dose . The reproductive system is also vulnerable to the toxic effects of pesticides. Evidence is mounting that exposure to chemical pollutants and pesticides in the womb or in early childhood can interfere with normal

sexual development and may be contributing to declining male reproductive health in the industrialized world (Carlson,1992 and Colburn 1993).We do know, however, that several currently used pesticides commonly found in food mimic the female hormone estrogen, and others clearly interfere with normal hormone function. In addition, recent work shows that the effect of estrogenic pesticides is additive, and that exposure to estrogenic mixtures at low levels can cause an effect produced by a single chemical, only when administered at a higher dose (Soto,1994).

A mother's lifelong exposure to estrogens is the best indicator of exposure and risk to the child, particularly for estrogens that accumulate in the body. According to a recent editorial in the British medical journal, The Lancet (1995) "The various facets of declining male reproductive health seem to have a common origin in childhood, and defects that may be induced in the current birth cohort by xeno-oestrogens [estrogens from sources other than the human body]. A similar observation (*Vankar,2001*) was made by us among the residents of Kasaragod village, Kerala, India, where endosulphan was being sprayed on cashew plantations for the last 18 years. Mother's lifelong exposure.to very high concentration of endosulphan, caused more and more children in that village born with cerebral palsy, low immunity and other congenital deformities.

MATERIALS AND METHODS

The study included the following parameters: Sampling, extraction of pesticides, analysis of multi-residues, simultaneously percent recovery of pesticides and spiking analysis for each pesticide. The standards for the pesticides chosen for study were obtained from RDH Laborchemikalien GmbH & Co. KG D-30918 Seelze through Promochem India pvt. ltd, Bangalore India.

Organochlorines and Organophosphates: standards 5 ppm
Aldrin, α, β, χ, δ-BHC, DDD, DDT, Dieldrin, α, β-Endosulfan, Ethion
Methyl parathion,Dimethoate and Malathion.

50g of the sample was taken and the pesticides were extracted for 8 hrs at the rate of 6-7 cycles per hour, in 250 ml n-hexane in a soxhlet extractor (EPA, 3540C). The extract obtained was cooled, filtered and concentrated in vacuum in a rotary evaporator. Hexane layer after concentration by rotary evaporation and final sample were prepared in 2ml of hexane (high purity grade).

1.0μl of the sample thus prepared was injected and analysed for the presence of fifteen pesticides, by Gas-Chromatograph (Trace GC) with the selective electron-capture detector (ECD), it allows the detection of contaminants at trace level concentrations in the lower ppb range in the presence of a multitude of compounds extracted from the matrix to which these detectors do not respond. The GC condition and the column specifications. are Capillary Column- DB-17, Oven condition is Ramp: 140 °C for 3min, 10 °C /min, 265°Cfor 10 min, column length :30 m, ID 0.25 mm, Injector temperature used is 275 °C, Detector temperature used is:250°C and Carrier gas: N_2 –2 ml/min.
Recovery and Spiking experiments were done as mentioned above, generally with every ten set of extraction one recovery experiment was performed so for this analysis

particularly three recovery experiment were set and spiking was performed only in that condition when some residues were present above MRL values (optional). The reproducibility of results for all the pesticides was 96.4% and above for all the samples.

Table 1. Brands of infant food in this statement

Infant Formula	Manufacturer	Manufacturing Date.	Date of Expiry	Batch No.
Cerelac Wheat	Nestle India	Sep'2000	Sep'2001	AMEEBJ
Cerelac Rice	Nestle India	Oct'2000	Oct'2001	AIKCDS
Cerelac Honey	Nestle India	June'2000	June'2001	ADPBBJ
Cerelac Orange	Nestle India	Nov'2000	Nov'2001	INALHADS
Cerelac banana	Nestle India	Mar'2001	Mar'2002	INPP2ADS
Cerelac Apple	Nestle India	Mar'2001	Mar'2002	INPRDADS
Cerelac Veg	Nestle India	Sep'2000	Sep'2001	AMSD8J
Nestum Rice	Nestle India	Mar'2001	Mar'2002	INPR6808
Lactogen	Nestle India	Mar'2001	June'2002	INPRBD3J
Dexolac	Wockhardt	Oct'2000	Dec'2001	90027

RESULTS AND DISCUSSION

The analysis of ten different products of infant formula is given in the Table-2. It shows the presence of more than 5 pesticides at least in each ranging from 0.04 ppb –335.87 ppb. β-BHC, α-endosulphan, malathion, dimetheoate, dieldrin, aldrin were present in most of the samples. Methyl parathion was present only in dexolac while ethion was absent in all the samples. DDE, DDD and DDT were present in small quantities in about 7-8 samples The presence of these pesticides were confirmed by GC-MS. The mass fragments matched with the pesticide's fragmentation pattern. GC-MS analysis of the infant food samples have confirmed the presence of aldrin, β-BHC, malathion, DDE, DDT and methyl parathion in addition to α and χ-BHC. The BHC isomers were identified by their relative retention times as well as by selective ion monitoring at 221,181, 113 and 97 mass unit characteristic to BHC isomers. The presence of β-BHC in large quantities, along with α, χ-BHC, reflects partial isomerization of χ-BHC on residue ageing. Similarly for DDT, DDD, DDE the main peaks are 352, 330, 318, 291 and, 235, while for methyl parathion263, 231, 168, 154 and 139 are specific peaks. In malathion the characteristic peaks identified were 330, 174, 172, 157 and 88, which matched in the infant food samples as well.

The toxicological significance of these residues is not known, but is cause for concern. In our study, 15 pesticides were detected in the 8 infant foods tested. Cerelac honey,

Table 2. Analytical result of different brands of infant food

NAME	PESTICIDE RESIDUES (ppb)												
Infant Formula	1	2	3	4	5	6	7	8	9	10	11	12	13
Cerelac Wheat	0.05	1.92	245.44	1.00	1.64	21.67	ND	ND	2.01	3.78	ND	4.28	26.76
Cerelac Rice	1.07	1.34	30.26	1.5	0.21	3.00	3.00	2.30	2.91	ND	1.31	ND	ND
Cerelac Honey	6.90	0.40	335.87	2.04	0.40	0.05	1.27	ND	1.39	ND	1.11	1.75	11.51
Cerelac Orange	9.00	1.1	23.00	0.8	1.7	0.9	1.2	1.0	1.6	ND	1.3	2.1	12.7
Cerelac banana	ND	0.27	59.28	0.45	ND	ND	0.48	ND	0.7	ND	0.71	ND	1.0
Cerelac Apple	ND	0.7	ND	0.3	1.4	ND	ND	ND	ND	ND	0.6	9.6	15.2
Cerelac Vegeta.	0.49	1.6	ND	1.0	1.1	0.6	0.7	ND	1.4	ND	ND	ND	12.00
Lactogen	0.97	0.69	9.62	ND	ND	ND	ND	ND	ND	ND	ND	ND	19.16
Dexolac*	ND	0.04	72.78	ND	ND	ND	1.17	ND	1.27	ND	1.18	0.707	4.06
Nestum Rice	ND	0.7	89.6	ND	1.0	0.62	0.76	ND	0.78	ND	1.2	2.0	2.7

- Methyl Parathion was present in this sample at1.8 ppb, ND=Not detected ** The maximum cumulative pesticide residues allowed for infant food is 10 ppb. 1.= α-HCH , 2.= χ-HCH , 3.= β-HCH ,4.= δ-HCH ,5.= Aldrin, 6.= α-Endosulphan ,7.= DDE , 8.= Dieldrin , 9.= DDD ,10.= β- Endosulphan , 11.=DDT , 12.= Dimethoate and13.= Malathion

wheat, vegetable and Nestum rice contained more pesticides and at higher levels, than cereal and fruits cerelac. The WHO (1986) has proposed an acceptable daily intake (ADI) of 20 μg/Kg body weight for adults for DDT compounds, 10μg/Kg for χ-BHC, malathion and endosulphan. If these ADI values are applied to infants , whose mean body weight is 5Kg, the mean concentration will exceed 50μg/Kg per pesticide, assuming a mean intake of 0.5 Kg of infant solid food. This would be far more than the ADI allowed for an adult. For BHC and endosulphan, residues should not exceed 0.5μg/g and 1.0μg/g according to Codex Alimentarius, (1986).The concentrations of DDT, DDE and DDD were lower , while the concentration of endosulphan was the highest in Cerelac Wheat. Methyl parathion was observed only in Dexolac. All the infant food are prepared in water, which is also contaminated by some of these pesticides. If contamination occurred greater than the levels recommended by WHO, it will enhance the pesticide contents intake further.

As such there is no specific study conducted in the field of commercially available infant formula and infant milk preparation in India till date. Few studies are there, which shows the presence of very high levels of DDT and BHC in human breast milk, infant formula and food items. A recent study by the Punjab Agricultural University in India found that babies in the cotton-growing region of the Punjab were consuming 24 times as much DDT residue as is considered safe.

Pesticide contamination of breast milk has been found even in remote villages in India. Studies have shown that women in developing countries suffer the greatest exposure to pesticide residues and ultimately through breast feeding it enters in the infant body. The present study gives an insight to this very important area of infant food's contamination. Government needs to make stringent rules for the producers of infant food to minimize the pesticide residues in this class of food item. Our data show that world needs pesticide safety standards for infants and young children because infants and young children are exposed to pesticides as soon as they begin to eat food. Some of the pesticides found by this study and in separate studies by the FDA are toxic to delicate organ systems, such as the nervous and endocrine system.

Current standards for pesticides in food do not specifically account for the special vulnerability of infants. They do not account for the additive or potentially greater than additive toxicity of pesticide combinations in baby food and they do not account for the additive effects of pesticide exposure from sources such as contaminated tap water used to reconstitute infant formula or juice, or home and garden use of pesticides.

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