Comparison of Methods for Preparing and Analyzing Samples for Chemicals of High Concern to Children in Children's Clothing and Other Products

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Overview

The Washington State Department of Ecology (Ecology) regularly conducts studies to ensure manufacturer compliance with Washington State's Children's Safe Products Act (CSPA – RCW 70.240). During 2014 and 2015, Ecology conducted a study to measure frequently reported chemicals of high concern to children (CHCCs) in children's clothing, footwear, and accessories (Mathieu and Sekerak, 2015). Ecology tested samples of the children's products for metals (antimony, arsenic, cadmium, cobalt, lead, mercury, and molybdenum), phthalates (DEHP, BBP, DEP, DnHP, DIDP, DINP, DMP, DBP, and DnOP[1]), ethylene glycol, methyl-ethyl ketone (MEK), styrene, octamethylcyclotetrasiloxane (D4), and 4-nonylphenol.

During the process of this study, Ecology's product testing team identified a need to develop guidance for consistency in preparing and analyzing consumer products. Little guidance is available in the literature or from government agencies on the preparation of various consumer product matrices prior to analysis using cryomilling procedures. Cryomilling is the process of reducing a sample to very small particle sizes (~5-50 microns) by lowering the product to cryogenic temperatures and mechanically milling it with a stainless steel magnetic shaker. This process provides a homogenous, finely divided solids sample for efficient extraction.

Fabric is a matrix that may be easily digested and/or extracted, and might not require cryomilling prior to metals analysis. While cryomilling provides a representative, homogenous sample that is more efficient for extraction, it also increases the time and cost of analysis. Simpler methods, such as cutting to a fine size using hand scissors, may achieve the same analytical results. Since little guidance is available in the literature or from other federal or state agencies, the most appropriate preparation method remains an open question. To assess whether the cryomilling method would yield different analytical results from the hand-cutting method, Ecology reanalyzed a subset of fabric samples from the original project, treating each sample with both preparation methods prior to analysis. This comparison was limited to metal analytes.

Ecology also identified a need to ensure consistency in the analysis of consumer products for phthalates. The U.S. Consumer Products Safety Commission (CPSC) has published Method CPSC-CH-C1001-09.3 (hereafter referred to as the CPSC method) for the preparation and analysis of phthalates in children's toys and child care articles (CPSC, 2010). However, according to this method, users may follow the extraction and analysis outlined in CPSC-CH-C1001-09.3 or several alternative extraction and analysis methods. Ecology's Manchester Environmental Laboratory (MEL) uses EPA Method 3546 and modifications of EPA Method 8270D (hereafter referred to collectively as SW3546) for extraction and analysis, respectively, which are allowed under CPSC-CH-C1001-09.3. To assess differences between the methods, Ecology re-analyzed phthalates in one of the samples from the original project – a plastic handbag – as well as two standard reference materials (SRM) using both the CPSC and SW3546 methods.

Details of the plan for comparing metals preparation methods and phthalate extraction methods may be found in *Addendum 1 to Quality Assurance Project Plan: Chemicals of High Concern in Children's Clothing, Footwear, and Accessories* (Mathieu, 2015).

[1]DEHP = di-2-ethylhexyl phthalate; BBP = butyl benzyl phthalate; DEP = diethyl phthalate; DnHP = di-n-hexyl phthalate; DIDP = diisodecyl phthalate; DINP = diisononyl phthalate; DMP = dimethyl phthalate; DBP = dibutyl phthalate; DnOP = Di-n-octyl phthalate.

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Methods

Sample Preparation and Study Design

Five archive samples of fabric from the original CHCC project were selected for analysis of metals (Table 1). All had detectable concentrations of at least one target metal reported in the original CHCC project (antimony and/or cobalt, molybdenum). No archive material with detections of arsenic, cadmium, lead, or mercury was available from the original project.

Ecology headquarters staff cut each fabric sample into 2 cm x 2 cm pieces, placed them in certified 8 oz. glass jars, and sent them to MEL via courier. MEL staff then divided each sample into two equally-weighted subsamples. Each subsample was either (1) cryomilled or (2) cut to 2 mm x 2 mm pieces, using stainless steel scissors. Subsamples were then split by the laboratory into seven aliquots of each treatment type. The 14 aliquots of each sample were then analyzed for the target metals analyte list using EPA 3052/EPA 6020A. Figure 1a displays the preparation and analysis scheme for metals.

Table 1. Concentrations of Metals and Phthalates in Samples Analyzed during the Original Study and Selected for Analysis in the Present Study.

Analyte	Fabric (n=5)	Handbag (n=1)	PVC SRM[1]	PE SRM[2]
Metals (mg/kg)				
Antimony	**5 - 180**	NA	NA	NA
Arsenic	<1	NA	NA	NA
Cadmium	<1	NA	NA	NA
Cobalt	<1 - **340**	NA	NA	NA
Lead	<1	NA	NA	NA
Mercury	<0.02	NA	NA	NA
Molybdenum	<1 - **1.6**	NA	NA	NA
Phthalates (mg/kg)				
DEHP	NA	**1,400**	3,000 ± 363	3,000 ± 363
BBP	NA	<24	2,970 ± 359	3,000 ± 363
DEP	NA	<24	3,000 ± 363	3,000 ± 363
DIDP	NA	<49	30,000 ± 3,630	30,000 ± 3,630
DINP	NA	<49	30,000 ± 3,630	30,000 ± 3,630
DMP	NA	<24	3,010 ± 364	3,000 ± 363
DBP	NA	<120	3,000 ± 363	3,000 ± 363
DnOP	NA	<64,000	3,000 ± 363	3,000 ± 363

SRMs were not analyzed in the original study, concentrations shown are certified values ± uncertainty.
Detected values in bold.
NA = not analyzed.
[1] SPEX CertiPrep CRM-PVC001.
[2] SPEX CertiPrep CRM-PE001.

For phthalates, only one archive children's product (handbag) sample from the original CHCC study contained enough sample material for additional analyses. This sample, along with two SRMs, was analyzed for the suite of phthalates. Since phthalates may be a concern in both polyvinyl chloride (PVC) and polyethylene (PE) matrices, SRMs representing each matrix were analyzed.

The handbag sample was hand-cut by Ecology headquarters staff into 2 cm x 2 cm pieces, placed in a certified 4 oz. glass jar, and sent to MEL. MEL staff cryomilled then divided the sample into 14 aliquots. Seven sample aliquots along with seven aliquots of each SRM (sold in powdered form and therefore do not require cryomilling) were analyzed for the target phthalate list using SW3546. The same number of sample and SRM aliquots were also analyzed following the CPSC method. The CPSC method uses tetrahydrofuran (THF) as the primary extraction solvent whereas SW3546 uses an acetone–hexane mix. Both extracts were analyzed by a modification of EPA 8270D; more detail can be found in the project plan (Mathieu, 2015). Figure 1b displays the preparation and analysis scheme for phthalates.

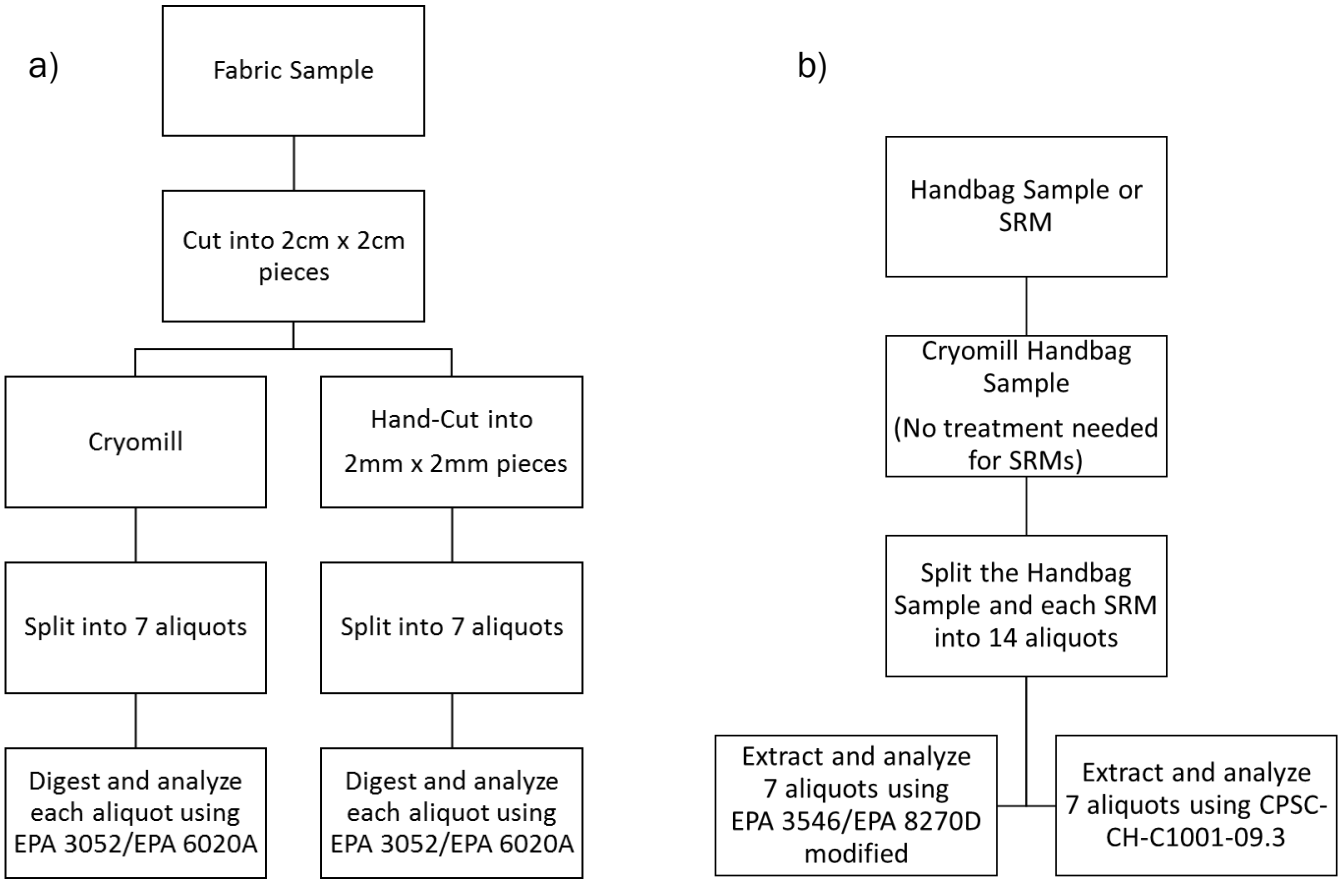

Figure 1. Sample Preparation and Analysis Scheme for (a) Metals and (b) Phthalates.

Data Quality

Quality control and quality assurance tests were within acceptance limits with the following exceptions. Molybdenum was detected in method blanks above acceptance limits and some samples were qualified as estimates (J qualifier) as a result. Two phthalates – DBP and DnOP – exceeded initial calibration upper control limits during analysis of the SRMs by EPA 8270D and were qualified (J).

DIDP and DMP in the PVC SRM were "tentatively identified" in one replicate each using SW3546 and CPSC methods, respectively. The resulting values were considered an approximation (NJ qualifier) and were not used in the calculation of summary statistics.

Results

Comparison of Methods for Metals

Of the seven metals analyzed in fabric, only antimony, cobalt, and molybdenum were detected (Table 2). Arsenic, cadmium, and lead were not detected using either preparation method at a reporting limit of 1 mg/kg; mercury was not detected at a reporting limit of 0.02 mg/kg. Reporting limits for the two methods were identical for each metal. Molybdenum was rarely detected (four of 35 analyses for cryomilling, two of 35 analyses for hand-cutting; reporting limit of 1 mg/kg) and method comparisons are therefore not meaningful for this metal. Sample results for all metals were similar to those found in the original study (Mathieu and Sekerak, 2015).

Antimony was detected in all replicates for all samples prepared by both methods. Mean concentrations measured in samples prepared by using the hand-cutting method were slightly higher than those prepared by cryomilling (Table 2). Replicate variability was higher in results from hand-cut samples, although coefficients of variation (CV, standard deviation divided by the mean) were small in all cases (range = 0.5 – 9.7%).

Cobalt was detected in all replicates analyzed from two of the samples, but not detected in any of the remaining three samples. Like antimony, hand-cut samples yielded slightly higher concentrations. High replicate precision was found for all cobalt analyses (CV = 0.4 – 2.0%).

Table 2. Concentrations of Metals in Fabric Samples (mg/kg; mean ± standard deviation of seven replicates).

Metal	Prep Method	Fabric Sample				
		ON-2-14-1	RE-2-5-1	KL-1-9-3	ON-2-2-6	TG-11-20-1
Antimony	Cryomill	**132 ± 1.7**	**4.6 ± 0.23**	**172 ± 1.7**	**150 ± 0.76**	**179 ± 1.9**
	Handcut	**137 ± 1.8**	**4.6 ± 0.44**	**179 ± 1.4**	**154 ± 8.3**	**181 ± 11**
Arsenic	Cryomill	U (1.00)	U (1.00)	U (1.00)	U (1.00)	U (1.00)
	Handcut	U (1.00)	U (1.00)	U (1.00)	U (1.00)	U (1.00)
Cadmium	Cryomill	U (1.00)	U (1.00)	U (1.00)	U (1.00)	U (1.00)
	Handcut	U (1.00)	U (1.00)	U (1.00)	U (1.00)	U (1.00)
Cobalt	Cryomill	**13.1 ± 0.11**	**355 ± 1.4**	U (1.00)	U (1.00)	U (1.00)
	Handcut	**13.2 ± 0.26**	**365 ± 5.3**	U (1.00)	U (1.00)	U (1.00)
Lead	Cryomill	U (1.00)	U (1.00)	U (1.00)	U (1.00)	U (1.00)
	Handcut	U (1.00)	U (1.00)	U (1.00)	U (1.00)	U (1.00)
Mercury	Cryomill	U (0.020)	U (0.020)	U (0.020)	U (0.020)	U (0.020)
	Handcut	U (0.020)	U (0.020)	U (0.020)	U (0.020)	U (0.020)
Molybdenum	Cryomill	U (1.00)	U (1.00)	U (1.00)	**1.15 ± 0.09* J**	**2.03****
	Handcut	U (1.00)	U (1.00)	**2.05****	**1.51****	U (1.00)

Detected values in **bold.**
J=estimated values due to blank contamination.
U=not detected at or above reporting limit in parentheses.
*.mean ± standard deviation of three detected results.
** single detected result.

Results

Comparison of Methods for Phthalates

DEHP was the only phthalate detected in the single consumer product tested; the other seven phthalates were not detected at reporting limits of 24.2 – 35.4 mg/kg using SW3546, and 368 – 750 mg/kg using the CPSC method (Table 3). Higher concentrations of DEHP were found in the handbag material when tested using SW3546 compared with the CPSC method; replicate precision was good for both methods (CVs = 3.9 – 6.3%).

Table 3. Concentrations of Phthalates in a Handbag Sample and Standard Reference Materials (mg/kg; mean ± standard deviation of seven replicates).

Phthalate	Method	Handbag Sample	SRMs	
		TG-11-15-1	CRM-PVC001*	CRM-PE001**
DEHP	SW3546	**1,370 ± 53**	**2,250 ± 494**	**2,150 ± 131**
	CPSC	**1,230 ± 77**	**2,460 ± 152**	**2,670 ± 198**
BBP	SW3546	U (24.2 – 25.4)	**2,050 ± 412**	**2,740 ± 112**
	CPSC	U (368 - 375)	**2,580 ± 64**	**2,710 ± 147**
DEP	SW3546	U (24.2 – 25.4)	**2,590 ± 513**	**2,900 ± 103**
	CPSC	U (368 - 375)	**2,650 ± 79**	**2,540 ± 66**
DIDP	SW3546	U (24.2 – 25.4)	**21,100 ± 4,390**	**24,200 ± 1,450**
	CPSC	U (735 - 750)	**28,500 ± 380**	**29,400 ± 2,650**
DINP	SW3546	U (24.2 – 25.4)	**21,800 ± 3,690**	**22,400 ± 681**
	CPSC	U (735 - 750)	**26,800 ± 610**	**26,900 ± 2,350**
DMP	SW3546	U (24.2 – 25.4)	**2,250 ± 441**	**2,570 ± 170**
	CPSC	U (368 - 375)	**2,090 ± 123**	**1,920 ± 198**
DBP	SW3546	U (24.2 – 25.4)	**2,330 ± 515 J**	**2,380 ± 53 J**
	CPSC	U (368 - 375)	**2,510 ± 101**	**2,720 ± 178**
DnOP	SW3546	U (24.2 – 25.4)	**2,420 ± 485 J**	**2,300 ± 200 J**
	CPSC	U (368 - 375)	**2,500 ± 132**	**2,540 ± 164**

Detected values in **bold.**
J=estimated values due to exceedance of calibration control limits.
* Certified concentrations are 30,000 ± 3,630 mg/kg for DIDP and DINP, 2,970 ± 359 for BBP, 3,010 ± DMP, and 3,000 ± 3,630 mg/kg for all other phthalates.
** Certified concentrations are 30,000 ± 3,630 mg/kg for DIDP and DINP, and 3,000 ± 3,630 mg/kg for all other phthalates.
U=not detected at or above reporting limit in parentheses.

Analysis of SRMs showed mixed results using the two methods, but concentrations were generally closer to certified values using the CPSC method compared with SW3546. For the PVC SRM, average recovery of the certified concentrations using the CPSC method was 85% versus 75% recovery for SW3546. Only one phthalate – DMP – had higher recovery using SW3546. Precision was also better using the CPSC method (Table 3), as displayed in the error bars in Figure 2.

Overall recovery of certified phthalate concentrations in the PE SRM was 84%, with the CPSC method recovering 86% of the phthalate concentrations on average and SW3546 recovering 82% (Figure 3). Both methods had very high precision in the percent recovery, with standard errors of the means rarely exceeding 3%.

Results

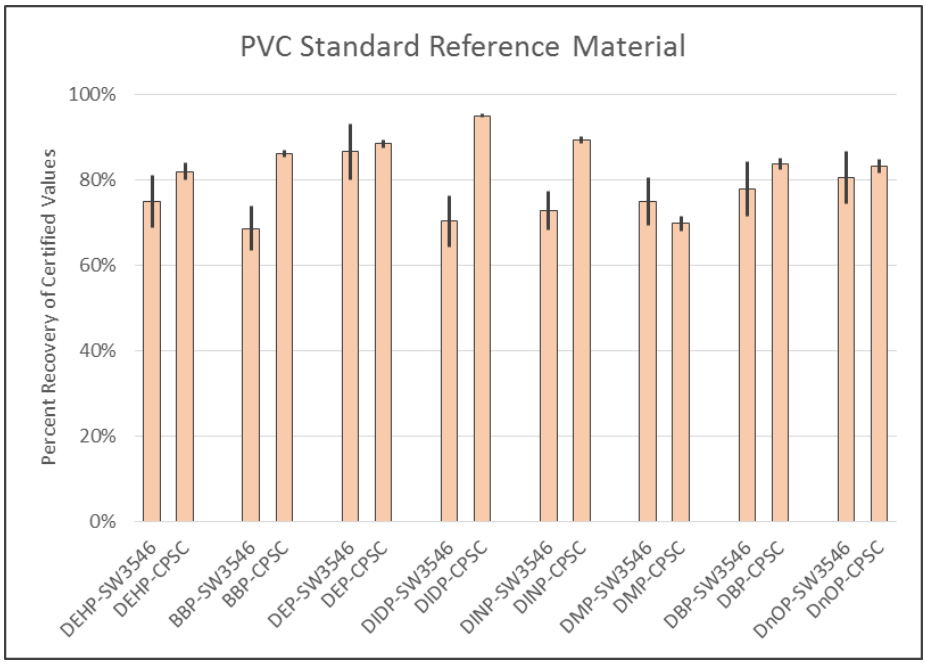

Figure 2. Percent Recoveries of the Phthalate Concentrations in PVC Standard Reference Material.

Bars and lines are means and standard errors, respectively. Suffix -SW3546 indicates Method EPA 3546/8270D; suffix -CPSC indicates Method CPSC-CH-C1001-09.3.

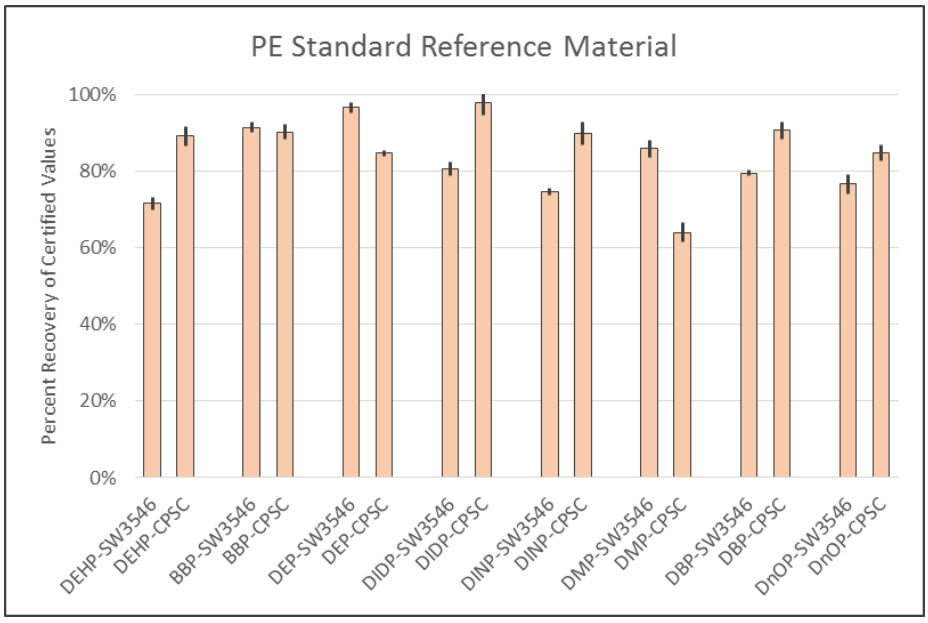

Figure 3. Percent Recoveries of Phthalate Concentrations in PE Standard Reference Material.

Bars and lines are means and standard errors, respectively. Suffix -SW3546 indicates Method EPA 3546/8270D; suffix -CPSC indicates Method CPSC-C1001-09.3.

Discussion

During 2014 and 2015, Ecology evaluated children's products for the presence of select chemicals from Washington State's CHCC list to support enforcement of the Children's Safe Products Act. During the process of this study, Ecology's product testing team identified a need to develop guidance for consistency in the preparation and analysis of consumer products.

Metals

Cryomilling is the current method used to prepare materials for metals analysis, although simpler methods, such as cutting to a fine size using hand scissors, may achieve the same results. In order to test for differences using the two preparation methods, five samples of fabric were analyzed for metals after each were prepared using both the cryomill and hand-cutting methods.

Results were not comparable for most tested metals, since concentrations were below reporting limits, as was the case in the original study. For antimony (detected in all five samples) and cobalt (detected in two of five samples), concentrations measured in samples prepared by using the hand-cutting method were slightly higher than those prepared by cryomilling. Within-sample precision (seven replicate analyses for each sample/prep method) was good for antimony and cobalt results (CVs <10% and ≤ 2%, respectively). Overall, the cryomilling method yielded slightly higher precision (CVs = 0.4 – 5.0%) than the hand-cutting method (CVs = 0.8 – 9.7%).

The similar analysis results yielded by these two preparation methods should not be taken as conclusive evidence that there is no difference between them. To begin, only one of the seven metals tested was consistently detected, bringing to question what the two methods might yield for detectable levels of other metals. Second, the sample size was too small to conduct any sort of hypothesis testing. Although 35 results were generated for each metal for each method, the sample size for each method was only five since replicate samples do not represent samples of a population. Therefore, parametric tests were not feasible since testing for distribution with such a small sample size has very little power, and even the non-parametric Wilcoxon test cannot be tested at a significance level smaller than α=0.20 (two-tailed). Additionally, the samples tested for comparison of these two methods were limited to fabrics. Testing for preference of one preparation method over another should include other materials such as plastic or metallic products.

Phthalates

For phthalates, testing of one product component (handbag) and two SRMs (one PVC and one PE) was done to compare methods conducted routinely by MEL with those published by CPSC. Differences are primarily concerned with the extraction process rather than sample preparation or analysis of the extract. All samples were prepared by cryomilling and all analyses were done using modifications of EPA 8270D (the SW3546 and CPSC methods use slightly different modifications of EPA 8270D). However, the extraction steps for the two methods involve different primary extraction solvents; the CPSC method uses tetrahydrofuran (THF) as the primary extraction solvent whereas EPA 3546 uses an acetone–hexane mix.

It is not clear if the two methods yielded substantially different extraction efficiencies, but the differences in reporting limits were notable. The SW3546 method had reporting limits 15 – 30 times lower than those using the CPSC method. This may have been partially due to a smaller initial sample weight for the CPSC method. The CPSC method states "For samples larger than 0.05 g, add 10 ml of THF for every 0.1 g of sample (or a reasonable amount to dissolve sample)." MEL followed the CPSC method extraction protocol using a proportional sample size to solvent ratio. Following the method in this manner, the best reporting limits are likely to be in the 150 mg/kg range.

Phthalates (cont.)

However, other laboratories may choose to use a larger sample size and add only as much solvent as needed to dissolve the sample, which may achieve lower reporting limits.

Results of the SRM analyses suggested the CPSC method provides better recoveries for most phthalates. However, SW3546 yielded a higher concentration of DEHP (the only phthalate detected) in the handbag sample. Precision using the CPSC method was higher for the PVC SRM, while SW3546 generally had higher replicate precision in the PE SRM analysis.

No conclusions about method preference can reasonably be drawn from the data. The samples analyzed are not a full representation of the consumer product materials available, and the SRM analysis does not demonstrate consistently superior bias and precision of one method over another. However, the higher reporting limits for the CPSC method could be a problem when determining compliance with regulatory thresholds if the CPSC extraction method is to be followed using a proportional sample size to solvent ratio.

CSPA legislation requires that manufacturers report the presence of phthalates in children's products at levels greater than practical quantitation limits defined by Ecology (5.0 – 50 mg/kg) if the chemical was intentionally added to the product, or at 100 mg/kg or higher if present as a contaminant. Washington State law RCW 70.240.020 prohibits the sale or distribution of a children's product or product component containing phthalates, individually or in combination, at more than 1,000 ppm. Therefore, reporting limits need to be low enough to assess compliance with state law.

Summary and Conclusions

- Hand-cutting fabric into small (2mm x 2mm) pieces yielded slightly higher antimony and cobalt concentrations than when the same samples were prepared by cryomilling. However, sample sizes were too small (n=5) to estimate the probability that the differences were due to chance. No conclusions could be drawn about differences in preparation methods for other metals since they were not detected (arsenic, cadmium, lead, mercury) or rarely detected (molybdenum).
- Overall concentrations of phthalates in SRMs were found to be higher when the extraction and analysis prescribed in Method CPSC-CH-C1001-09.3 was used compared with the method routinely used by MEL (EPA 3546/EPA 8270D with modifications). However, this was not the case for all phthalates, and EPA 3546/EPA 8270D yielded higher concentrations of DEHP in handbag material, the only phthalate detected in the sole consumer product tested. Due to the small sample size (n=3 total), hypothesis testing could not be done to determine if differences are statistically significant.
- Reporting limits for individual phthalates were 350 – 750 mg/kg using Method CPSC-CH-C1001-09.3 compared with ~25 mg/kg using EPA 3546/8270D. While MEL's reporting limits for Method CPSC-CH-C1001-09.3 may be lowered to around 150 mg/kg, this would not be sufficiently low to assess compliance with RCW 70.240.020. However, MEL's reporting limits using the CPSC method are based on using a proportional sample size to solvent ratio during the extraction process. Lower reporting limits may be achieved by increasing the sample size and adding only enough solvent to dissolve the sample.

Recommendations

This report makes the following recommendations for Ecology's Product Testing Program to consider:

- Expand the number, types of samples, and analytes used to compare the cryomilling and hand-cutting preparation methods. Samples should include materials known to contain detectable concentrations of metals other than (or in addition to) antimony and cobalt. If hand-cutting may be desirable for preparation of samples intended for organic chemical analysis, these types of samples should also be included in future studies.
- When testing products to evaluate compliance with CSPA legislation, laboratories should use an analytical method that meets minimum acceptance limits defined by the study's quality assurance project plan and achieves reporting limits low enough to assess compliance. Data presented in this report show that the method currently employed by MEL – EPA3546/8270D – may meet these criteria. The CPSC method also meets acceptance limits, but should be followed in a manner that achieves lower reporting limits (i.e., appropriate sample-size-to-solvent ratio during the extraction process).
- Additional sampling and analysis of phthalates using different sample materials and methods should be considered to assess method performance. An inter-laboratory comparison study including multiple laboratories using various methods is also recommended.

References

CPSC, 2010. Test Method CPSC-CH-C1001-09.3 - Standard Operating Procedure for Determination of Phthalates. Consumer Products Safety Commission, Directorate for Laboratory Sciences, Gaithersburg, MD.

Mathieu, C., 2015. Addendum 1 to Quality Assurance Project Plan: Chemicals of High Concern to Children in Children's Clothing, Footwear, and Accessories. Pub. No. 15-03-114. Washington State Department of Ecology, Olympia, WA. https://fortress.wa.gov/ecy/publications/SummaryPages/1503114.html

Mathieu, C. and S. Sekerak, 2015. Chemicals of High Concern to Children in Children's Clothing, Footwear, and Accessories. Pub. No. 15-03-039. Washington State Department of Ecology, Olympia, WA. https://fortress.wa.gov/ecy/publications/SummaryPages/1503039.html

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Websites

Children's Safe Products Act: http://www.ecy.wa.gov/programs/hwtr/RTT/cspa/index.html

List of Chemicals of High Concern to Children: http://www.ecy.wa.gov/programs/hwtr/RTT/cspa/chcc.html