MULTI-RESIDUE METHOD FOR PESTICIDES AND POPS IN MILK AND CREAM USING COMPREHENSIVE TWO DIMENSIONAL GAS CHROMATOGRAPHY TIME-OF-FLIGHT MASS SPECTROMETRY

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Introduction

The determination of pesticides and POPs in foods with high fat content presents unique difficulties to analytical methods[1,2]. Often the analytes of interest are found associated with the lipid portion of food and as such considerable amounts of fats are recovered in the extract and must be removed before measurement. Methods need to be "fit for purpose" for tolerance enforcement in foods such as milk where action levels and tolerances exist for POPs (eg. BHCs 300 ng/g, heptachlor 50 ng/g, DDT 1250 ng/g fat). Tolerances are expressed on a fat weight basis which requires a correct fat determination to be done as well. Some pesticide methods in the literature have not demonstrated their effectiveness in the concentration range for US FDA action levels in milk while some others have[3]. A number of physical and chemical approaches have been developed including techniques such as solvent partitioning, extraction with polar solvents, absorption on solid phase sorbents or Gel permeation chromatography (GPC). These approaches have recently been reviewed[2].

Recently pesticide extraction and clean up has been streamlined by use of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method that uses smaller sample sizes, an excess of salts and acetonitrile to remove pesticides from fruit and vegetable matrices[4]. A portion of the extract is cleaned up using dispersive or column SPE materials. We have recently developed an effective procedure for dietary supplements that uses GPC and SPE clean up[5]. The method described in this paper combines the ease of a QuEChERS type extraction with effectiveness of this dietary supplement clean up for milk and cream[5]. The procedure was tested by fortifying 34 pesticides, their isomers and metabolites in whole milk at four levels.

In March of 1982, the State of Hawaii Department of Health (DOH) recalled dairy products on Oahu due to contamination with Heptachlor Epoxide (HE). On July 6, 1982, nearly four months after the initial recall, the DOH embargoed 120 plastic containers (45 lbs each) of cream contaminated with HE that was still being sold from a food broker's warehouse. The DOH tests of eleven cream containers revealed it contained HE at concentrations of 0.62 - 0.71 mg/kg, over twice the action level at that time. Four containers were kept for use in follow up studies of the exposed population. In 2003, at the conclusion of these studies, two samples were pulled from the cream and provided to a local private lab and the US Food and Drug Administration (FDA) for future testing[14]. On August 20, 2003 the local (Hawaii) lab found the HE concentration was 250 ug/kg and also found DDE at 45 ug/kg wet weight. The HE level exceeded the allowable level for disposal at the local Oahu landfill. The cream was declared a hazardous waste and properly shipped and disposed of in a facility in Washington State in November of 2003. The Hawaii Heptachlor Foundation and Education Fund (HHR&EF) paid for this disposal. The other sample was held at the US FDA CFSAN until March 2009 when it was tested. We report the HE concentration for the 1982 Oahu, Hawaii cream sample sent to US FDA using this alternative method.

Materials and Methods

Whole pasteurized milk was purchased in College Park for fortification studies. An archival cream sample kept frozen since 1982 was generously provided by the HHR&EF. The cream was collected during a Heptachlor contamination incident in Hawaii related to Heptachlor treated pineapple chop used in cattle feed. All samples and extracts were kept at -40^{o} or -20^{o} C respectively. Milk was thaw, remixed and 20 g portions were used for test extractions, except the Hawaii cream sample where 1.5 g portions were used.

Solvents used were all high purity pesticide grade from Fisher Scientific and the pesticides standard were all >99% provided by US EPA at Fort Meade, MD. Milk was weighed into 50 mL centrifuge bottles and fortified a pesticide standard mixture in acetone. The standards were vortexed into the milk for 1 min. and then 20 mL of a mixture of acetone/cyclohexane/ethylacetate (2:1:1) was added. Eight grams Mg_2SO_4+1.5 g NaCl was added. The mixture was shaken for 1 min. at 1000 strokes/min. on a Geno-grinder. After centrifuging for 5 min. at 4500 rpm, 14-16 mL of the upper solvent layer was removed. Lipids were determined gravimetrically using the entire extract for milk or a separate 4 mL aliquot for cream. The lipid results were compared with conventional liquid/liquid extraction of milk for fat found in FDA PAM 1[1] and to pressurized liquid extraction of freeze dried milk[3,7]. The fat was reconstituted in to 5mL in cyclohexane/ethyl acetate. The entire 5 mL extract was injected on to an express GPC column of 24 g S-X3 biobeads in 50:50 ethylacetate/cyclohexane ~5 mL/min. using a 22 min runtime. Pesticide containing fraction was reduced to approximately 1-2 mL and applied to a PSA (500mg)/carbon (250 mg) SPE column and eluted with 13 mL of 75% acetone/toluene. The entire extract was reduced to 1 mL in toluene and transferred to a vial containing 100 ng acenaphthene-D10, phenanthrene-D10 and chrysene-D12 internal standards.

The milk and cream extracts were measured for pesticide content using Pegasus 4D GCxGC TOFMS (LECO Corp. USA). Five micro-liters of each extract was injected at 0.88 µL/s into a Gerstel CIS4 PTV injector (Gerstel Gmbh & Co. KG) with solvent venting at 200 mL/min with an initial temperature of 40 C. Toluene was vented using stop flow (10 kPa) for 12 seconds and then the CIS4 was heated at 10°C/s to 280° C[8,10]. The columns used were a 5 M 0.25 deactivated retention gap (Restek corp. Bellefonte, PA USA) with either a 30 M x 0.25 mm id VF-5 ms (Varian Corp.) for the first dimension and a 2.2 M x 0.1 mm id BPX-50 (SGE Corp.) for the second dimension column or alternately, a combination of a HP-5 ms (30 M) and a 1.5 M 0.15 mm id BPX-50 column. Both column sets were found to be effective with pesticides and POPs separations when tested with fortified milk as suspected from previous reports[5,9,10,12]. Columns were connected with press fit glass universal connectors (Restek Corp.). A constant pressure of 379 kPa (55 psig) was maintained using the longer second column or 197 kPa or (29 psig) with the shorter column to provide more optimal flow for the first dimension at the initial temperature and for the second dimension at the final temperature[10,11]. The temperature program was 90° C 2 min. 10° C/min to 180° C 4°/min. to 290° C with a 35° C offset for the secondary oven and modulator offset was 50° C. The modulation period was 3 s with the spectral acquisition rate set to 200 Hz and the MCP detector at 1750 V[13]. Matrix match standards were prepared at 1, 2, 5, 10, 20, 50, 100 and 200 ng/mL. Injection speed was 0.88 µL/s. and the volume 5 µL for all samples and matrix matched standards.

Results and Discussion:

After centrifugation approximately 14-16 mL of extraction solvent was recovered from each fortified milk sample. The extraction solvent recovered had formed an upper layer above a plug of milk solids separating it from the saturated water and salt as in a typical QuEChERS extraction[5]. The lipid content of the extract accurately reflected the fraction of recovered extract for a 20 mL aliquot of whole milk and was comparable to other methods[1,3]. The co-extracted fats are 98% removed by the GPC without significant loss of the pesticides[5]. The pesticides eluted slightly earlier in the presence of co-extracted lipids than in mobile phase only, so the collection point needed to be adjusted slightly to 1 min earlier during the milk fortification tests. Table 1 provides the results for fortification at a range of concentrations above and well below the action levels for HE and DDE in milk and cream. Average recoveries were 84, 77, 66, 73, 72, 77% for 50, 10, 2, 1, 0.4 and 0.2 ppb respectively with a average relative standard deviations of <10% at all levels (Table 1). The LOQs were either 0.2 or 0.4 µg/kg wet weight (~6 or 12 µg/kg fat) (Table 1). HE recoveries averaged 79% with a standard deviation of ±4% at 12, 30, 60, 300 and 1500 µg/kg fat.

The Hawaii cream sample when thawed revealed that the solid and aqueous components of the cream had largely separated. The cream was otherwise in good condition for such an old sample having been stored at -20° C for 27 years. The solids (fat) portion was sampled for analysis. The analytical portion was combined with 18.5 mL of HPLC grade water and analyzed through the procedure like a whole milk test portion. Greater than 19 mL of the 20 mLs of extraction solvent added was recovered. Only an 8 mL aliquot was evaporated to <5 mL for clean up and determination of the HE concentration and a 4 mL portion was evaporated and weighed for fat

determination. HE and DDE were found at 270 µg/kg fat and 49 µg/kg wet weight respectively. The lipid content was 72%±1.5 in the solid portion of the cream extracted by this method. The mean HE and DDE concentrations were therefore 380 and 68 µg/kg fat, respectively calculated from the milk matrix matched standard curve (Table 2).

The HE concentration in the cream from the reserved batch was in general agreement with the amount reported by a private lab in 2003. Measurements on 11 batches of cream by DOH from the same supplier in 1982 gave an averaged of 670 µg/kg. The values measured in 2003 and 2009 are somewhat lower possibly reflecting sampling differences or perhaps degradation during the 20 year storage at -20 C. Only a few measurements were available on the contaminated milk and cream sampled in 1982 or before[14]. Little is known about the duration or magnitude of the HE exposure on Oahu from milk consumption. US EPA through their radiation monitoring program had been collecting milk from Oahu during the same time period. The EPA had in their archives milk from as far back as 1980 that showed elevated HE concentrations. No samples were available earlier than 1980. HE contamination of Hawaiian milk is known to extend to at least to July 1980 or perhaps earlier, 18 month prior to the first measurements done by the Hawaiian DOH. In addition, no through study of any additional contaminants presence in the milk was ever performed in the intervening years from the first collection in 1982. Our study has demonstrated that only HE and DDE were present in the milk at this time and there is no evidence of other persistent contaminants above our LODs.

Table 1. Cow's milk fortification recoveries for pesticides fortified in milk at 0.2 0.4, 1 2, 10 and 50 µg/kg wet weight; ND = not deconvoluted, BST = below similarity threshold for library match. Bold indicates a POP

	0.2	0.4	1	2	10	50
Pesticide name (LOQ)	n=4	n=4	n=4	n=4	n=4	n=2
Aldrin (0.2)	69±12	68±5	69±5	76±7	72±1	83±1
BHC-alpha (0.2)	69±6	57±6	70±6	72±5	73±4	87±7
BHC-beta+lindane (0.2)	68±9	60±5	62±3	68±9	71±2	98±5
Chlordane-cis (0.2)	84±12	69±5	68±4	63±4	70±1	84±0.8
Chlordane-trans (0.2)	82±6	79±4	74±4	61±6	61±3	82±3
Dacthal (0.2)	81±9	75±6	75±3	70±3	76±1	86±1
DDD-o,p' (0.2)	52±5	72±4	67±7	62±6	72±1	ND
DDD-p,p' +DDT-o,p' (0.2)	58±10	67±6	66±8	62±6	69±3	93±2
DDE-o,p' (0.2)	69±8	73±5	56±7	63±6	79±2	88±2
DDE-p,p' (0.2)	65±13	80±10	75±7	64±4	79±1	102±1
DDT-p,p' (0.4)	80±18	79±7	66±8	64±5	83±2	77±3
Dieldrin (0.2)	90±10	79±11	87±8	73±6	85±1	96±4
Endosulfan I (0.4)	110±18	71±11	73±24	66±9	80±3	90±0
Endosulfan II (0.4)	43±12	80±10	73±7	ND	70±4	94±0.5
Endrin (0.2)	93±6	78±10	88±13	74±8	86±0.6	94±2
Fonofos (0.4)	87±13	86±6	71±2	67±9	70±2	80±1
Heptachlor (0.4)	64±27	74±9	71±4	70±6	76±2	74±1
Heptachlor epoxide (0.2)	101±9	84±7	77±3	76±6	78±2	79±0
Hexachlorobenzene (0.2)	76±9	59±12	74±7	52±10	79±1	67±1
Mirex (0.2)	86±5	70±9	102±11	72±5	96±6	119±16
Pentachloroaniline (0.2)	72±6	75±5	68±4	59±5	72±4	78±1
Pentachlorobenzene (0.4)	BST	ND	58±2	ND	73±4	68±3
Pentachlorobenzonitrile (0.2)	76±8	63±8	63±7	60±9	68±7	73±3
Pentachlorothioanisole (0.2)	87±13	61±4	62±9	54±3	66±8	86±2

Quintozene (0.4)	83±17	67±8	92±7	60±9	100±7	68±2
Tecnazene (0.2)	64±2	ND	88±4	74±8	96±6	64±0.9
Tetrachloroaniline-2,3,5,6 (0.2)	81±5	76±5	79±4	64±14	83±5	68±3
mean±stdev	77±10	72±8	73±7	66±7	77±3	84±3

Table 2. Hawaiian cream collected on Oahu in 1982 during a Heptachlor contamination of pineapple chop used in cow feed. Concentrations are in µg/kg fat.

Test portion	1	2	3	mean±stdev
Heptachlor Epoxide	406	377	349	380±29
DDE-p,p'	63	85	55	68±16

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