DEPARTMENT OF HEALTH & HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION

Public Health Service

Memorandum

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From Chemist, Indirect Additives Laboratory, HFS-248

Subject Migration Data for Bisphenol-A from Can Enamels to Infant Formula Concentrates

To Gregory Diachenko, Director, Division of Product Manufacture and Use, HFS-245

The recent interest in estrogen mimics has prompted the Indirect Additives Laboratory to develop an analytical protocol for analysis of bisphenol-A (BPA) in canned foods including infant formula. Since it is well known that BPA is a reactant for the preparation of epoxy enamels used in can coatings, a limited survey of canned infant formula concentrates has been conducted. The purpose of the survey is to quantitate the amount of BPA that has migrated into the formula from the can coatings. It was initially decided that 3 cans, each representing a different lot of each formula manufacturer would be tested.

The Office of Special Nutritionals (HFS-456) has advised that at the present time there are [redacted] Each manufacturer has several formulations of milk and soy based products. The variety of formulations range from those fortified with nutrients like iron to products intended for consumption by different age groups. Both soy and milk based formulas contained between 7% and 8% fat (wt./vol.) according to the labels. All of the cans in the limited survey are made to contain 384 mL (except as noted) and are of 2 or 3 piece, flat top/bottom design. (Only the [redacted] cans have pull tops.) Of the [redacted] three each of the cans and contents are tested with the exceptions of [redacted] 4 cans and [redacted] 1 can.

The analysis of each of the cans and it's contents involves four different techniques. The interior surface of each can is inspected visually for color and appearance of the coating. Microscopic analysis is used for any surface or sub-surface features which may be of interest (film thickness when possible). Specular reflectance fourier transform infrared spectroscopy is used for identification of the type of coating. The contents of each can are analyzed for BPA by HPLC using the protocol described in Appendix A.

Visual inspection shows the BPA-epoxy based food contact surfaces to range from yellow to brown in color. Two piece can sides have a generally cloudy or "flat" appearance, while three piece can sides and ends have a striated appearance which also shows up in microscopic analysis.

Microscopic inspection of the food contact surfaces reveals 2 general categories of surfaces; smooth almost featureless and wavy or rippled surfaces. Most can coatings in the survey exhibit some entrapped bubbles (solvent?), of which, the three piece cans containing the [redacted] products are extreme examples. Microscopic inspections could not confirm more than one layer of enamel on any portion of the cans. Film thicknesses are difficult to gauge, but appear less than 10 microns on the portions of the surfaces examined.

Infrared analysis shows all cans to have at least a portion of their food contact surfaces coated with an epoxy or modified epoxy enamel based on BPA. All spectra are compared (manually and by matching algorithm to standards in the high resolution Hummel Polymer Library.

In this limited survey, the highest amount of BPA measured in any of the infant formula concentrates is 13.2 ng/mL. The lowest level is 0.1 ng/mL. (Levels of BPA in prepared formulas would be lower; directions on the labels call for a 1:1 dilution (v/v) with water.) Recoveries for milk based formulas average 86% while recoveries for soy based formulas average 104%. Recoveries are calculated by fortifying the formula (in duplicate) with an amount of BPA equal to the average amount quantitated in the same formula. The average BPA concentration was subtracted from the total BPA concentration in the spiked formula to determine the net amount of BPA recovered. The limit of detection for the instrument (LOD_I) is 0.9 ng/mL. Generally, concentration steps by as much as a factor of 30 are performed before instrumental analysis for BPA. The limit of quantitation with a 30 fold concentration ($LOQ'_{conc.}$) is 0.3 ng/mL. See Table 1. for the amount in ppb (ng/mL) of BPA found in each of the formulas analyzed.

At this time the objective of the limited survey of canned infant formula concentrates has been met. Three cans, each representing a different lot of the [redacted] manufacturers in the United States have been analyzed and the migrated BPA in the formula quantitated. In addition, an in-house infrared spectral library of BPA epoxy based can enamels has been started. A comparison of the infrared data with the HPLC data strongly suggests that there may be greater migration of BPA to formula from modified epoxy coatings relative to the un-modified BPA based epoxy coatings.

[redacted]

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[redacted]

APPENDIX A

PROCEDURE OUTLINE:

Dilute an aliquot (usually 10 mL to 30mL) of formula to 100 mL with HPLC grade water. Pre-condition an ENVICHROM-P 6 mL solid phase extraction cartridge with 10 mL of HPLC water. Allow the diluted formula to flow from a 100 mL buret through the SPE cartridge at a rate of 15 to 20 mL per minute. Rinse the buret and cartridge with 15 mL of water afterwards. Rinse SPE cartridge with 15 mL of UV grade hexane. Elute the BPA from the cartridge with 15 mL of chloroform. Evaporate chloroform until only a few microliters remain. Redissolve with a known volume (usually 1 to 4 mL depending on the concentration of BPA in the sample) of 1:1:1 (v/v) acetonitrile, methanol and water. Blend thoroughly on a vortex mixer and analyze by HPLC using fluorescence detection. The fluorescence excitation and emission wavelengths were 235 nm and 317 nm respectively. Determine recoveries by fortifying the aliquot of formula with BPA prior to dilution with water.

The absolute $LOD_{conc.}$ for this protocol can be as low as 0.03 ppb depending matrix effects, levels below 0.1 ng/mL have been measured. The Waters fluorescence detector now being used in place of the Shimadzu has a LOD_I of 0.9 ppb (0.045 ng for a 50 uL injection). The sensitivity of the analysis can be adjusted with the volume of the aliquot of formula and/or the volume of 1:1:1 ACN, MeOH and H_2O used to redissolve the dried chloroform eluate.

TABLE 1.
Amount of BPA in Infant Formula Concentrates

[redacted]

Mfc.	Can Style	Lot No.	Formula Type	BPA[a] ppb	BPA[a] ng/cm²	Rec.	Lid Color	Lid Coating	Side Color	Side Coating
A	2	MKJ42	Milk	13.2	11.6	75%	Yellow	epoxy+	Gold	ep/estr
A	2	MJJ04	Milk	12.1	10.6	90%	Yellow	epoxy+	Gold	ep/estr
E	3	ALJ24	Milk	9.5	8.5	90%	Gold	ep/co	Gold	ep/ma
B	2	AK27C	Milk	8.3	7.4	79%	Yellow	epoxy	Gold	ep/co
A	3	CJJ88	Milk	5.1	4.5	83%	Yellow	epoxy	Gold	epoxy+
B	3	1C23C	Milk	4.8	4.2	67%	Yellow	epoxy	Brown	epoxy
C[b]	3	73509	Soy	4.5	4.0	104%	Yellow	epoxy	Gold	ep/fa
D	3	5293	Soy	3.9	3.5	109%	Yellow	epoxy	Gld/ylw	epoxy
B	3	2AO6C	Milk	3.6	3.2	95%	Yellow	epoxy	Brown	epoxy
C	2	10523	Milk	1.5	10.1[c]	80%	Yellow	epoxy	Brn/gry	PVC
C	2	10590	Milk	1.3	8.8[c]	89%	Yellow	epoxy	Brn/gry	PVC
C	2	06799	Soy	1.3	8.8[c]	100%	Yellow	epoxy	Brn/gry	PVC
D	3	5193	Milk	0.7	0.6	106%	Yellow	epoxy	Yellow	epoxy
D	3	5319	Milk	0.1[d]	0.09	97%	Yellow	epoxy	Yellow	epoxy

a.) BPA concentrations in ppb (ng/mL) were NOT corrected for recoveries. The average recoveries for milk and soy based formulas were 86% and 104% respectively.
b.) Tim Begley's 945 mL (large) can approx. 3 years old.
c.) Area is calculated for the epoxy coated surfaces only.
d.) Injected solution below LOQ_I; $LOD_{conc.}$ = 0.03 ng/mL, $LOQ_{conc.}$ = 0.3 ng/mL.
Key for abbreviations:
ep = epoxy, fa = fatty acid, co = castor oil, ma = melamine, estr = ester