Status Report

Review of Metals in the Toy Safety Standard,
ASTM F 963
03/14/2012

Dominique W. Johnson, MPH, Toxicologist
Directorate for Health Sciences
Phone: 301-504-7597
E-mail: djohnson@cpsc.gov

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Status Report

UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
4330 EAST WEST HIGHWAY
BETHESDA, MARYLAND 20814

Memorandum

Date: 03/14/2012

TO : Mary Ann Danello, Ph.D., Associate Executive Director, Directorate for Health Sciences

THROUGH: Lori E. Saltzman, M.S., Director, Division of Health Sciences

FROM : Dominique W. Johnson, MPH, Toxicologist, Division of Health Sciences

SUBJECT : Status Report: Review of Metals in the Toy Safety Standard ASTM F 963[1]

I. Background

On August 14, 2008, the President signed into law the Consumer Product Safety Improvement Act (CPSIA) of 2008 (P.L. 110-314). Section 106(d)(1) of the CPSIA requires the U.S. Consumer Product Safety Commission (CPSC or the Commission) to ". . . examine and assess the effectiveness of ASTM F 963 . . . and shall assess the adequacy of such standards in protecting children from safety hazards. . . ."

The toy safety standard, ASTM F 963-07, was made a mandatory CPSC standard by the CPSIA. Under ASTM F 963-07, section 4.3 provides migration limits for eight heavy metals that may be in toy materials. These metals are: Antimony (Sb), Arsenic (As), Barium (Ba), Cadmium (Cd), Chromium (Cr), Lead (Pb), Mercury (Hg), and Selenium (Se).

In an effort to begin assessing the effectiveness of the requirements for heavy metals in the toy safety standard, CPSC staff contracted with Versar, Inc., to review the toxicity literature from the years 2000 to 2010, on all of the metals listed in the standard, except lead,[2] and to evaluate any toxicity data that may influence the determination of the effectiveness of the current safety limits. In addition, Versar, Inc., was asked to develop an acceptable daily intake (ADI) value for each metal. The ADI is the amount of a chemical that a person may be exposed to on a daily basis without the chemical posing a significant risk of adverse health effects. An ADI is based on the study reporting the lowest exposure levels associated with adverse effects or a dose that was not associated with an adverse effect and is estimated using specified uncertainty factors to account, for example, for extrapolation from animal studies to humans or other deficiencies in knowledge.

[1] These comments are those of the CPSC staff and have not been reviewed or approved by, and may not necessarily reflect the views of, the Commission.
[2] Lead was excluded because it is addressed separately in section 101 of the CPSIA.

II. Discussion

A. Derivation of ASTM F 963-07 Values

CPSC staff believes that the migration limits in ASTM F 963-07 came directly from the European Standard EN 71-3, based on the following information reviewed by staff. A report by the Netherlands National Institute for Public Health and the Environment (RIVM, 2006), indicates that permissible levels of bioavailable elements from toys were derived by the Science Advisory Committee in the European Union (EU) in 1985. These permissible levels were then specified in the EU's Toy Safety Directive (Council Directive 88/378/EEC, 1988) and subsequently incorporated into European Standard EN 71-3 (European Standard EN 71-3, 1994). As documented in the RIVM (2006) report, the permissible levels were derived from estimated adult weekly dietary intake levels for each element. The adult dietary intake level was then reduced by half to estimate a child's dietary intake. An additional adjustment was made to derive the allowable daily intake level that may be contributed by toys.[3] This adjustment was between 0.1 and 10 percent of the estimated children's dietary intake levels. These permissible intake levels were converted to migration levels in milligrams per kilogram (mg/kg) of toy material, with the assumption of a daily intake of 8 mg of toy material (8 mg/day), and assuming an average weight of a child to be 12 kg.[4]

CPSC staff believes that the values for the migration limits in ASTM F 963-07 come directly from the European Standard EN 71-3, based in part on the fact that the limits specified in ASTM F 963-07 and EN71-3 are identical. It should be noted that the scope of the migration limits of the two standards differ in that ASTM F 963-07 currently applies only to paints and surface coatings, while EN 71-3 applies to toy substrate materials, such as plastic and metal, in addition to paints and surface coatings.

B. Steps Taken by CPSC Staff

In order to compare the existing values in ASTM F 963-07 and those derived from Versar, Versar was charged with reviewing only non-cancer data for all the metals. This is because the reports that formed the basis for ASTM F 963, the Netherlands National Institute for Public Health and the Environment (RIVM, 2006, and the previous European Toy Safety Standard (Council Directive 88/378/EEC, 1988), derived their values from non-cancer endpoints.

Staff systematically analyzed the ADIs developed by Versar, compared the derived values to the permissible intake levels (ingestion levels that have been determined to pose little to no risk of adverse effects) provided in the EU's toy safety directive, and drafted a staff document that addressed the comparison to each chemical. Because the values from Versar and the EU directive were derived under different assumptions and procedures, staff adjusted the derived ADIs in the same way that the EU's limits were handled, in order to compare the "existing"

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[3] The reduction of the dietary intake value by half, and reduction to between 0.1 and 10 percent of the value to derive the contribution of toys to the dietary intake level, appear to be based not on any specific data, but rather, on assumptions deemed reasonable by the report's authors. CPSC staff found insufficient information in the RIVM report for evaluating the validity of these assumptions.

[4] The assumed 12 kg body weight for children was reported in the RIVM (2006), but was not further documented. Twelve kg is equivalent to about 26.5 pounds, and using reasonably current data, corresponds to children who are approximately 1 year old (Ogden *et al.,* 2004).

ASTM F 963 values with "new" values. Staff converted the Versar ADIs to daily intake levels in milligrams per day (mg/day) by applying the same percentage of allowed daily intake for exposure from toys as was done in the European analysis. Thus, staff multiplied the ADI by the percent allowed for toys (0.1 %–10%), and multiplied the result by the average weight of a 1-year-old child (12 kg).

Staff recognizes that the use of different approaches could result in different values. CPSC staff and contractors used a risk-based approach. A risk-based approach to deriving ADIs is one in which well-conducted toxicity and epidemiology studies are used to identify the lowest exposure doses that are associated with adverse health effects. The dietary intake approach that forms the basis of the EU toy safety standard and the current ASTM F 963-07 standard, considers data about typical exposures to the chemicals of interest within the population and then, through assumptions and adjustments, derives permissible daily limits.

These approaches have clear differences. For example, the dietary approach looks at typical exposures, not the possible adverse effects that may occur from those exposures, and does not adjust the values using uncertainty factors. The ADI is defined as the amount of a chemical that a person may be exposed to on a daily basis without the chemical posing a significant risk of adverse health effects. The dietary approach can, but does not necessarily, result in an exposure level that is associated with adverse effects (*i.e.*, typical dietary exposures may actually be associated with a risk for adverse effects). Further, the dietary approach may not account for specific forms of a chemical that are likely used in toys or other products and that may have differing levels of toxicity. However, the goal in this case was to make the same adjustments to a derived-exposure limit, regardless of the method used to derive it, in order to facilitate the comparison of new values with the existing values in ASTM F 963-07.

In an effort to determine the migration of metals from toys, CPSC staff also requested that the contractor review current data showing migration of metals from children's toys. However, little or no data for metals used in toys or migration of metals from children's toys was found, see VERSAR report in Tab A. This lack of data was also noted in the RIVM report (2006). Staff believes that additional testing will be required to identify the use of metals in children's toys and to quantify the potential migration of the metals from children's toys.

In February 2011, CPSC staff initiated an external scientific peer review of the staff document and the Versar contract report related to the review and analysis of the metals in the ASTM F 963-07 toy safety standard. Two reviewers were selected by Versar, and three reviewers from other U.S. federal agencies were selected by CPSC staff. Tab C summarizes the peer review comments and provides CPSC staff's responses to those comments. There were 61 comments received during the peer-review process. Comments were in the areas of general information and understanding, key studies and endpoints used, comparison of dietary and risk-based approaches, the use of uncertainty factors, relating findings to recommended dietary amounts, and other current standards for heavy metals. Staff revised the CPSC staff metals document (Tab B), as appropriate, based on the peer-review comments. The Versar contract report did not require any revisions.

Attached is the final report provided by Versar, Inc., on four of the metals of interest: arsenic (As), chromium (Cr), mercury (Hg), and selenium (Se) (Tab A).[5] The remaining metals included in ASTM F 963 were not included in the review by Versar for the following reasons:

- Lead is addressed separately in section 101 of the CPSIA.
- Initial review of available information for antimony and barium indicated a lack of new data. Thus, a full review by Versar was not necessary. Instead, staff developed its own reports for these two elements (Tab B).
- As part of other agency work on cadmium, staff prepared its own review and analysis for this element (Williams, 2010). Included in this status report is a summary of that review and analysis (Tab B).

III. Conclusion

CPSC staff used the information developed by Versar, peer reviewers' comments, and existing staff assessments to derive intake limits using recent toxicity data for each of the metals listed in ASTM F 963.

Table 1 contains a comparison of the existing intake limits in the EU Toy Safety Standard and ASTM F 963, with the corresponding intake limits based on the ADIs developed by Versar or CPSC staff. Many of the derived intake limits are lower than the existing ASTM F 963-07 intake limits, although antimony and barium limits are higher. This is due, in part, to the different sources of data, as well as different assumptions and procedures that were used to derive the values, as discussed above.

Table 1: Current F 963 Intake Limits and Calculated Values Resulting from Use of Modifying Factors

	Existing Standard ASTM F 963	**Values Resulting from Current Review**	
Metal	**Intake Limit (µg/day)**	**ADI (µg/kg/day)**	**Intake Limit* (µg/day)**
Antimony (Sb)	0.2	6	7.2
Arsenic (As)	0.1	0.03	0.00036
Barium (Ba)	25	200	120
Cadmium (Cd)	0.6	0.1	0.06
Chromium (Cr)	0.3	0.4	0.05
Mercury (Hg)	0.5	0.001	0.001
Selenium (Se)	5	1	1
*Includes all modifying factors used in the derivation of the current ASTM F 963-07 standard.			

The dietary approach used to derive the ASTM F 963-07 intake limits is not the approach normally used by staff. Rather than adjusting the derived exposure limit with an additional factor, staff would base the daily intake limit on the derived ADI and the weight of a child. Thus, using the ADIs from the current review, and the weight of a small child (*i.e.*, 12 kg; same as the current standard), staff estimated the corresponding intake limits. Table 2 shows the intake limits derived using staff's preferred approach.

[5] All comments and recommendations received during CPSC clearance and review will be reviewed and addressed by Versar.

Table 2: Current F 963 Intake Limits and CPSC's Calculated Intake Limits

Metal	Current ASTM F 963 Intake Limit (µg/day)	ADI (µg/kg/day)	Intake Limit[#] (µg/day)
Antimony (Sb)	0.2	6	72
Arsenic (As)	0.1	0.03	0.36
Barium (Ba)	25	200	2400
Cadmium (Cd)	0.6	0.1	1.2
Chromium (Cr)	0.3	0.4	4.8
Mercury (Hg)	0.5	0.001	0.012
Selenium (Se)	5	1	12
#Derived using standard CPSC staff approach.			

With the exception of mercury, the existing ASTM intake limits are considerably lower (*i.e.*, more protective) than the limits from the current analysis. Staff supports additional consideration of the ASTM F 963 heavy metal limits, particularly for mercury because the current analysis suggests the need for reducing the mercury limit. One of the issues that would need to be considered concerns the test method and whether limitations exist as to the levels of mercury that can be detected. Another consideration is the current status of the use or presence of mercury or mercury compounds in children's toys. Staff's review of limited testing data shows that mercury or mercury compounds are not currently being used in the manufacture of children's toys. Thus, the use of mercury in children's toys is not an immediate concern, and implementing a change to the mercury limit in the standard would not necessarily increase the health protectiveness of the standard. Therefore, staff finds that the existing intake limits of ASTM F 963-07 are sufficiently protective of children using toys that conform to the standard.

CPSC staff is aware that the European Union (EU) released an updated toy safety directive in 2009, and that the changes to the European toy safety standards pertaining to heavy metals are to become effective in 2013. The updated EU toy safety directive will include significant changes to the intake levels for heavy metals (see Appendix B table 8). CPSC staff has worked closely, and will continue to work closely with ASTM on revisions to the ASTM F 963 toy safety standard, as well as consider information from new or updated international standards. Staff will take into consideration the interests in harmonizing with other international standards, in addition to providing an appropriate level of safety for consumers.

As an example of the cooperation between ASTM and staff, CPSC staff recently participated in a working group under the ASTM F15.22 toy safety subcommittee that was charged with developing an amendment to the ASTM F 963-07 toy safety standard to address cadmium in toys. The working group considered changes to the standard that would expand the requirements for toys with cadmium and other chemicals. The revised standard was approved by ASTM on December 1, 2011, and published shortly thereafter.

The revised standard includes certain requirements for cadmium in toys, as well as the other chemical elements that previously had only been restricted in paints and surface coatings. In addition to the limits for paints and surface coatings, the revised standard limits these chemicals in plastics, metal, glass, and ceramic toys and parts of toys. These new requirements align with the similar portions of the European EN 71-3 toy safety standard. CPSC staff supports the changes made during the ASTM F 963 subcommittee review process and actively encouraged expanding the scope of the heavy metals section to include materials other than paints and coatings. Products subject to this new section of the standard include toys and parts of toys that

are intended to be mouthed, toys for children under 6 years of age, and toys and parts of toys that are small parts (*i.e.*, that fit into the test fixture specified at 16 CFR part 1501).

This Status Report is part of the staff's congressionally mandated activity of examining and assessing the effectiveness of the ASTM F 963. The 2007 version of the F 963 standard was the current version at the time of this mandate. Since the ASTM work and staff's activities related to this Status Report were done concurrently, the analyses presented here could not be considered by the ASTM subcommittee in development of the ASTM F 963-11. As discussed above the staff finds that the existing intake limits of ASTM F 963-07 are sufficiently protective of children using toys that conform to the standard. Consequently, staff also finds the migration limits of F 963-11 are sufficiently protective of children since the limits did not change. In fact, staff finds F 963-11, with respect to the heavy metals, to be a more robust standard because it expands the types of products and materials subject to the standard.

While the revised standard includes a number of new requirements, staff and the working group focused on cadmium content or the potential for exposure to cadmium because of the recent work by CPSC staff, which showed the potential for harm to children who are exposed to excess cadmium. The new requirements address the potential for harm from chronic exposure to cadmium (*i.e.*, exposures that occur over time, such as from repeated mouthing of a product by a child), and acute exposures to cadmium (*i.e.*, exposures that happen within a very short period of time, such as when a child swallows a small part of a toy).

IV. **Staff Recommendation**

Staff finds that the existing intake limits in ASTM F 963-07 and EN 71-3 are sufficiently protective of children who use toys that conform to the current standard. In developing their standard, the EU did consider known toxicity information about the elements being reviewed. Using this information, policy makers chose to reduce the percentage contribution of a particular element in a toy to the daily intake for that element. Although their methods were based on average daily intake, CPSC staff believes that the use of toxicity information to make adjustments in developing their standard provides sufficient protection from adverse effects due to toxicity from metals in toys. The current levels in ASTM F 963-07 generally provide a lower limit compared to the intake limits that resulted from the ADIs derived by CPSC staff and the contractor, using the risk-based approach. While the revised standard is based on the existing intake limits, the requirements have been expanded to include more products. CPSC staff supports the changes made to the ASTM F 963. Therefore, at this time, staff is not recommending any changes to the migration limit section of ASTM F 963-07 (section 4.3), but we do recommend that the Commission and staff continue to work closely with ASTM on improvements to the toy safety standard and continue to assess any changes in ASTM or other international safety standards.

Works Cited

Council Directive 88/378/EEC (1988) On the approximation of the laws of the Member States concerning the safety of toys. Official Journal L 187, 16/7/1988, P 1–13. 3 May 1988.

European Standard EN 71-3 (1994) Safety of Toys—Part 3: Migration of certain elements.

National Institute for Public Health and the Environment (RIVM). (2006). *Chemicals in Toys RIVM/SIR Revised Advisory Report 0010278A02.* The Netherlands: RIVM.

Ogden CL, Fryar CD, Carroll MD, Flegal KM (2004) Mean bodyweight, height, and body mass index, United States 1960–2002. Advance data from vital and health statistics; no. 347. Hyattsville, Maryland: National Center for Health Statistics.

Williams DJ (2010) Toxicity review of Cadmium. U.S. Consumer Product Safety Commission, Bethesda, MD 20814. August 2010

TAB A: Versar Report on ASTM F-963 Heavy Metals

TAB A

FINAL DRAFT

REVIEW OF TOXICITY DATA AND ASSESSMENT OF SEVEN METALS

Contract No. CPSC-D-06-0006
Task Order 008

Prepared for:

Ms. Dominique Williams
U.S. Consumer Product Safety Commission
4330 East West Highway
Bethesda, MD 20814

Prepared by:

Versar, Inc.
Exposure and Risk Assessment Division
6850 Versar Center
Springfield, VA 22151

and

SRC, Inc.
7502 Round Pond Road
North Syracuse, NY 13212

July 16, 2010

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1.0 INTRODUCTION

1.1 PURPOSE

This document provides a summary of the most recent toxicological data and a toxicity assessment for the metals identified in the ASTM International-implemented soluble metal content restrictions in the F963 voluntary standard for toy safety [except for lead]. These metals are the following: antimony, arsenic, barium, cadmium, chromium, mercury, and selenium. This document provides toxicity assessments for arsenic, chromium, mercury and selenium, only. Toxicity assessments were not developed for antimony because of the lack of recent data. In addition cadmium was not assessed because the Consumer Product Safety Commission (CPSC or Commission) staff prepared that assessment because it required a more in-depth review than what is provided for the other four metals. Because there is potential ingestion exposure to the soluble migratable metals, recent data on the migration of the selected metals from toys are also provided in this document, where available. The primary purpose of the review was to determine if recent toxicological data are available that may justify derivation of acceptable daily intakes (ADIs) that might alter the current soluble metals standard.

1.2 BACKGROUND

Using the European Union's EN 71-3 Safety of Toys standard as a guideline, the ASTM-International implemented soluble migratable metal restrictions in the F963 voluntary standard for toy safety. The standard assumes repeated exposure by ingestion of 8 mg/day (mean value) of toy material. The permissible levels of bioavailable elements from toys were derived in a 1985 advice by the Scientific Advisory Committee in the European Union and were based on adult weekly dietary intakes (RIVM, 2006). The EN 71-3 levels were converted to migration limits in mg/kg of toy material, assuming that a child ingests 8 mg of toy material per day (RIVM 2006). Routes of ingestion of toy material include: direct ingestion and also licking, sucking, mouthing, and hand-to-mouth behavior; these activities could result in the migration of the metals from the toy material matrix thru saliva.

In addition to the EU standard established in 1985, RIVM (2006) developed a risk-based methodology to assess the safety of exposure to chemicals in toys to derive a more health-based standard for toys. The emphasis of the methodology was inorganic elements in toys intended to be put in the mouth. RIVM (2006) noted that their proposed methodology can be used to derive migration and content limit values for elements in toys and to perform safety assessments of elements in toys. RIVM (2006) derived new migration limit values for 16 elements, including those metals addressed in this document, and recommended proposed migration limit values. A new European Union Toy Safety Directive (2009) gives consideration to the proposed migration

limits of RIVM (2006). The Directive was adopted on July 19, 2009, but it does not come into full effect until July 19, 2013.

Under the Consumer Product Safety Improvement Act (CPSIA) (2008), F963 was adopted as a rule by the Consumer Product Safety Commission and became a mandatory safety standard in February 2009 (CPSIA, 2008). The CPSIA also required the Commission to evaluate the adequacy of the requirements that address hazards associated with small magnets; toxic substances; toys with spherical ends; hemispheric-shaped objects; cords, straps, and elastics; and battery-operated toys. Section 4.3.5.2 of F963, pertaining to toxic substances, prohibits the use of seven metals (in addition to lead) in paint and similar surface-coating materials when the soluble metal content exceeds levels ranging from 25 ppm to 1000 ppm. The specific level for each metal is provided later in Section 2.0. Under the CPSIA, lead is restricted by a total content limitation. In consideration of this, and to begin evaluating Section 4.3.5.2 for possible update and incorporation of more recent scientific data, the Commission staff initiated efforts to obtain updated information on metal toxicity.

1.3 APPROACH

The objectives of this effort were to review and assess the toxicological data on the metals identified in ASTM F963 specified above to determine if there were potential updates or needed changes to the CPSC's mandatory standard. To achieve these objectives, the following sequential steps occurred:

- A literature search was conducted to identify available data from the year 2000 to present on the chosen metals (in inorganic form) for acute and chronic toxicity (repeated exposure oral toxicity) studies, and data relating to migration from products, toxicological effects, and suggested maximum daily intake, focusing primarily on toxicity information relating to human health. The primary databases searched were PubMed and the Agency for Toxic Substance and Disease Registry (ATSDR) toxicological profiles and chemical evaluation documents. The ATSDR toxicological profiles and chemical evaluation documents were used as a starting point for the search. Other databases, such as TOXLINE, were used to augment the primary data search.

- The literature search results (titles, authors, abstracts, and other citation information) were incorporated into a separate EndNote database for each metal. Approximately 13,000 references were identified.

- The abstracts in the EndNote databases were reviewed to determine if they included data that potentially could affect the ADIs for each metal. The references were primarily grouped in the metals' respective EndNote databases according to "All References"; "May Impact" (references that potentially could affect the acceptable daily intake (ADI)); and "Key Studies." These grouping are shown by metal in Table 1-1.

Table 1-1. Number of References Identified by Metal

	All References	May Impact	Key Studies
Antimony	326	25	1
Arsenic	3018	197	30
Barium	469	16	0
Cadmium	2186	158	14
Chromium	1285	73	15
Mercury	4784	33	6
Selenium	3141	76	7

- Copies of the full references were retrieved for any reference that was determined to possibly impact the ADI, and PDFs of the references were incorporated into the EndNote database.

- The "May Impact" studies were reviewed to select the key studies—the ones believed likely to influence a change in F963, Section 4.3 mandatory standard of the CPSC.

- Summaries of key studies were prepared for each metal and are provided in Appendix A.

- The key studies were reviewed for use in the toxicity assessment and potential derivation of ADIs based on new/current non-cancer dose-response data and the No Observed (Adverse) Effect Levels (NO(A)EL) data found. NOAEL and Lowest Observed Affect (LOAEL) values were identified, and the ADIs for the metals were developed using the NOAEL and LOAEL approach based on the supporting data. The ADI development for chromium and mercury was based on animal toxicity data and for arsenic and selenium was based on human epidemiology data.

- Toxicity assessments and ADIs were developed for four metals (arsenic, cadmium, mercury, and selenium) and are provided herein. Toxicity assessments and ADIs were not developed for barium and antimony because no new data were found that potentially could influence the ADI. In addition cadmium was not assessed because the CPSC required a more in-depth review than what is provided in this document for the other four metals.

1.4 ORGANIZATION

The sections of this document are organized as follows:

- Section 2 provides information on migration of the selected metals from products, specifically toys.
- Sections 3, 4, 5, and 6 provide the available recent toxicity information and a calculated ADI based on the available supporting data for arsenic, chromium, mercury, and selenium, respectively.
- Appendix A provides summaries of each article that was selected as a key study.

1.5 REFERENCES

RIVM. (2006). Chemicals in toys: a general methodology for assessment of chemical safety of toys with a focus on elements. RIVM/SIR Revised Advisory Report 0010278A02. Revised Final Version, October 12, 2006.

CPSIA. 2008. Consumer Product Safety Improvement Act. Bethesda, MD: U.S. Consumer Product Safety Commission.

SECTION 2.0 MIGRATION OF METALS FROM TOYS

2.1 BACKGROUND

ASTM International, formerly known as the American Society for Testing and Materials ASTM), has provided the maximum amount of soluble migrated element from toy material in F963, Section 4.3.5 as a provision for toy safety. The values from F963 are shown below.

Table 2-1. Current F963 Migration Limit for Each Metal

Element	Migration Limit (mg/kg product)
Antimony (Sb)	60
Arsenic (As)	25
Barium (Ba)	1000
Cadmium (Cd)	75
Chromium (Cr)	60
Mercury (Hg)	60
Selenium (Se)	500

2.2 RECENT MIGRATION DATA

A literature search was conducted to obtain the most recent data (year 2000+) on the migration of the identified seven metals from products, specifically toys. Minimal data were found for studies conducted to determine leaching of metals from products such as ceramic ware, jewelry, crystal glassware, plastic bottles, and stainless steel cookware.

Recent data on the migration of metals from toys and/or levels of metals in toys were identified in studies that were conducted in Japan and India. These studies are briefly summarized and presented below.

Kawamura, et al. (2006) investigated levels of 8 metals (antimony, arsenic, barium, cadmium, chromium, lead, mercury, and selenium) in 45 baby toys and 10 paints. The toys were made of polyvinyl chloride. Kawamura, et al. (2006) reported that the samples contained the following levels:

- Barium (0.3–3,700 mg/kg) (all samples);
- Cadmium (0.2–26 mg/kg) (several samples);
- Chromium (0.5–280 mg/kg) (several samples);
- Lead (1.5 – 1,300 mg/kg); and
- Antimony (5.3 mg/kg) (1 sample).

Additionally, the samples were evaluated using the migration test of International Organization for Standardization, ISO 8124-3 (ISO, 1997). Barium, cadmium, chromium, and lead migrated from some of the samples but at limits lower than the migration limits required by the ISO 8124-3 Method (Safety of Toys: Part 3; Migration of Certain Elements)(Kawamura, et al., 2006). It should be noted that the summary of Kawamura, et al. (2006) is from the abstract

and not the article. The article text is provided only in Japanese. Actual data values were provided in tables, but text describing the tables was provided in Japanese only.

Kawamura, et al. (2009) compared the migration tests of cadmium and lead from paint film on baby toys set out in the Japanese Food Sanitation Law (official standard) and ISO 8124-3. Vinyl chloride resin enamel and acrylic resin enamel containing 1,000 mg/kg cadmium and lead on a dried basis were painted on glass plates, soaked in water, and tested. Cadmium and lead were found to be below the limit of determination (<0.1 µg/mL) (Kawamura, et al., 2009). However, when the solvent was changed to four percent acetic acid or 0.07 mol/L HCl, 0.3-2.3 µg/mL cadmium and lead migrated from the acrylic resin enamel, but no migration was observed from the vinyl chloride resin enamel (Kawamura, et al., 2009). The tests were conducted according to the ISO method, where the paint was scratched from the glass plates and the powder was soaked in 0.07 mol/L HCl at 37 degrees C for 1 hour with and without shaking. The migration of Cd and Pb reached 310 to 910 mg/kg (i.e., 3.5–12 times more than the migration limits). Cadmium migrated more extensively than lead, and both migrated more readily from the acrylic resin enamel than from the vinyl chloride enamel (Kawamura, et al., 2009). It should be noted that this summary is based on the abstract provided for an article that is written in Japanese.

Kumar and Pastore (2006) investigated levels of lead and cadmium in soft toys in India. This investigation was conducted because a large number of toys are imported into India with little or no quality control and because the small children and infants chew and play with soft plastic toys (Kumar and Pastore, 2006). A total of 111 non-branded PVC toys and soft toys were randomly purchased in cities in India and tested to determine lead and cadmium levels at the National Accreditation Board for Testing and Calibration Laboratories in India. Of the 111 total samples, 77 PVC and 11 non-PVC samples were analyzed for cadmium and lead. Both metals were found to be present in all samples at varying concentrations. Kumar and Pastore (2006) reported that average cadmium concentrations ranged from 15.71–26.53 ppm with maximum concentrations ranging from 11.6–188 ppm. The minimum concentrations ranged from 0.03–0.16 ppm.

2.3 REFERENCES

ISO. (1997). ISO Standard 8124-3 Safety of Toys Part 3: Migration of Certain Elements, International Organization for Standardization, 1997.

Kawamura Y., C. Kawasaki, S. Mine, M. Mutsuga et al. (2006). Contents of eight harmful elements in baby toys and their migration tests. Food Hyg. Saf. Sci., 47, 51–57.

Kawamura Y, M. Mutsuga, T. Yamauchi T, S.Ueda, K. Tanamoto et al. (2009). Migration tests of cadmium and lead from paint film of baby toys. Food Hyg. Saf. Sci., 50, 93–96.

Kumar A., P. Pastore. (2006). Toying with toxics. An investigation of lead and cadmium in soft toys in three cities in India. Toxics Link: Delhi, Mumbai, Chennai, India (August 2006). New Delhi–110014, India. (www.toxicslink.org)

3.0 REVIEW OF SELECTED KEY STUDIES AND TOXICITY ASSESSMENT FOR ARSENIC (As)

3.1 SUMMARY

The existing standard for arsenic is 0.1 µg As/day from toys, which corresponds to a daily dose of 8E-06 mg As/kg-day from toys for a 12 kg child. An ADI of 3E-05 mg As/kg-day is derived herein based on a chronic LOAEL of 0.001 mg As/kg-day for skin lesions in an exposed human population and an uncertainty factor of 30 to adjust from a LOAEL to a NOAEL (10) and protect sensitive populations (3). Applying the same modifying factors that were used to derive the existing standard, the permissible intake from toys based on this ADI would be 0.00036 µg As/day. This comparison suggests that the ADI would be approximately three hundredfold lower than the existing standard.

3.2 EXISTING STANDARD FOR ARSENIC

Existing standards for children's intake of metals from toys, originally published in EU 12964 EN and EN 71-3, are based on estimated levels of metals in the diet and allowable relative source contributions from toys, ranging from 0.1–10 percent based on the chemical's toxicity (RIVM, 2006). For arsenic, the existing standard is 0.1 µg As/day from toys, based on an assumed children's dietary intake of 700 µg As/week (calculated as 50 percent of the measured adult intake of 1400 µg As/week), and an allowable contribution from toys of 0.1 percent (700 µg As/week ÷ 7 days/week × 0.1% = 0.1 µg As/day) (RIVM, 2006). The allowable contribution for arsenic from toys was reduced to 0.1 percent from the starting value of 10 percent due to known carcinogenicity of arsenic by the oral route. Assuming a body weight of 12 kg (RIVM, 2006), the permissible intake of 0.1 µg As/day corresponds to a daily dose of 0.008 µg As/kg-day or 8E-06 mg As/kg-day from toys.

3.3 REVIEW OF SELECTED KEY STUDIES FOR ARSENIC

RIVM (2006) reviewed the existing toxicity data and assessments available for arsenic. Arsenic can occur in various organic and inorganic forms that differ in physical and chemical properties, occurrence, and toxicity. While organic forms of arsenic exhibit very low toxicity, numerous epidemiological studies have identified adverse health effects associated with ingestion of inorganic arsenic in human populations. For the current assessment, epidemiological literature on the non-cancer effects of inorganic arsenic published since 2000 was reviewed. The database includes a large number of studies, many of which quantified exposure to inorganic arsenic in the exposed populations. Some of these studies were designed to assess traditional endpoints associated with arsenic exposure (e.g., dermal lesions), but several

of the studies evaluated other endpoints (e.g., intellectual ability, blood pressure, heart disease mortality), including reproductive and developmental endpoints (e.g., neonatal and infant death, spontaneous abortion, stillbirth, anemia during pregnancy, erectile dysfunction). The selected studies and their associated NOAEL/LOAEL values, where applicable, are summarized in Table 3-1. The most sensitive effects generally were found at similar levels (within roughly an order of magnitude) in all studies (with midpoints of NOAEL and LOAEL ranges varying from 0.0006–0.006 and 0.001–0.016 mg As/kg-day, respectively). LOAELs for increased risk of skin lesions were observed in several studies at estimated midpoint doses ranging from about 0.001 to 0.004 mg As/kg-day (Ashan et al., 2006; Rahman et al., 2006: McDonald et al., 2006; 2007; Guo et al. 2006 a, b). LOAELs for cardiovascular effects were 0.001 mg As/kg-day for increased blood pressure (Kwok et al., 2007) and ≥ 0.009 mg As/kg-day for increased risk of heart disease mortality (Wade et al., 2009). Other studies identified LOAELs for: decreased intellectual function (0.007 mg As/kg-day, Wasserman et al., 2004); increased risk of infant or neonatal death (0.006 mg As/kg-day, Milton et al., 2005; 0.016 mg As/kg-day, Rahman et al., 2007; > 0.001 mg As/kg-day, Myers et al., 2009); increased risk of stillbirth (≥ 0.004 mg As/kg-day, Cherry et al., 2008); increased prevalence of anemia in pregnant women (0.002 mg As/kg-day, Hopenhayn et al., 2006); and increased risk of erectile dysfunction (>0.004 mg As/kg-day, Hsieh et al., 2008).

The most reliable estimates of exposure are from the Ahsan et al. (2006) and Rahman et al. (2006) studies of dermal lesions, which estimated mean chronic (≥5 years) arsenic drinking water exposure for each individual, and the Rahman et al. (2007) study of pregnancy outcome, which estimated exposure of each individual specifically during pregnancy. All of these were large studies that grouped subjects by exposure quintile, and two of them (Ahsan et al., 2006; Rahman et al., 2007) reported median exposure levels for each quintile. Taking the median value (or midpoint where the median was not reported) as representative of the quintile, the Ahsan et al. (2006) and Rahman et al. (2006) studies reported LOAEL values of 0.001 and 0.002 mg As/kg-day, respectively, for increased risk of skin lesions (melanosis and/or hyperkeratosis). A NOAEL was not identified in either study. The LOAEL of 0.001 mg As/kg-day was the lowest in the database, along with the study by Kwok et al. (2007) that reported increased blood pressure in postpartum women at the same estimated exposure level (also without a NOAEL). In comparison, the Rahman et al. (2007) study of pregnancy outcome found effects only at higher doses. In this study, increased risk of infant death was the most sensitive endpoint, with a NOAEL of 0.006 mg As/kg-day and LOAEL of 0.016 mg As/kg-day.

3.4 TOXICITY ASSESSMENT FOR ARSENIC

An ADI for inorganic arsenic can be derived from the chronic LOAEL of 0.001 mg As/kg-day for skin lesions (Ahsan et al., 2006). There is abundant evidence in the reviewed studies, as well as the older literature, for skin lesions as a sensitive endpoint for inorganic arsenic. There is also a large database of supporting studies for other effects, including adverse reproductive outcomes, at only slightly higher doses. A NOAEL for dermal lesions was not identified in the Ahsan et al. (2006) study. Taking the LOAEL of 0.001 mg As/kg-day (Ahsan et al., 2006) as the point of departure (POD), and applying an uncertainty factor of 30 (10 for adjustment from a LOAEL to a NOAEL and 3 for human variability), results in an ADI for inorganic arsenic of 3E-05 mg As/kg-day. Use of a partial uncertainty factor of 3, rather than a full factor of 10 to protect sensitive individuals, is consistent with treatment of the older data in existing assessments by the U.S. Environmental Protection Agency (1993) and ATSDR (2007).

3.5 COMPARISON OF ADI TO EXISTING TOY STANDARD FOR ARSENIC

The ADI of 3E-05 mg As/kg-day derived here applies to total daily intake of inorganic arsenic from all sources. The existing permissible intake for arsenic in the European toy safety standard is 0.1 µg As/day, which refers specifically to intake from toys. In order to compare these, we applied the same modifying factors that were used to derive the toy standard. Therefore, a source allocation of 0.1 percent was applied to the ADI and a bodyweight of 12 kg was used to calculate a permissible intake level that is directly comparable to the existing value (3E-05 mg As/kg-day × 0.1% x 12 kg = 3.6E-07 mg As/day from toys). Comparison of the permissible intake of arsenic from toys based on the ADI derived here (3.6E-07 mg As/day or 0.00036 µg As/day) and the existing permissible intake from toys (0.1 µg As/day) suggests that the ADI would be approximately three hundredfold lower than the existing standard.

3.6 REFERENCES

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Table 3-1. Summary of Oral Non-cancer Dose-Response Information for Inorganic Arsenic in Humans

Study population	Exposure	NOAEL (mg As/kg-day)	LOAEL (mg As/kg-day)	Responses at the LOAEL	Comments	Reference
Chronic exposure						
Cross-sectional survey of 11,746 adults in Bangladesh	Subjects sorted into 5 quintiles based on TWA As concentration in drinking water for at least 5 years: 0.1-8.0 (median = 1.8), 8.1-40 (23), 40.1-91 (62), 91.1-175 (125), or 175.1-864 (255) µg As/L [≈ <0.0004 (0.00008), 0.0004-0.002 (0.001), 0.002-0.005 (0.003), 0.005-0.009 (0.006), or 0.009-0.04 (0.01) mg As/kg-day][a]	NA	0.0004-0.002 Median = 0.001	Increased risk of skin lesions	Dose-dependent increase in prevalence of skin lesions (melanosis and/or hyperkeratosis). Prevalence OR (adjusted for age, sex, BMI, education, smoking, sun exposure, and land ownership) significantly increased in 2nd (1.38, 95% CI 1.20-2.94), 3rd (3.32, 95% CI 2.18-5.05), 4th (3.78, 95% CI 2.50-5.71), and 5th (5.70, 95% CI 3.80-8.55) quintiles relative to 1st quintile. Same results for exposure quantified as cumulative As dose (mg As) or urinary As (µg As/g creatinine).	Ahsan et al., 2006
Case-control study (504 cases with skin lesions and 1,830 controls, roughly half males and half females) in Bangladesh	Cases and controls sorted into 5 quintiles based on chronic mean As exposure: <10, 10-49, 50-149, 150-299, or ≥300 µg As/L in drinking water for at least 6 months (≈ <0.0007, 0.0007-0.004, 0.004-0.009, 0.009-0.02, or ≥0.02 mg As/kg-day)[b]	NA	0.0007-0.004 Midpoint = 0.002	Increased risk of skin lesions (males)	Chronic mean drinking water exposure to As was significantly ($p<0.01$) higher in cases (♂ 200 µg/L, ♀ 211 µg/L) than in controls (♂ 143 µg/L, ♀ 155 µg/L). There were significant dose-related increases in skin lesion risk in males and females with increasing exposure to As ($p < 0.001$). OR significantly increased in males at 2nd quintile (3.25, 95% CI 1.43-7.38) and above and females at 3rd quintile (3.06, 95% CI 1.39-6.74) and above, relative to the 1st quintile.	Rahman et al., 2006
Cross-sectional survey of 13,705 women in Bangladesh	Participants sorted into 4 groups by mean As concentrations in village well water: ≤5, 6-10, 11-50, or >50 µg As/L (≈ ≤0.0004, 0.0004-0.0007, 0.0007-0.004, or >0.004 mg As/kg-day)[b]	0.0004-0.0007 Midpoint = 0.0006	0.0007-0.004 Midpoint = 0.002	Increased risk of skin lesions	Prevalence of skin lesions in village increased with As concentration in village well water. Prevalence ratio significantly increased in 3rd (2.10; 95% CI 1.03-4.28) and 4th (22.05; 95% CI 11.24-43.27) groups relative to 1st group.	McDonald et al., 2006
Case-control study (155 women with skin lesions and 155 controls matched for village and age) in Bangladesh	Cases and controls sorted into 3 groups based on As levels in drinking water wells: 0-10, 11-50, or >51 µg As/L (≈ 0-0.0007, 0.0007-0.004 or >0.004 mg As/kg-day)[b]	0.0007-0.004 Midpoint = 0.002	>0.004	Increased risk of skin lesions	OR for skin lesions significantly increased in 3rd group (2.96, 95% CI 1.02-8.59) relative to 1st group. Nonsignificant increase in 2nd group relative to 1st group (1.33, 95% CI 0.77-2.28).	McDonald et al., 2007

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Study population	Exposure	NOAEL (mg As/kg-day)	LOAEL (mg As/kg-day)	Responses at the LOAEL	Comments	Reference
Cross-sectional study of 109 subjects in As affected village and 32 subjects from control village in Mongolia	Subjects sorted into 4 groups based on As levels in drinking water wells used for at least 6 months: <50, 51-99, 100-149, or >150 µg As/L (≈ <0.001, 0.001- 0.003, 0.003-0.004, or >0.004 mg As/kg-day)[c]	NA	0.001-0.003 Midpoint = 0.002	Increased risk of skin lesions	Prevalence of skin lesions (keratosis, pigmentation, and/or depigmentation) increased with As exposure. Prevalence OR (adjusted for age, sex, smoking, duration of exposure) significantly increased in 2nd (15.5, 95% CI 1.53-248.7), 3rd (16.10, 95% CI 3.73-69.93), and 4th (25.70, 95% CI 6.43-102.87) groups relative to 1st group.	Guo et al., 2006a
Cross sectional study of 448 adult residents of a single village in Mongolia	Subjects sorted into 4 groups based on As levels in drinking water wells: <50, 51-199, 200-499, or >500 µg As/L (≈ <0.001, 0.001- 0.006, 0.006-0.01 or ≥0.01 mg As/kg-day)[c]	NA	0.001-0.006 Midpoint = 0.004	Increased risk of skin pigment disorder	Prevalence of skin lesions increased with As water level, primarily due to increase in prevalence of pigment disorders. Prevalence of keratosis was very high in all groups, including lowest exposure group, and did not increase further with exposure level. Prevalence OR for pigment disorders (adjusted for age, sex, and smoking) significantly increased in 2nd (2.25, 95% CI 1.3-83.24), 3rd (10.97, 95% CI 1.50-79.95), and 4th (10.00, 95% CI 1.39-71.77) groups relative to 1st group.	Guo et al., 2006b
Cross-sectional survey of 241 children (109 boys and 132 girls) aged 4-15 in 2 rural villages in Bangladesh	Participants sorted into tertiles based on urinary As excretion (mean values of 108, 239, 631 µg As/g creatinine).	NA (631 µg As/ g creatinine)	NA	NA	Dermal lesions in 141/241 (59%) children. Lesion incidence did not vary with tertile of As exposure, as assessed by urinary As excretion (55-60% in each tertile). There was a significant trend for increasing proportion of children with low BMI with increasing tertile of As in urine, but no significant pair wise differences.	Watanabe et al., 2007
Cross-sectional study of 479 adults (220 men and 259 women) in Taiwan	Participants sorted into 3 groups of cumulative As exposure (0, 0.1-15.4, ≥15.4 mg As/L × yr).	NA (≥15.4 mg As/L × yr)	NA	NA	Nonsignificant increase in risk of peripheral vascular disease with increasing cumulative As exposure (adjusted OR of 3.41[95% CI 0.74-15.78] and 4.62[95% CI 0.96-22.21] for 2nd and 3rd groups, respectively, versus 1st group).	Tseng et al., 2005

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Study population	Exposure	NOAEL (mg As/kg-day)	LOAEL (mg As/kg-day)	Responses at the LOAEL	Comments	Reference
Cross-sectional study in 8790 recently pregnant women in Mongolia	Subjects sorted into 4 groups based on As levels in drinking water wells: <20, 21-50, 51-100, or >100 µg As/L (≈ <0.0007, 0.0007-0.002, 0.002-0.004, or >0.004 mg As/kg-day)[d]	NA	0.0007-0.002 Midpoint = 0.001	Increased systolic and diastolic blood pressure	Systolic and diastolic blood pressure (6 weeks postpartum) increased with increasing As concentration in drinking water. The increases were significant for exposure groups 2 (systolic increased by 1.88 mm Hg, diastolic by 2.11 mm Hg), 3 (systolic 3.90 mm Hg, diastolic 2.74 mm Hg), and 4 (systolic 6.83 mm Hg, diastolic 3.08 mm Hg), relative to group 1 (adjusted for age and weight).	Kwok et al., 2007
Retrospective mortality study of 572 deaths in a single village in Mongolia	Subjects sorted into 5 groups based on As exposure in drinking water: <5, 5.1-20, 20.1-100, 100.1-300, or ≥300 µg As/L (≈ <0.0001, 0.0001-0.006, 0.006-0.003, 0.003-0.009, or ≥0.009mg As/kg-day)[c]	0.003-0.009 Midpoint = 0.006	≥0.009	Increased risk of heart disease mortality	Incidence rate ratio (adjusted for age, sex, smoking, alcohol and farm work) significantly increased for mortality due to all causes (3.39, 95% CI 1.32-8.69), cancer (6.25, 95% CI 1.08-36.22), and heart disease (5.08, 95% CI 1.45-17.81) in highest exposure group (relative to low exposure group) among residents exposed since before 1990.	Wade et al., 2009
Cross-sectional study of 117 children (62 boys and 55 girls) aged 12-14 in Taiwan	Children sorted into 3 groups based on current As exposure in drinking water: ≤10, 10.1-50, or >50 µg As/L (≈ <0.0003, 0.0003- 0.001, or >0.001 mg As/kg-day)[e] or cumulative As dose: ≤50, 50.1-100, >100 mg As.	NA (50.1-100 mg As)	NA (>100 mg As)	Increased risk of slow sural nerve sensory action potential	Measured nerve conduction velocity (NCV). No difference in NCV between ≤50 and >50 ug As/L current exposure, but sural nerve sensory action potential (SAP) decreased at >100 mg As versus ≤100 mg As cumulative dose. OR for slow sural SAP (adjusted for gender and height) showed nonsignificant increase for 3rd group versus 1st group (2.4; 95% CI 0.7-8.1) based on current exposure and significant increase for 3rd group versus 1st group (2.9; 95% CI 1.1-7.5) based on cumulative exposure.	Tseng et al., 2006
Cross-sectional study of 201 children (98 boys and 103 girls) aged 9.5-10.5 in Bangladesh	Children placed in quartiles based on current As exposure: <5.5, 5.6-50, 50.1-176, or 177-790 µg As/L in drinking water (≈ <0.0003, 0.0003-0.003, 0.003-0.01, or 0.01-0.05 mg As/kg-day)[f]	0.0003-0.003 Midpoint = 0.002	0.003-0.01 Midpoint = 0.007	Decreased intellectual function	After adjustment for sociodemographic covariates and water Mn concentrations, there was a dose-related reduction in intellectual function (measured via modified Wechsler Intelligence Scale) with increasing As exposure that was statistically significant in the 3rd and 4th quartiles versus the 1st quartile.	Wasserman et al., 2004

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Study population	Exposure	NOAEL (mg As/kg-day)	LOAEL (mg As/kg-day)	Responses at the LOAEL	Comments	Reference
Cross-sectional study of adults (223 exposed and 160 controls) in Taiwan	Participants sorted into 3 groups based on cumulative As exposure (<0.1, 0.1-15, ≥15.1 mg As/L × yr).	NA	NA (0.1-15 mg As/L × yr)	Increased risk of pterygium based on cumulative As exposure	Prevalence of pterygium (disfiguring disease that can lead to blindness) increased with cumulative As exposure and with duration of well water consumption. Significantly increased OR for both 2nd (2.04; 95% CI 1.04-3.99) and 3rd (2.88; 95% CI 1.42-5.83) groups versus 1st group, even after adjustment for age, sex, working in sunlight and working in sand (other significant risk factors).	Lin et al., 2008
Reproductive and developmental toxicity						
Study of pregnancy outcome in 29,134 women in Bangladesh	Subjects sorted into 5 quintiles based on As concentration in drinking water during pregnancy: <10 (median <1), 10-166 (77), 167-276 (225), 277-408 (340), or ≥409 (515) µg As/L [≈ <0.0007 (<0.00007), 0.0007-0.012 (0.006), 0.012-0.020 (0.016), 0.020-0.030 (0.025), or ≥0.030 (0.037) mg As/kg-day][b]	0.0007-0.012 Median = 0.006	0.012-0.020 Median = 0.016	Increased risk of infant death	Increased risk of infant (age < 12 mo.) death with increasing As concentration in maternal drinking water, significant versus quintile 1 in quintiles 3 (RR=1.19, 95% CI 1.00-1.42), 4 (RR=1.29, 95% CI 1.08-1.53), and 5 (RR=1.19, 95% CI 1.00-1.41). Increased fetal loss (stillbirth) in quintile 4 (RR=1.14, 95% CI 1.01-1.30) relative to quintile 1.	Rahman et al., 2007
Study of pregnancy outcome in 533 women in Bangladesh	Subjects sorted into 3 groups based on As levels in drinking water wells: <50, 51-100, or >100 µg As/L (≈ <0.004, 0.004-0.007, or >0.007 mg As/kg-day)[b]	NA	0.004-0.007 Midpoint = 0.006	Increased risk of spontaneous abortion and neonatal death	Increased risk of spontaneous abortion in exposure groups 2 (OR=2.4, 95% CI 1.2-5.1) and 3 (OR=2.5, 95% CI 1.5-4.4) relative to group 1 (adjusted for height and history of hypertension and diabetes). Increased risk of neonatal (age <28 days) death in group 2 (OR=2.7, 95% CI 1.1-6.73) and stillbirth in group 3 (OR=2.9, 95% CI 1.5-5.9).	Milton et al., 2005
Study of pregnancy outcome in 30,984 women in Bangladesh	Subjects sorted into 3 groups based on As levels in drinking water: <10, 10-50, or >50 µg As/L (≈ <0.0007, 0.0007-0.004 or >0.004 mg As/kg-day)[b]	0.0007-0.004 Midpoint = 0.002	≥0.004	Increased risk of stillbirth	Stillbirths increased with As exposure level. OR for stillbirth was nonsignificantly increased in group 2 (1.23, 95% CI 0.87-1.74) and significantly increased in group 3 (1.80, 95% CI 1.14-2.86) relative to group 1 in multivariate analysis.	Cherry et al., 2008

Table 3-1. Summary of Oral Non-cancer Dose-Response Information for Inorganic Arsenic in Humans

Study population	Exposure	NOAEL (mg As/kg-day)	LOAEL (mg As/kg-day)	Responses at the LOAEL	Comments	Reference
Study of pregnancy outcome in 9890 women in Mongolia	Subjects sorted into 4 groups based on As levels in drinking water wells: <20, 21-50, 51-100, or >100 µg As/L (≈ <0.0006, 0.0006-0.001, 0.001-0.003, or >0.003 mg As/kg-day)[c]	NA	>0.001	Increased risk of neonatal death	Increased risk of neonatal (age <28 days) death (OR=2.01, 95% CI 1.12-3.59, adjusted for adequacy of prenatal care) in exposure groups 3+4 (combined) versus groups 1+2 (combined). No evident relationship between maternal As exposure in drinking water and preterm birth, stillbirth, or birth weight.	Myers et al., 2009
Prospective cohort study of 844 infant-mother pairs (424 from an As-exposed city and 420 from a low-As city) in Chile	Subjects sorted into 2 groups based on As levels in city drinking water: <1 or 40 µg As/L (approximately <0.00004 or 0.002 mg As/kg-day)[g]	0.002	NA	NA	There was a non-significant decrease in birth weight in infants of women from the As-exposed city (-57 g, 95% CI -123 - 9) in multivariate analysis.	Hopenhayn et al., 2003
Prospective cohort study of 810 mothers (407 from an As-exposed city and 403 from a low-As city) in Chile	Subjects sorted into 2 groups based on As levels in city drinking water: <1 or 40 µg As/L (approximately <0.00004 or 0.002 mg As/kg-day)[g]	NA	0.002	Increased prevalence of anemia in pregnant women	Prevalence of anemia (Hgb < 11 g/dL) was higher in pregnant women from As-exposed city than low-As city, and disparity increased with trimester (11.0%, 28.7%, 49.4% versus 6.8%, 12.4%, 17.0% in multivariate model). Differences were statistically significant in 2nd and 3rd trimesters.	Hopenhayn et al., 2006
Cross-sectional study of men ≥50 years of age (66 from As-endemic area and 111 others) in Taiwan	Subjects sorted into 2 groups based on As levels in drinking water: ≤50 or >50 ppb As (≈ <0.004 or >0.004 mg As/kg-day)[h]	NA	>0.004	Increased risk of erectile dysfunction	Increased risk of erectile dysfunction (ED) in high exposure group relative to low exposure group (OR=3.0, 95% CI 1.0-9.2) after adjusting for serum testosterone level and traditional ED risk factors. ED graded as severe was more strongly associated with As exposure in same model (OR=7.5, 95% CI 1.8-30.9).	Hsieh et al., 2008

NA = not applicable

[a]Based on 2.75L/day midpoint estimate for water consumption (reported in paper) and 55 kg body weight (assumed by analogy to Taiwan cohort).
[b]Based on 4L/day midpoint estimate for water consumption (reported by Biswas et al., 1998) and 55 kg body weight (assumed by analogy to Taiwan cohort).
[c]Based on default water consumption of 2L/day and body weight of 70 kg (U.S. EPA, 1988) in absence of specific data for Mongolian cohort.
[d]Based on default water consumption of 2L/day (U.S. EPA, 1988) and mean body weight of 55 kg (reported in paper).
[e]Based on 90th percentile water consumption of 1.3L/day and mean body weight of 51 kg for children aged 12–14 years (U.S. EPA, 1997).
[f]Based on 90th percentile water consumption of 1.3L/day for children aged 10 years (U.S. EPA, 1997) and mean body weight of 22 kg (reported in paper).
[gf]Based on water consumption of 2.3L/day (reported in paper) and 60 kg body weight (estimated from BMI reported in paper).
[h]Based on water consumption of 4.5L/day and 55 kg body weight for Taiwan cohort (U.S. EPA, 1993).

4.0 REVIEW OF SELECTED KEY STUDIES AND TOXICITY ASSESSMENT FOR CHROMIUM (Cr)

4.1 SUMMARY

The existing standard for chromium is 0.3 µg Cr/day from toys, which corresponds to a daily dose of 2.5E-05 mg Cr/kg-day from toys for a 12 kg child. An ADI of 4E-04 mg Cr/kg-day for hexavalent chromium, the form most likely to occur in toys, is derived herein based on a chronic LOAEL of 0.4 mg Cr/kg-day for gastrointestinal tract lesions in mice in a chronic drinking water study and an uncertainty factor of 1000 to extrapolate from mice to humans (10), adjust from a LOAEL to a NOAEL (10), and protect sensitive populations (10). Applying the same modifying factors that were used to derive the existing standard, the permissible intake of hexavalent chromium from toys based on this ADI would be 0.05 µg Cr/day. This comparison suggests that the ADI would be approximately sixfold lower than the existing standard.

4.2 EXISTING STANDARD FOR CHROMIUM

Existing standards for children's intake of metals from toys, originally published in EU 12964 EN and EN 71-3, are based on estimated levels of metals in the diet and allowable relative source contributions from toys, ranging from 0.1–10 percent based on the chemical's toxicity (RIVM, 2006). For chromium, the existing standard is 0.3 µg Cr/day from toys, based on an assumed child's dietary intake of 200 µg Cr/week (calculated as 50 percent of the measured adult intake of 400 µg Cr/week), and an allowable contribution from toys of 1 percent (200 µg Cr/week ÷ 7 days/week × 1% = 0.3 µg Cr/day) (RIVM, 2006). The allowable contribution for chromium from toys was reduced to 1 percent from the starting value of 10 percent due to known carcinogenicity and mutagenicity of chromium in humans by the inhalation route. Assuming a body weight of 12 kg (RIVM, 2006), the permissible intake of 0.3 µg Cr/day corresponds to a daily dose of 0.025 µg Cr/kg-day or 2.5E-05 mg Cr/kg-day from toys.

4.3 REVIEW OF SELECTED KEY STUDIES FOR CHROMIUM

RIVM (2006) reviewed the existing toxicity data and available assessments for chromium. Chromium is found in trivalent and hexavalent forms in the environment. Naturally occurring chromium is almost always found in trivalent forms, while chromium used in industrial applications typically is limited to hexavalent forms. The toxicities of trivalent and hexavalent chromium differ considerably, the latter being far more potent (RIVM, 2006). For the current assessment, toxicology literature on trivalent and hexavalent chromium published since 2000 was reviewed.

4.4 TRIVALENT CHROMIUM (Cr3+)

The database for trivalent chromium since 2000 includes subchronic (Shara et al., 2005; NTP 2008a), chronic (Shara et al., 2007; NTP, 2008a), and developmental (Staniek et al., 2009) toxicity studies in rats and/or mice that were considered potential candidates for development of toxicity values. These studies are summarized in Table 4-1. No significant non-cancer effects were seen in any of the studies, which is consistent with the previously existing database for trivalent chromium. NOAELs and doses tested were 1–3 orders of magnitude higher for the NTP (2008a) studies than the other studies because of differences in bioavailability of the specific forms of trivalent chromium used (all dietary studies). The material tested in the NTP (2008a) studies, chromium picolinate monohydrate, is a crystalline solid that is slightly soluble in water and is lipid soluble; it is reportedly more bioavailable than comparable unchelated forms of trivalent chromium. NTP (2008a) cited studies showing that absorption of this material through the gut was 2.8 percent in humans and comparable to that for chromic chloride in rats (NTP, 2008a). A study comparing the two materials found that liver and kidney concentrations of chromium were two- to sixfold higher in rats fed chromium picolinate versus chromic chloride at identical doses in the diet (Anderson et al., 1997). The materials tested by Shara et al. (2005, 2007) and Staniek and Krejpcio (2009), niacin-bound chromium complex and chromium propionate cation, respectively, are well absorbed and highly bioavailable. Absorption efficiency was reported to be 40–60 percent for chromium propionate cation (Staniek and Krejpcio, 2009).

4.5 HEXAVALENT CHROMIUM (Cr6+)

The database for hexavalent chromium since 2000 includes subchronic toxicity studies (two studies in rats and two studies in mice), chronic toxicity studies (one study in rats and one study in mice), and reproductive toxicity studies (several in monkeys and rabbits) that were considered potential candidates for development of toxicity values. These studies are summarized in Table 4-2. LOAEL values identified from subchronic and chronic toxicity studies ranged from 0.4 to 3.1 mg Cr/kg-day and were associated with liver, kidney, hematological, and lymphatic effects (Stout et al., 2009; NTP, 2007, 2008b; Acharya et al., 2001). LOAELs for reproductive (testicular) effects were in the range of 1.8 to 5.4 mg Cr/kg-day (Yousef et al., 2006; Aruldhas et al., 2006, 2005, 2004; Subramanian et al., 2006). The lowest LOAEL, 0.4 mg Cr/kg-day, was identified for epithelial hyperplasia in the duodenum, histiocytic infiltration of the liver and mesenteric lymph node, and cytoplasmic alterations in the pancreatic acini from a chronic toxicity study in mice administered sodium dichromate dihydrate in the drinking water for two years (Stout et al., 2009; NTP, 2008b).

4.6 TOXICITY ASSESSMENT FOR CHROMIUM

4.6.1 Trivalent chromium (Cr3+)

None of the available studies on trivalent chromium found effects at any of the doses tested. All of the more recent studies were conducted with relatively well-absorbed forms of trivalent chromium. The chromium picolinate monohydrate studied by NTP (2008a) is of similar bioavailability as chromic chloride. Bioavailabilities of the niacin-bound chromium complex and chromium propionate cation studied by Shara et al. (2005, 2007) and Staniek and Krejpcio (2009), respectively, are much higher still.

A tentative ADI for soluble trivalent chromium can be derived from the 52-week NOAEL of 1.6–3.0 mg Cr/kg-day reported by Shara et al. (2007), although there is considerable uncertainty in deriving an ADI based on a free-standing NOAEL. This study included only one dose level, but used an adequate number of animals and investigated a wide array of endpoints. This NOAEL is supported by the results of the subchronic (Shara et al., 2005) and developmental toxicity (Staniek and Krejpcio, 2009) studies. Taking the low end of the male-female range (1.6 mg Cr/kg-day) as the POD, and applying an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability), results in a tentative ADI for soluble trivalent chromium of 0.016 or 1.6E-02 mg Cr/kg-day.

Additional uncertainty factors for extrapolation across durations (this was a 52-week study) and database weaknesses (lack of reproduction toxicity studies) could be considered as well.

4.6.2 Hexavalent chromium (Cr6+)

An ADI for hexavalent chromium can be derived from the chronic LOAEL of 0.4 mg Cr/kg-day for lesions (including epithelial hyperplasia in the duodenum, histiocytic infiltration of the liver and mesenteric lymph node, and cytoplasmic alterations in the pancreatic acini) in mice administered sodium dichromate dihydrate in the drinking water for two years (Stout et al., 2009; NTP, 2008b). A corresponding NOAEL was not identified. This study included testing of large numbers of mice of both sexes at multiple dose levels and featured evaluation of a comprehensive array of endpoints. This LOAEL is supported by similar effects in rats in a companion chronic experiment (Stout et al., 2009; NTP, 2008b) and in both rats and mice in precursor subchronic experiments (NTP, 2007). Taking the LOAEL of 0.4 mg Cr/kg-day as the POD, and applying an uncertainty factor of 1000 (10 for extrapolation from animals to humans, 10 for human variability, and 10 for adjustment from a LOAEL to a NOAEL), results in an ADI for hexavalent chromium of 0.0004 or 4E-04 mg Cr/kg-day.

4.6.3 Comparison of ADI to Existing Toy Standard for Chromium

The ADIs of 0.016 mg Cr/kg-day for soluble trivalent chromium and 4E-04 mg Cr/kg-day for hexavalent chromium derived here; apply to total daily intake from all sources. The existing permissible intake for chromium in the European toy safety standard is 0.3 µg Cr/day, which refers specifically to intake from toys and does not differentiate among chromium species. Because chromium in toys is most likely to be hexavalent, we used the ADI for hexavalent chromium as the basis for comparison and applied the same modifying factors that were used to derive the toy standard. Therefore, a source allocation of 1 percent was applied to the ADI for hexavalent chromium and a bodyweight of 12 kg was used to calculate a permissible intake level that is directly comparable to the existing value (4E-04 mg Cr/kg-day × 1% x 12 kg = 5E-05 mg Cr/day from toys). Comparison of the permissible intake of hexavalent chromium from toys based on the ADI derived here (5E-05 mg Cr/day or 0.05 µg Cr/day) and the existing permissible intake from toys (0.3 µg Cr/day) suggests that the ADI for hexavalent chromium would be approximately sixfold lower than the existing standard.

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Table 4-1. Summary of Oral Noncancer Dose-Response Information for Trivalent Chromium (Cr3+)

Species and study type (n/sex/group)	Exposure [report doses, route, frequency, duration]	NOAEL (mg Cr/kg-day)	LOAEL (mg Cr/kg-day)	Responses at the LOAEL	Comments	Reference
Subchronic toxicity						
Sprague-Dawley rat 18/sex/group (6/sex/group at each time point)	0, 5, 50, or 125 ppm niacin-bound chromium (providing 0, 0.2, 2, or 5 mg Cr/day) in the diet for 30, 60, or 90 days (≈ 0, 0.5-0.8, 5-8, or 11-19 mg Cr/kg-day)[a]	11-19	NA	NA	No significant effects on survival, clinical signs, body weight, feed or water intake, hematology, clinical chemistry, organ weights, hepatic lipid peroxidation, hepatic DNA fragmentation, or gross or microscopic pathology.	Shara et al., 2005
F344/N rat 10/sex/group	0, 80, 240, 2000, 10,000 or 50,000 ppm chromium picolinate monohydrate in the diet for 90 days (≈ 0, 0.8, 2.4, 19, 95, or 506 mg Cr/kg-day)[b]	506	NA	NA	No significant effects on survival, clinical signs, body weight, feed intake, hematology, clinical chemistry, organ weights, sperm parameters (♂) or estrus cycling (♀), or gross or microscopic pathology.	NTP, 2008a
B6C3F$_1$ mouse 10/sex/group	0, 80, 240, 2000, 10,000 or 50,000 ppm chromium picolinate monohydrate in the diet for 90 days (≈ 0, 2, 5-6, 44-54, 212-274, or 1090-1419 mg Cr/kg-day)[b]	1090-1419	NA	NA	No significant effects on survival, clinical signs, body weight, feed intake, hematology, clinical chemistry, organ weights, sperm parameters (♂) or estrus cycling (♀), or gross or microscopic pathology.	NTP, 2008a
Chronic toxicity						
Sprague-Dawley rat 18/sex/group (6/sex/group at each time point)	0 or 25 ppm niacin-bound chromium (providing 0 or 1 mg Cr/day) in the diet for 26, 39 or 52 weeks (≈ 0 or 1.6-3.0 mg Cr/kg-day)[a]	1.6-3.0	NA	NA	The body weights of treated rats were reduced by ~10% in females at 39 weeks, and ~13% in males at 52 weeks, but were otherwise within 10% of controls at weeks 26, 39 and 52. The researchers did not consider the effect on body weight to be adverse. No significant effects on survival, clinical signs, body weight, feed or water intake, hematology, clinical chemistry, organ weights, hepatic lipid peroxidation, hepatic DNA fragmentation, or gross or microscopic pathology.	Shara et al., 2007
F344/N rat 50/sex/group	0, 2000, 10,000 or 50,000 ppm chromium picolinate monohydrate in the diet for 105 weeks (≈ 0, 11-12, 55-61, or 286-314 mg Cr/kg-day)[b]	286-314	NA	NA	No significant effects on survival, clinical signs, body weight, feed intake, or noncancer lesions.	NTP, 2008a
B6C3F$_1$ mouse 50/sex/group	0, 2000, 10,000 or 50,000 ppm chromium picolinate monohydrate in the diet for 105 weeks (≈ 0, 30, 143, or 727-783 mg Cr/kg-day)[b]	727-783	NA	NA	No significant effects on survival, clinical signs, body weight, feed intake, or noncancer lesions.	NTP, 2008a

Table 4-1. Summary of Oral Noncancer Dose-Response Information for Trivalent Chromium (Cr3+)

Species and study type (n/sex/group)	Exposure [report doses, route, frequency, duration]	NOAEL (mg Cr/kg-day)	LOAEL (mg Cr/kg-day)	Responses at the LOAEL	Comments	Reference
Reproductive and developmental toxicity						
Wistar albino rat 20 females/group	0 or 7.2 mg Cr/kg-day (administered as chromium propionate cation) in the diet on GD 0 to GD 21	7.2	NA	NA	No significant effects on body weight, feed intake, clinical chemistry, or organ weights of dams; pregnancy outcome, litter size; or fetal body and organ weights, or morphology. The control diet contained 0.02 mg Cr/kg-day.	Staniek et al., 2009

[a]Daily chromium doses in mg Cr/day converted to mg Cr/kg-day by dividing by body weights reported in the study. Ranges are shown where body weights for males and females differed.
[b]Daily chromium picolinate monohydrate doses in mg/kg-day reported by the researchers converted to mg Cr/kg-day by multiplying by ratio of molecular weight of chromium to molecular weight of chromium picolinate monohydrate (52/436 = 0.119). Ranges are shown where chromium picolinate monohydrate doses in mg/kg-day reported by the researchers differed for males and females.

Table 4-2. Summary of Oral Noncancer Dose-Response Information for Hexavalent Chromium (Cr6+)

Species and study type (n/sex/group)	Exposure [report doses, route, frequency, duration]	NOAEL (mg Cr/kg-day)	LOAEL (mg Cr/kg-day)	Responses at the LOAEL	Comments	Reference
Subchronic toxicity						
Wistar rat 5-6 males/group	0 or 25 ppm potassium dichromate in the drinking water for 22 weeks (≈ 0 or 1.3 mg Cr/kg-day)[a]	NA	1.3	Increased serum AST and ALT, decreased liver glycogen and triglycerides, increased liver cholesterol, histopathological alterations in the liver (altered hepatic structure and damage to hepatocytes) and kidneys (damage to the renal tubules and Bowman's capsule).	Body weights and food and water consumption were reportedly monitored, but data were not shown.	Acharya et al., 2001
F344/N rat 10/sex/group	0, 62.5, 125, 250, 500, or 1,000 mg/L sodium dichromate dihydrate in the drinking water for 13 weeks (≈ 0, 1.7, 3.5, 5.9, 11.2, or 20.9 mg Cr/kg-day)[b]	NA	1.7	Microcytic hypochromic anemia (decreased hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin; increased RBC), increased serum ALT and SDH, increased incidence of histiocytic infiltration in the pancreatic lymph nodes	Histiocytic infiltration in the liver and duodenum at higher doses.	NTP, 2007
$B6C3F_1$ mouse 10/sex/group	0, 62.5, 125, 250, 500, or 1,000 mg/L sodium dichromate dihydrate in the drinking water for 13 weeks (≈ 0, 3.1, 5.2, 9.1, 15.7, or 27.9 mg Cr/kg-day)[b]	NA	3.1	Erythrocyte microcytosis (decreased mean cell volume and mean cell hemoglobin); increased incidence of epithelial hyperplasia in the duodenum	Erythrocyte microcytosis less severe than in rats; histiocytic infiltration of the duodenum and mesenteric lymph node at higher doses.	NTP, 2007
$B6C3F_1$ mouse 10 males/group BALB/c mouse 10 males/group am3-C57BL/6 mouse 5 males/group	0, 62.5, 125, or 250 mg/L sodium dichromate dihydrate in the drinking water for 13 weeks (≈ 0, 2.8, 5.2, or 8.7 mg Cr/kg-day)[b]	NA	2.8	Eryrthrocyte microcytosis (decreased mean cell volume and mean cell hemoglobin); histiocytic infiltration and/or epithelial hyperplasia of the duodenum, secretory depletion in the pancreas, glycogen depletion of the liver; body weight reduced >10% in am3-C57BL/6 mice	Similar effects in all 3 strains, although effective dose levels varied.	NTP, 2007

Table 4-2. Summary of Oral Noncancer Dose-Response Information for Hexavalent Chromium (Cr6+)

Species and study type (n/sex/group)	Exposure [report doses, route, frequency, duration]	NOAEL (mg Cr/kg-day)	LOAEL (mg Cr/kg-day)	Responses at the LOAEL	Comments	Reference
Chronic toxicity						
F344 rat 50/sex/group	0, 14.3, 57.3, 172, or 516 mg/L sodium dichromate dihydrate in the drinking water for 2 years (≈ 0, 0.2, 0.8, 2.1-2.4, or 5.9-7.0 mg Cr/kg-day)[b]	0.2	0.8	Microcytic hypochromic anemia (decreased hemoglobin, hematocrit, mean cell volume, mean cell hemoglobin; increased RBC); increased serum ALT and decreased serum ALP; histiocytic infiltration of the liver, duodenum, and mesenteric lymph node; also chronic inflammation and fatty changes in the liver and hemorrhage of the mesenteric lymph node		NTP, 2008b; Stout et al., 2009
B6C3F1 mouse 50/sex/group	Males: 0, 14.3, 28.6, 85.7, or 257.4 mg/L sodium dichromate dihydrate in the drinking water for 2 years (≈ 0, 0.4, 0.9, 2.4, or 5.9 mg Cr/kg-day)[b] Females: 0, 14.3, 57.3, 172, or 516 mg/L (≈ 0, 0.4, 1.4, 3.1, or 8.7 mg Cr/kg-day)[b]	NA	0.4	Epithelial hyperplasia in the duodenum, histiocytic infiltration of the liver and mesenteric lymph node, cytoplasmic alterations in the pancreatic acini	Eryrthrocyte microcytosis (decreased mean cell volume and mean cell hemoglobin) and histiocytic infiltration of the duodenum, jejunum, and pancreatic lymph node at higher doses.	NTP, 2008b; Stout et al., 2009
Reproductive toxicity						
Bonnet monkey 3 adult males/group	0, 50, 100, 200, or 400 ppm Cr (administered as potassium dichromate) in drinking water for 6 months (≈ 0, 2.7, 5.4, 10.8, or 21.7 mg Cr/kg-day)[c]	2.7	5.4	Decreased sperm count and motility; decreased activities of superoxide dismutase and catalase in seminal plasma and sperm; increased H_2O_2 concentration in seminal plasma and sperm		Subramanian et al., 2006

Table 4-2. Summary of Oral Noncancer Dose-Response Information for Hexavalent Chromium (Cr6+)

Species and study type (n/sex/group)	Exposure [report doses, route, frequency, duration]	NOAEL (mg Cr/kg-day)	LOAEL (mg Cr/kg-day)	Responses at the LOAEL	Comments	Reference
Bonnet monkey 3 adult males/group	0, 100, 200, or 400 ppm Cr (administered as potassium dichromate) in drinking water for 6 months (≈ 0, 5.4, 10.8, or 21.7 mg Cr/kg-day)[c]	NA	5.4	Histopathological changes in the epididymis (including ductal obstruction and the formation of microcanals)		Aruldhas et al., 2004
Bonnet monkey 6 adult males/group	0, 100, 200, or 400 ppm Cr (administered as potassium dichromate) in drinking water for 6 months (≈ 0, 5.4, 10.8, or 21.7 mg Cr/kg-day)[c]	NA	5.4	Decreased testis weight, decreased activities of testicular enzymes (superoxide dismutase, catalase, glutathione reductase, and G-6-PDH), decreased concentrations of antioxidants (glutathione, vitamins A, C and E), increased concentrations of H_2O_2 and OH^- in the testis, histopathological changes in the testes (including disorganized seminiferous tubules, depletion of germ cells, and hyperplasia of Leydig cells)		Aruldhas et al., 2005
Bonnet monkey 3 adult males/group	0, 100, 200, or 400 ppm Cr (administered as potassium dichromate) in drinking water for 6 months (≈ 0, 5.4, 10.8, or 21.7 mg Cr/kg-day)[c]	NA	5.4	Histopathological changes in the epididymis (increased numbers of basal cells and intraepithelial macrophages with accumulation of sperm-derived lipofuscin material)		Aruldhas et al., 2006
New Zealand white rabbit 6 males/group	0 or 5 mg/kg-day potassium dichromate via daily gavage for 10 weeks (≈ 0 or 1.8 mg Cr/kg-day)[d]	NA	1.8	Decreased testes and epididymis weights, decreased plasma testosterone concentration, alterations in semen characteristics (including decreases in sperm concentration, motility, and percent normal sperm, and increases in dead sperm and initial pH), and increased TBARS and decreased GST in the seminal plasma		Yousef et al., 2006

Table 4-2. Summary of Oral Noncancer Dose-Response Information for Hexavalent Chromium (Cr6+)

Species and study type (n/sex/group)	Exposure [report doses, route, frequency, duration]	NOAEL (mg Cr/kg-day)	LOAEL (mg Cr/kg-day)	Responses at the LOAEL	Comments	Reference

[a]Doses of potassium dichromate were calculated using default body weight and water consumption data for male Wistar rats in a subchronic duration study. Doses of potassium dichromate were converted to doses of Cr6+ by multiplying by the ratio of molecular weight chromium/molecular weight potassium dichromate (104/294=0.354).

[b]Doses in mg Cr/kg-day reported by researchers. Ranges are shown where doses reported by the researchers differed for males and females.

[c]It is not entirely clear whether reported concentrations are for Cr or potassium dichromate, although it appears to be the former. Assuming concentrations were for Cr, doses of Cr were calculated using default body weight and water consumption values for mature rhesus monkeys (no values for bonnet monkeys). If reported concentrations were for potassium dichromate, Cr doses would be approximately 35% of the doses shown (≈ 0, 0.96, 1.9, 3.8, or 7.6 mg Cr/kg-day).

[d]Doses of potassium dichromate were converted to doses of Cr6+ by multiplying by the ratio of molecular weight chromium/molecular weight potassium dichromate (104/294=0.354). The researchers reported that the 5 mg/kg dose "contains 3.6 mg chromium (VI)" but it is not clear how this number was calculated or exactly what it means.

5.0 REVIEW OF SELECTED KEY STUDIES AND TOXICITY ASSESSMENT FOR MERCURY (Hg)

5.1 SUMMARY

The existing standard for mercury is 0.5 µg Hg/day from toys, which corresponds to a daily dose of 4E-05 mg Hg/kg-day from toys for a 12 kg child. An ADI of 1E-06 mg Hg/kg-day is derived herein based on a LOAEL of 0.001 mg Hg/kg-day for testicular lesions in male rats exposed in the drinking water for three months and an uncertainty factor of 1000 to extrapolate from rats to humans, adjust from a LOAEL to a NOAEL, and protect sensitive populations. Applying the same modifying factors that were used to derive the existing standard, the permissible intake from toys based on this ADI would be 0.001 µg Hg/day. This comparison suggests that the ADI would be approximately five hundredfold lower than the existing standard.

5.2 EXISTING STANDARD FOR MERCURY

Existing standards for children's intake of metals from toys, originally published in EU 12964 EN and EN 71-3, are based on estimated levels of metals in the diet and allowable relative source contributions from toys, ranging from 0.1-10 percent based on the chemical's toxicity (RIVM, 2006). For mercury, the existing standard is 0.5 µg Hg/day from toys, based on an assumed child's dietary intake of 35 µg Hg/week (calculated as 50 percent of the measured adult intake of 70 µg Hg/week), and an allowable contribution from toys of 10 percent (35 µg Hg/week ÷ 7 days/week × 10% = 0.5 µg Hg/day) (RIVM, 2006). Assuming a body weight of 12 kg (RIVM, 2006), the permissible intake of 0.5 µg Hg/day corresponds to a daily dose of 0.04 µg Hg/kg-day or 4E-05 mg Hg/kg-day from toys.

5.3 REVIEW OF SELECTED KEY STUDIES FOR MERCURY

RIVM (2006) reviewed the existing toxicity data and assessments available for mercury. Mercury can occur as a metal (element), inorganic salt, or organic compound (e.g., methylmercury). Only inorganic forms are considered relevant for toy-related exposures (RIVM, 2006). For the current assessment, toxicology literature on inorganic mercury published since 2000 was reviewed. Six studies were identified that include data for noncancer effects following subchronic or chronic oral exposure to inorganic mercury at lowdose levels. All of these studies were designed to assess reproductive endpoints in rodents. These studies observed effects on reproductive performance in rats and mice, including reduced fertility, implantations and fetal viability, as well as testicular degeneration and decreases in sperm counts and motility in males. Two of these studies (Atkinson et al., 2001 and Khan et al., 2004) also observed changes in kidney weights of parental animals (without accompanying histopathology or clinical

chemistry changes). These studies and the NOAEL/LOAEL values identified are summarized in Table 5-1. LOAEL values were 0.001 mg Hg/kg-day for testicular lesions in males, 0.18 mg Hg/kg-day for reduced fertility in breeding trials, and 1.5 mg Hg/kg-day for reduced implantations and hormonal effects in females.

At the lowest LOAEL of 0.001 mg Hg/kg-day, Penna et al. (2009) observed morphological and ultrastructural changes in the testes and epididymis of male rats maintained on drinking water containing mercuric chloride ($HgCl_2$) for up to three months. Testicular and epididymal lesions observed in these rats increased in incidence/severity with increasing dose and exposure duration. Testes and epididymis weights did not differ significantly from controls at the doses used in this study. Degenerative testicular lesions were also observed at higher doses in other studies of rats (Boujbiha et al., 2009) and mice (Orisakwe et al., 2001). In these higher-dose studies, the testicular lesions were accompanied by decreases in testes weight, reduced sperm counts, and reduced production of viable embryos in mating trials.

5.4 TOXICITY ASSESSMENT FOR MERCURY

An ADI for mercury can be derived from the LOAEL of 0.001 mg Hg/kg-day for testicular effects in the study by Penna et al. (2009). This endpoint is supported by the other studies in Table 5-1 showing testicular and reproductive effects of inorganic mercury at higher doses. Taking the LOAEL of 0.001 mg Hg/kg-day (Penna et al., 2009) as the point of departure (POD) (no corresponding NOAEL was identified), and applying an uncertainty factor of 1,000 (10 for extrapolation from animals to humans, 10 for human variability and 10 for adjustment from a LOAEL to a NOAEL), results in an ADI for inorganic mercury of 1E-06 mg Hg/kg-day. Additional uncertainty factors for extrapolation from subchronic to chronic duration and for database weaknesses (lack of developmental toxicity studies) could be considered as well.

5.5 COMPARISON OF ADI TO EXISTING TOY STANDARD FOR MERCURY

The ADI of 1E-06 mg Hg/kg-day derived here applies to total daily intake of inorganic mercury from all sources. The existing permissible intake for mercury in the European toy safety standard is 0.5 µg Hg/day, which refers specifically to intake from toys. In order to compare these, we applied the same modifying factors that were used to derive the toy standard. Therefore, a source allocation of 10 percent was applied to the ADI, and a bodyweight of 12 kg was used to calculate a permissible intake level that is directly comparable to the existing value (1E-06 mg Hg/kg-day × 10% x 12 kg = 1E-06 mg Hg/day from toys). Comparison of the permissible intake of inorganic mercury from toys based on the ADI derived here (1E-06 mg Hg/day or 0.001 µg Hg/day) and the existing permissible intake from toys (0.5 µg Hg/day) suggests that the ADI would be approximately five hundredfold lower than the existing standard.

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Table 5-1. Summary of Key Studies for Mercury

Reference	Species/ Group size	Mercury Form/ Route/ Duration	Dose Compound (mg/kg-day)	Dose Hg (mg Hg/ kg-day)	NOAEL	LOAEL	Effects at LOAEL	Additional effects at higher doses	Additional Comments
Atkinson et al., 2001	Sprague-Dawley rats (F0: 20/sex/ group, F1: 15-25/ sex/group)	$HgCl_2$ via gavage 7 days/week pre-mating through lactation for F0 and F1 generations	M: 0, 0.5, 1, 1.5[a] F: 0, 0.75, 1.5, 2.5[b]	M: 0, 0.37, 0.74, 1.1 F: 0, 0.55, 1.1, 1.8	NA	0.37 (M) 0.55 (F)	↓number pregnant, ↓fertility index, ↓mean # live pups/litter, ↓live birth index, ↓implant efficiencies, ↑kidney weight	↓testes and seminal vesicle weight; ↓body weight; ↓pup 4-day survival index,; ↑clinical signs and mortality in F0 adults	No histopathology conducted; effects in first generation appeared to moderate in second generation (effects observed at higher doses, if at all)
Khan et al., 2004	C57BL/6 mice (25/sex/ group)	$HgCl_2$ via gavage 7 days/week pre-mating through lactation	0, 0.25, 0.5, 1.0	0, 0.18, 0.37, 0.74	NA	0.18	↓fertility index, ↑kidney weight (M)	↓live birth index, ↑kidney weight (F)	Planned F1 breeding not performed due to low fertility in all F0 treated groups; no significant effects on clinical chemistry or histopathology
Heath et al., 2009	Female Sprague-Dawley rats (20/group)	$HgCl_2$ via gavage 7 days/week for 60 days prior to mating with unexposed males	0, 1, 2	0, 0.74, 1.5	0.74	1.5	↓total implantations; ↑non-viable implantations; ↓plasma progesterone levels; ↑pituitary luteinizing hormone (LH) levels	NA	Dams sacrificed on gestation day (GD) 13; ↓body weight (<10%) at all doses
Boujbiha et al., 2009	Male Wistar rats (44/group)	$HgCl_2$ in the drinking water for up to 90 days (groups of 6 rats sacrificed periodically throughout the study)	0, 4, 8	0, 3, 6	NA	3	Degenerative histological changes in seminiferous tubules; ↓epididymal sperm count and motility; ↓viable embryos per litter in mating trials with untreated females	More severe degenerative changes in seminiferous tubules; ↓ mating index in mating trials with untreated females	6 rats from each dose group used for mating trials with untreated females (1 ♂:2 ♀)

Table 5-1. Summary of Key Studies for Mercury

Reference	Species/ Group size	Mercury Form/ Route/ Duration	Dose Compound (mg/kg-day)	Dose Hg (mg Hg/ kg-day)	NOAEL	LOAEL	Effects at LOAEL	Additional effects at higher doses	Additional Comments
Penna et al., 2009	Male Sprague-Dawley rats (15/group)	$HgCl_2$ in the drinking water for up to 3 months (groups of 5 rats sacrificed periodically throughout the study)	NA	0, 0.001, 0.005, 0.01	NA	0.001	Degenerative histological changes in seminiferous tubules and epididymis that progressed with time (rated "moderate" at low dose after 3 months, indicating alterations in 20–50% of the seminiferous tubules in tissue sections from more than half of the total number of treated rats)	Increased incidence/ severity of degenerative changes in seminiferous tubules and epididymis	Dose reported as µg Hg/rat-day by researchers - divided by reported body weights to give values used here; no effects on body, testes, or epididymis weights
Orisakwe et al., 2001	Male CD-1 mice (5/group)	$HgCl_2$ in drinking water for 12 weeks	NA	0, 0.65	NA	0.65	↓water intake, ↓body weight gain, ↓testes weight, ↓epididymal sperm count, testicular necrosis, disintegration of spermatocytes from basement membrane	NA	Dose reported as mg Hg/kg-day by researchers; co-exposure to zinc partially mitigated testicular effects of Hg

[a]Reduced from 2.0 mg/kg-day after 43 days (still pre-mating period) due to overt toxicity (clinical signs); not included in F1 parental generation due to insufficient number of F1 offspring.
[b]Reduced from 3.0 mg/kg-day after 27 days (cohabitation period) due to overt toxicity (clinical signs, mortality); not included in F1 parental generation due to insufficient number of F1 offspring.

6.0 REVIEW OF SELECTED KEY STUDIES AND TOXICITY ASSESSMENT FOR SELENIUM (Se)

6.1 SUMMARY

The existing standard for selenium is 5 µg Se/day from toys, which corresponds to a daily dose of 4E-04 mg Se/kg-day from toys for a 12 kg child. An ADI of 0.001 mg Se/kg-day is derived herein based on a chronic LOAEL of 0.003 mg Se/kg-day for increased risk of diabetes in an exposed human population and an uncertainty factor of 3 to adjust from a LOAEL to a NOAEL and protect sensitive populations. Applying the same modifying factors that were used to derive the existing standard, the permissible intake from toys based on this ADI would be 1 µg Se/day. This comparison suggests that the ADI would be approximately fivefold lower than the existing standard.

6.2 EXISTING STANDARD FOR SELENIUM

Existing standards for children's intake of metals from toys, originally published in EU 12964 EN and EN 71-3, are based on estimated levels of metals in the diet and allowable relative source contributions from toys, ranging from 0.1–10 percent, based on the chemical's toxicity (RIVM, 2006). For selenium, the existing standard is 5 µg Se/day from toys, based on an assumed children's dietary intake of 350 µg Se/week (calculated as 50 percent of the measured adult intake of 700 µg Se/week), and an allowable contribution from toys of 10 percent (350 µg Se/week ÷ 7 days/week × 10% = 5 µg Se/day) (RIVM, 2006). Assuming a body weight of 12 kg (RIVM, 2006), the permissible intake of 5 µg Se/day corresponds to a daily dose of 0.4 µg Se/kg-day or 4E-04 mg Se/kg-day from toys.

6.3 REVIEW OF SELECTED KEY STUDIES FOR SELENIUM

RIVM (2006) reviewed the existing toxicity data and assessments available for selenium. For the current assessment, toxicology literature on selenium published since 2000 was reviewed. Several recent epidemiological studies and clinical trials were located that reported positive associations between high selenium status and increased risk of type 2 diabetes (Bleys et al., 2007; Laclaustra et al., 2009a; Lippman et al., 2009; Stranges et al., 2007), hyperlipidemia (Bleys et al., 2008), and hypertension (Laclaustra et al., 2009b) in (primarily) U.S. adults. These studies are summarized in Table 6-1. Among these studies, the only ones with measured oral intake data were clinical trials that showed increased risk of diabetes, alopecia, and dermatitis in subjects given 200 µg Se/day in dietary supplements for a number of years (Stranges et al., 2007; Lippman et al., 2009). NOAELs were not identified in either study. The other studies measured

exposure in terms of blood levels. Effects in these studies were found at blood levels similar to those measured in the Stranges et al. (2007) supplementation trial.

Human and animal studies providing supporting information on a possible role of selenium in contributing to development of diabetes and metabolic syndrome (i.e., hyperlipidemia, hypertension and other risk factors that occur together and increase the risk for type 2 diabetes, coronary artery disease, and stroke), data on a possible protective role of selenium in diabetes, and hypotheses on pro- and anti-diabetic mechanisms of selenium, are comprehensively reviewed by Mueller et al. (2009).

The key studies in Table 6-1 and supporting data reviewed by Mueller et al. (2009) collectively suggest that a high selenium status resulting from normal diets or from long-term dietary supplementation with selenium may increase the risk of developing type 2 diabetes. The key studies used large numbers of subjects and showed increases in diabetes risk that were generally dose-related and statistically significant. Cross-sectional evaluations of adult participants in National Health and Nutrition Examination Surveys (NHANES) found that the prevalence of diabetes was increased at serum selenium levels as low as 124-138 μg Se/L, as shown by corresponding multivariable-adjusted odds ratios (ORs) of 1.57-3.18 (Bleys et al., 2007; Laclaustra et al., 2009a). The adjusted OR for diabetes increased to 3.65 and 7.64 in NHANES participants with serum levels of 134-146 and ≥147 μg Se/L, respectively (Laclaustra et al., 2009a). Evaluations of NHANES adults also found increased serum lipid concentrations (total, HDL and LDL cholesterols, triacyglycerols and apolipoproteins A-I and B) at ≥135 μg Se/L serum (Bleys et al., 2008), and increases in blood pressure levels at ≥140 μg Se/L serum and hypertension prevalence at ≥122 μg Se/L (Laclaustra et al., 2009b). Selenium intake data were not reported in the NHANES studies, although similar blood levels (>121.6 μg Se/L) and risks of type 2 diabetes [adjusted hazard ratios (HRs) of 1.55-2.70] were found in a clinical trial of adults who had long-term dietary supplementation with 200 μg Se/day (Stranges et al., 2007). Another clinical trial found that long-term dietary supplementation with 200 μg Se/day caused only a nonsignificant increase in relative risk (RR) of diabetes, although small statistically significant increases were found for dermatitis (RR=1.17) and alopecia (RR=1.28), which are two known adverse effects of selenium (Lippman et al., 2009).

6.4 TOXICITY ASSESSMENT FOR SELENIUM

The increased risk of diabetes and related effects in U.S. populations detected in recent epidemiological studies and clinical trials provides a sensitive endpoint for derivation of toxicity values for selenium. A chronic oral LOAEL of 200 μg Se/day can be identified for type 2 diabetes based on the results of the Stranges et al. (2007) clinical trial. The diabetes increase in this study was found at selenium blood concentrations that appear to be consistent with the levels

associated with increased diabetes, serum lipids, and hypertension in the NHANES studies (Bleys et al., 2007, 2008; Laclaustra et al., 2009a, 2009b). Limited additional support for a 200 µg Se/day oral LOAEL is provided by the small increases in alopecia and dermatitis at this intake level observed by Lippman et al. (2009), although 200 µg Se/day caused only a nonsignificant increase in diabetes in the Lippman et al. (2009) study. The LOAEL of 200 µg Se/day may be close to the threshold for these effects, because the risks for diabetes and other effects at this level were low, and because diabetes was clearly increased in only one of the two studies with 200 µg Se/day supplementation.

Derivation of an ADI for selenium based on the 200 µg Se/day LOAEL for diabetes is a possibility that would need to be evaluated considering various factors, particularly the proximity of the LOAEL and ADI to the selenium RDA of 55 µg/day (NAS, 2000) and typical 30-220 µg/day range of U.S. selenium intakes from diet and supplements (Laclaustra et al., 2009a, 2009b; Stranges et al., 2007).

Given the proximity of the LOAEL of 200 µg Se/day (0.003 mg Se/kg-day) to the RDA and typical U.S. selenium uptakes, an uncertainty factor (UF) for an assessment based on this LOAEL is not likely to exceed 3. Applying a UF of 3 to the LOAEL of 0.003 mg Se/kg-day would yield an ADI of 0.001 or 1E-03 mg Se/kg-day.

6.5 COMPARISON OF ADI TO EXISTING TOY STANDARD FOR SELENIUM

The ADI of 0.001 mg Se/kg-day derived here applies to total daily intake of selenium from all sources. The existing permissible intake for selenium in the European toy safety standard is 5 µg Se/day, which refers specifically to intake from toys. In order to compare these, we applied the same modifying factors that were used to derive the toy standard. Therefore, a source allocation of 10 percent was applied to the ADI, and a bodyweight of 12 kg was used to calculate a permissible intake level that is directly comparable to the existing value (0.001 mg Se/kg-day × 10% x 12 kg = 0.001 mg Se/day from toys). Comparison of the permissible intake of selenium from toys based on the ADI derived here (0.001 mg Se/day or 1 µg Se/day) and the existing permissible intake from toys (5 µg Se/day) suggests that the ADI would be approximately fivefold lower than the existing standard.

6.6 REFERENCES

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Stranges, S., J. R. Marshall, et al. (2007). Effects of long-term selenium supplementation on the incidence of type 2 diabetes: a randomized trial. Annals of Internal Medicine 147(4): 217–23.

Table 6-1. Key Human Studies Suggesting Adverse Effects of Selenium on Diabetes, Hyperlipidemia and Hypertension

Study Design	Critical Effects	NOAEL	LOAEL	Reference
Randomized, double-blind, placebo controlled clinical trial[1] evaluated the effect of Se dietary supplementation on incidence of type 2 diabetes. 1202 adult men and women ingested 0 or 200 µg Se/day (n = 602 and 600, respectively) in tablets for a mean duration of 7.7 years.	Diabetes was diagnosed in 58 Se and 39 placebo recipients; the adjusted hazard ratio (HR) was 1.55 (95% CI 1.03-2.33, p = 0.03). An exposure-response gradient was found across 3 tertiles of plasma Se levels, although the risk was significantly increased only in the highest tertile (>121.6 ng/ml) when compared to the lowest tertile (≤ 105.2 ng/ml) with an adjusted HR of 2.70 (95% CI 1.30-5.61, p = 0.008).	NA	200 µg Se/day, 122 µg Se/L plasma	Stranges et al. (2007)
Randomized, double-blind, placebo-controlled clinical trial[2] evaluated the effect of Se dietary supplementation on incidence of type 2 diabetes. Adult men ingested 0 or 200 µg Se/day (n = 8696 and 8752, respectively) in capsules for a median duration of 5.46 years.	The relative risk (RR) of diabetes was slightly but not statistically significantly increased in the Se group compared to placebo (RR = 1.07, 95% CI 0.94-1.22, p=0.16). Evaluation of adverse effects known to be associated with Se showed small and statistically significantly increased risks of alopecia (RR = 1.28, 95% CI 1.01-1.62, p<0.01) and dermatitis (grades 1-2) (RR = 1.17, 95% CI 1.00-1.35, p<0.01) in the Se group.	NA	200 µg Se/day	Lippman et al. (2009)
Cross-sectional study of the association between serum Se concentrations and type 2 diabetes in 8876 adult men and women participants in NHANES III[3].	Increased prevalence of diabetes in serum Se quintile 5 (≥ 137.66 ng/ml) compared to quintile 1 (< 111.62 ng/ml) as shown by an adjusted odds ratio (OR) of 1.57 (95% CI 1.16-2.13).	NA	138 µg Se/L serum	Bleys et al. (2007)
Cross-sectional study of the association between serum Se concentrations and diabetes in 917 adult men and women participants in NHANES 2003-2004. Associations between serum Se levels and fasting plasma glucose and glycosylated hemoglobin levels (biomarkers of diabetes) were also evaluated.	Mean serum Se levels were significantly higher in participants with diabetes than without diabetes (143.7 vs. 136.4 µg/L, p=0.001). Comparisons of serum Se quartiles 2, 3 and 4 (124-133, 134-146 and ≥147 µg/L) with the lowest quartile (<124 µg/L) showed increased ORs for diabetes of 3.18 (95% CI 1.01-9.96), 3.65 (95% CI 1.31-10.16) and 7.64 (95% CI 3.34-17.46), respectively. ORs for diabetes, plasma glucose and glycosylated hemoglobin had significant increasing linear trends.	NA	124 µg Se/L serum	Laclaustra et al. (2009a)

Table 6-1. Key Human Studies Suggesting Adverse Effects of Selenium on Diabetes, Hyperlipidemia and Hypertension

Study Design	Critical Effects	NOAEL	LOAEL	Reference
Cross-sectional study of the association between concentrations of serum Se and serum lipids in 5452 adult men and women participants in NHANES III[3].	Serum concentrations of cholesterol (total, LDL and HDL), apolipoproteins (B and A-I), and triacylglycerols were significantly increased in the highest quartile of serum Se compared to the lowest quartile (≥134.7 and <113.7 ng/mL, respectively). Levels of all serum lipids and apolipoproteins had significant increasing trends, as did ratios of LDL:HDL cholesterol and apo B:apo A-I.	NA	135 µg Se/L serum	Bleys et al. (2008)
Cross-sectional study of the association between serum Se concentrations and prevalence of hypertension in 2638 adult men and women participants in NHANES 2003-2004.	Mean serum Se levels were significantly higher in participants with hypertension than without hypertension (138.3 vs. 136.1 µg/L). Statistically significant and clinically important increases in blood pressure levels (systolic, diastolic and pulse) occurred in the two highest quintiles of serum Se compared to the lowest quintile (≥140 µg/L and <122 µg/L, respectively). The prevalence of hypertension was significantly increased in quintiles 2, 3, 4 and 5 (122-131, 132-139, 140-149 and ≥150 µg/L) compared to the lowest quintile (<122 µg/L); ORs were 1.40, 1.26, 2.00 and 1.73 and showed a significant increasing trend.	NA	122 µg Se/L serum	Laclaustra et al. (2009b)

NA = not available
[1]Nutritional Prevention of Cancer (NPC) trial (1980s-early 1990s).
[2]Selenium and Vitamin E Cancer Prevention Trial (SELECT) (2001-2004).
[3]Third National Health and Nutrition Examination Survey (NHANES) (1988-1994).

APPENDIX A

Key Study Summaries

ANTIMONY

Valli, V., R. Poon, I. Chu, S. Gupta, B. Thomas. (2000). Comment. Subchronic/chronic toxicity of antimony potassium tartrate. Regulatory Toxicology and Pharmacology 32:337–338.

Lynch, B., C. Capen, E. Nestmann, G. Veenstra, J. Deyo. (2000). Comment. Reply to Comment. Regulatory Toxicology and Pharmacology 32:9339–340.

Lynch, B., C. Capen, E. Nestmann, G. Veenstra, J. Deyo. (1999). Review of subchronic/chronic toxicity of antimony potassium tartrate. Regulatory Toxicology and Pharmacology 30:9–17.

Poon, R., I. Chu, P. Lecavalier, V.E. Valli, W. Foster, S. Gupta, B. Thomas. (1998). Effects of Antimony on rats following 90-day exposure via drinking water. Food and Chemical Toxicology 36:21–35.

Valli et al. (2000) commented on a prior review article of Lynch et al. (1999), where Lynch et al. (1999) had commented on a 1998 study of Poon et al (1998). Lynch et al. (1999) concluded that a NOAEL of 0.5 ppm for antimony potassium tartrate for environmental exposure as reported by Poon et al. (1998) was not supported by the available data and suggested that a NOEAL of 50 ppm would be more appropriate.

Lynch et al. (1999) suggested that the 0.5 ppm NOAEL was based on mild biological effects that were reversible and not necessarily indicative of overt toxicity. Valli et al. (2000) refutes this, claiming that while grading of histopathological changes was of a subjective nature, the validity of the system had been verified in previous studies. Furthermore, they note that adverse reactions should not be interpreted as physiological rather than toxic simple because they are reversible. Lynch et al. (2000) noted that Valli et al. (2000) did not provide any additional information that would support that the subtle changes reported in Poon et al. (1998) were adverse; thus, the authors continue to conclude that the changes were adaptive and physiological in nature, making them unsuitable data on which to base the NOAEL.

Lynch et al. (1999) noted that hepatic anisokaryosis and nuclear hyperchomicity, effects that Poon et al. (1998) based the 0.5 ppm NOAEL on, are a part of normal variation in hepatocytes in young rats. Valli et al. (2000) acknowledges this variation, but argues that it does not negate the findings because these effects in the exposed population were compared to a control group. Poon et al. (1998) provided evidence of tissue injury and presence of antimony in the spleen. Lynch et al. (1999) criticized these findings, again suggesting that results could be due to natural variation and/or physiological changes that could be influenced by necropsy procedures. Valli et al. (2000) refuted this claim again, stating that effects of natural variation were accounted for by comparing exposed and control populations. Furthermore, Valli et al. (2000) noted that appropriate controls and handling methods were followed during necropsy.

Valli et al. (2000) criticized Lynch et al. (1999) for failing to mention the toxicological significance of effects (increased organ weights) occurring at a dose of 1.5 ppm reported in previous literature. Lynch et al. (2000) replied that these organ weight changes were not mentioned because the original author attributed them to variable fluctuation and thus, were not biologically significant.

Valli et al. (2000) criticized Lynch et al. (1999) for favoring the results of a 13-week intraperitoneal study done by NTP over the Poon et al. (1998) study during their literature review. The authors' indicated that rats in the NTP study were dosed intraperitoneal three times a week and thus is not as relevant to the mode of antimony exposure in humans (via drinking water) which occurs many times daily. Lynch et al. (2000) responded that they agree that the Poon et al. (1998) study provides the more appropriate data for establishing drinking water criteria. They further noted that while Poon et al. (1998) is more relevant, it does not change their opinion that the Poon data was over interpreted, and the data from the NTP study was utilized to support this opinion.

ARSENIC

Ahsan, H., Y. Chen, F. Parvez, L. Zablotska, M. Argos, I. Hussain, H. Momotaj, D. Levy, Z. Cheng, V. Slavkovich, A. van Geen, G. Howe, and J. Graziano. (2006). Arsenic Exposure from Drinking Water and Risk of Premalignant Skin Lesions in Bangladesh: Baseline Results from the Health Effects of Arsenic Longitudinal Study. American Journal of Epidemiology 163(12):1138–1148.

Ahsan et al. (2006) evaluated dose-response relations between arsenic exposure from drinking water and premalignant skin lesions. A population exposed to the full-dose range of arsenic (0.1–864 μg/liter) was identified in a 25-km^2 area near Araihazar, Bangladesh, that had not been subject to prior arsenic testing or other arsenic-related research/mitigation activities. In 2000, all 5,966 tube wells in the study area were tested for arsenic, and the users of the wells were identified through an interview process. Between 2000 and 2002, 11,746 participants (5,042 men and 6,704 women) were recruited into the Health Effects of Arsenic Longitudinal Study. The participants were married, between the ages of 18 and 75 years old, had resided in the study area for at least five years, and were a primary user of one of the tube wells tested. Eighty-nine percent of study participants (n = 10,494) shared tube wells with 0 to 5 other study participants, while the remaining 14 percent shared their wells with 6 to 13 others. The participants were interviewed and clinically assessed for skin lesions and other health conditions. Biologic samples (blood and urine) were also collected. Several measures of arsenic exposure were estimated for each participant, based on well-water arsenic concentration and usage patterns of the wells and on urinary arsenic concentration. The measures of arsenic exposure were time-weighted well arsenic concentration, cumulative arsenic index, and urinary creatinine-adjusted arsenic.

There were 714 confirmed cases of premalignant skin lesions identified in the cohort. Of these cases, 421 (337 men and 84 women) had only melanosis, while the remaining 293 (247 men and 46 women) had both hyperkeratosis and melanosis. Consistent dose-response effects were observed for all three arsenic exposure measures using different regression models. Also, prevalence odds ratios (PORs) of skin lesions increased with levels of arsenic exposure. Risk was significantly higher for the exposure group with 8.1 to 40 μg/liter than for the lowest exposure group (<8.1 μg/liter). Compared with drinking water containing <8.1 μg/liter of arsenic, drinking water containing 8.1 to 40.0, 40.1 to 91.0, 91.1 to 175.0, and 175.1 to 864.0 μg/liter of arsenic was associated with adjusted PORs of skin lesions of 1.91 (95% confidence interval (CI): 1.26, 2.89), 3.03 (95 percent CI: 2.05, 4.50), 3.71 (95 percent CI: 2.53, 5.44), and 5.39 (95 percent CI: 3.69, 7.86), respectively. Males were four times more likely than females to have skin lesions (POR = 4.15, 95 percent confidence interval: 3.27, 5.26). However, when the effects of arsenic exposure with age and body mass were assessed separately in men and women, patterns of prevalence odds ratios were similar between men and women; therefore, the results for only the overall study population are given. Older age was associated positively with risk of skin lesions. As compared to the youngest age group (<30 years), the risk of skin lesions increased nearly fivefold for participants in the oldest age group (<60 years). There was a general inverse trend regarding the association between body mass index and skin lesion risk. When arsenic exposure was held constant in the analysis, cigarette smoking, hookah smoking, and markers of socioeconomic status were also associated with the risk of skin lesions in this cohort.

Cherry, N., K. Shaikh, C. McDonald, Z. Chowdhury. (2008). Stillbirth in rural Bangladesh: arsenic exposure and other etiological factors: a report from Gonoshasthaya Kendra. Bulletin of the World Health Organization 86(3):172–177.

Cherry et al. (2008) primarily examined the epidemiological pattern of stillbirth made by arsenic contaminated hand pump wells in Bangladesh. The data were collected by Gonoshasthaya Kendra, a large nongovernmental organization providing health care to some 600 villages. The study used completed pregnancies and outcomes (n = 30984) for two calendar years, together with existing data on socioeconomic and health factors. The health care in these villages was administered from 16 geographical centers; information on the average arsenic concentration in each center was obtained from the National Hydrochemical Survey. Average arsenic concentrations ranged from <1 µg/l to 81 µg/l. After univariate analysis, a multivariate model was fitted, including all factors other than arsenic, with center as a random effect. The final model without arsenic included only factors significantly associated with stillbirth at $P < 0.05$. Arsenic exposure, as dummy variables contrasting 10 µg/l to < 50 µg/l, and ≥50 µg/l with < 10 µg/l, was then added to this best multilevel model to estimate any additional risk associated with concentrations at these two higher levels, having adjusted for potential confounders.

The overall stillbirth rate was 3.4 percent (1056/30984) and increased with estimated arsenic concentration (2.96 percent at <10 µg/l; 3.79 percent at 10 µg/l to <50 µg/l; 4.43% at ≥50 µg/l). Of the factors studied, 17 socioeconomic and health factors were considered to be related to risk of stillbirth. Cherry et al. (2008) noted that the effect of arsenic was estimated, allowing all factors significantly related to stillbirth; however, the dose response between the arsenic concentration and stillbirth was not diminished. The odds ratio estimated for arsenic was adjusted for these factors. After this adjustment, the odds ratios estimated for arsenic (with <10 µg/l as reference) remained raised: 1.23 (95 percent confidence interval, CI: 0.87–1.74) at 10 µg/l to <50 µg/l and 1.80 (95 percent CI: 1.14–2.86) at 50 µg/l or greater. Cherry et al. (2008) concluded that an increased risk of stillbirth is associated with arsenic contamination and noted that efforts are needed to protect women at high risk.

Chiou, H.Y, S.T. Chiou, Y.H. Hsu, Y.L. Chou, C.H. Tseng, M.L Wei, C.J Chen. (2001). Incidence of Transitional Cell Carcinoma and Arsenic in Drinking Water: A Follow-up Study of 8,102 Residents in an Arseniasis-endemic Area in Northeastern Taiwan. American Journal of Epidemiology 153(5):411–418.

Chiou et al. (2001) investigated the dose-response relation between long-term exposure to ingested arsenic through drinking well water and the incidence of bladder cancer in the arsenic endemic area of Lanyang Basin in northeastern Taiwan. In this area, arsenic levels in the well water varied from <0.15 μg/l (undetectable) to >3,000 μg/l. Each household in Lanyang Basin has its own well, and the wells have been in use for more than 50 years. Therefore, it was possible to assess individual exposure to inorganic arsenic in a much more precise way than previous studies.

Residents aged ≥40 years were recruited into the cohort with their informed consent. A total of 8,102 residents (4,056 men and 4,046 women) agreed to participate and were interviewed in their homes between October 1991 and September 1994. Data obtained from the interview included information on history of well water consumption, residential history, sociodemographic characteristics, cigarette smoking, alcohol consumption, physical activities, and history of sunlight exposure, as well as personal and family history of hypertension, diabetes mellitus, cerebrovascular disease, heart disease, and cancer. Estimation of each study subject's individual exposure to inorganic arsenic was based on the arsenic concentration in his or her own well water, collected during the home interview. Arsenic concentration was determined by hydride generation combined with atomic absorption spectrometry. The occurrence of urinary tract cancers was ascertained by follow-up interviews and by data linkage to community hospital records, the national death certification profile, and the cancer registry profile, through December 1996.

Cox's proportional hazards regression analysis was used to estimate multivariate-adjusted relative risks and 95 percent confidence intervals. During the follow-up period, 9 subjects were afflicted with bladder cancer, 8 were afflicted with kidney cancer, and 1 was afflicted with both bladder and kidney cancer. Among the 18 study subjects with urinary tract cancer, there were 17 with pathologic confirmation data and 11 with transitional cell carcinoma (TCC). The subjects' risk of developing cancers of the urinary organs was significantly higher than the risk in the general population of Taiwan (standardized incidence ratio (SIR) = 2.05; 95 percent confidence interval (CI): 1.22, 3.24). The SIR for bladder cancer was 1.96 (95 percent CI: 0.94, 3.61), while the SIR for kidney cancer was 2.82 (95 percent CI: 1.29, 5.36).

The incidence rates for cancers of the urinary organs and TCC were further analyzed by arsenic level in well water and duration of well water drinking. The incidence rates of urinary tract cancer and TCC for subjects who drank well water with arsenic levels of 10.0, 10.1–50.0, 50.1–100.0, and 100.0 μg/l were 37.6, 44.8, 66.4, and 134.1 per 100,000 and 12.5, 14.9, 66.4, and 114.9 per 100,000, respectively; these results show a dose-response relation. A significant dose-response relation between risk of cancers of the urinary organs, especially TCC, and indices of arsenic exposure was still observed after adjustment for age, sex, and cigarette smoking. The multivariate-adjusted relative risks of developing TCC were 1.9, 8.2, and 15.3 for arsenic

concentrations of 10.1–50.0, 50.1–100, and >100 μg/liter, respectively, compared with the referent level of ≤10.0 μg/liter.

When incidence rates for cancers of the urinary organs and TCC were analyzed by duration of drinking well water, no dose-response relation was observed. The incidence rates for urinary cancer were 61.1, 46.7, and 77.3 per 100,000 for persons who had drunk well water for <20.0, 20.1–39.9, and ≥40.0 years, respectively; the corresponding incidence rates for TCC were 0, 46.7, and 46.4 per 100,000, respectively.

Ferreccio, C., C. Gonzalez, V. Milosavjlevic, G. Marshall, A. Sancha, A. Smith. (2000). Lung Cancer and Arsenic Concentrations in Drinking Water in Chile. Epidemiology 11(6):673–679.

Ferreccio et al. (2000) investigated the relation between lung cancer and arsenic in drinking water in northern Chile in a case-control study involving patients diagnosed with lung cancer between 1994 and 1996, and frequency-matched hospital controls. The study identified 152 lung cancer cases and 419 controls (167 cancer controls and 252 noncancer controls). Participants were interviewed regarding drinking water sources, cigarette smoking, occupation, and other variables.

Three regions in northern Chile were included in the study area. The population in region II experienced high exposure to inorganic arsenic in past years from natural contamination of drinking water originating in the Andes Mountains. Water sources in regions I and III contained relatively little arsenic. Using lifetime residential histories, each participant was assigned the average water arsenic concentration for the county in which they resided for each year. Average arsenic water concentrations were calculated for the years 1930 to 1994. In addition, average arsenic water concentrations were calculated for the counties of residence for 1958 to 1970, when some of the highest exposures occurred. The population-weighted average arsenic concentration for region II in these years was 578 µg/l, much higher than the population-weighted average of 212 µg/l for the second highest concentration period, which was from 1971 through 1977. The combined control group had been exposed to an average arsenic concentration of 109 µg/liter in drinking water between 1930 and 1994, and an average of 280 µg/liter for the peak exposure period 1958 to 1970.

Lifetime (1930 to the present) average arsenic exposure was evaluated as a categorical variable with five exposure strata. Peak exposures on the basis of the average water concentration for each participant in 1958 to 1970 were evaluated. Lowest exposure categories were used as references to calculate odds ratios (ORs). Unconditional regression analyses were conducted using StataCorp statistical software, adjusting for age, sex, socioeconomic status, smoking, and working in a copper smelter.

Logistic regression analysis revealed a clear trend in lung cancer odds ratios and 95 percent confidence intervals (CIs) with increasing concentration of arsenic in drinking water, as follows: 1, 1.6 (95 percent CI = 0.5–5.3), 3.9 (95 percent CI = 1.2–12.3), 5.2 (95 percent CI = 2.3–11.7), and 8.9 (95 percent CI = 4.0 –19.6), for arsenic concentrations ranging from less than 10 µg/l to a 65-year average concentration of 200–400 µg/l. There was evidence of synergy between cigarette smoking and ingestion of arsenic in drinking water; the odds ratio for lung cancer was 32.0 (95% CI = 7.2–198.0) among smokers exposed to more than 200 µg/l of arsenic in drinking water (lifetime average) compared to nonsmokers exposed to less than 50 µg/l. Comparatively, the OR for nonsmokers in the highest arsenic exposure category was 8.0, and the OR for smokers in the lowest arsenic-exposure category was 6.1. Ferreccio et al. (2000) concluded that this study provides evidence that ingestion of inorganic arsenic is associated with human lung cancer.

Guo, X., Z. Liu, C. Huang1, L. You. (2006). Levels of Arsenic in Drinking-water and Cutaneous Lesions in Inner Mongolia. Journal of Health and Popular Nutrition 24(2):214-220.

Guo et al. (2006) investigated the relationship between levels of arsenic in drinking-water and cutaneous lesions in Inner Mongolia, PR China. The study examined the association between the prevalence of keratosis and pigment disorder and levels of arsenic exposure among villagers aged 18 years or older in the arsenic-affected village of Hetao Plain.

Subjects (n=448) were surveyed regarding the participants' residential history, socio-demographic conditions, sources of water at each residence, duration of tube-well usage, amount of water consumed daily, occupation, working conditions, and health-related lifestyle habits, such as smoking and alcohol consumption. After the interviews, subjects underwent a skin examination by a dermatologist; skin disorders were diagnosed using established clinical criteria. The study included 227 participants who were affected by cutaneous lesions (n=162 with keratosis and n=65 with both pigment disorder and keratosis) and 221 participants who were not affected by cutaneous lesions, as diagnosed in 1996 and 1998.

At the time of investigation, the geological team collected samples (n=106) from all wells in the village. The samples were analyzed for total concentration of arsenic using Ag-DDC analysis. The concentrations of arsenic in water in the village ranged from nondetectable to 1,354 μg/L. Most participants in the study started using tubule-type wells as a water source in the 1980s.

To evaluate the relationship between various cutaneous lesions and levels of arsenic in water, the prevalence of keratosis and pigment disorder was calculated separately. Arsenic-contamination levels in water were classified into four categories: secure level (<50 μg/L), low level (50–199 μg/L), middle level (200–499 μg/L), and high level (≥500 μg/L). Logistic regression analyses; adjusted for age, sex, and smoking; were performed to estimate odds ratios and their 95 percent confidence intervals for each level of arsenic.

Mean arsenic exposure levels increased from 207 μg/L for subjects without skin lesions (n=221), to 240 μg/L for subjects with keratosis (n=162), to 371 μg/L for subjects with pigment disorder (n=62). The ratios of cutaneous lesions increase from 34.2 percent for arsenic levels less than 50 μg/L to 66.7 percent for arsenic concentrations of ≥500 μg/L. The results from the logistic regression showed that, with the increase of arsenic concentration in water, the risk of pigment disorder also increased. The sex-, age- and smoking-adjusted odds ratios were 5.25 (95 percent CI 1.32-83.24), 10.97 (95 percent CI 1.50-79.95) and 10.00 (95 percent CI 1.39-71.77) (p=0.000) for 50–199, 200–499, and ≥500 μg/L, respectively. The association between risk of keratosis and levels of arsenic was not significant (p=0.346). The authors indicate that data suggests keratosis is an early feature of arsenic poisoning, and the development of pigment disorder depends on higher doses of arsenic intake rather than keratosis. The authors concluded that further studies are needed to confirm that cutaneous lesions and other adverse health effects occur at low levels of arsenic exposure.

Guo, X., Y. Fujino, X. Ye, J. Liu, T. Yoshimura, and Japan Inner Mongolia Arsenic Pollution Study Group. (2006). Association between Multi-level Inorganic Arsenic Exposure from Drinking Water and Skin Lesions in China. International Journal of Environmental Research and Public Health 3(3):262–267.

Guo et al. (2006) evaluated the association between multi-levels of inorganic arsenic exposure from drinking water and skin lesions in an arsenic-affected area in Inner Mongolia, China. The subjects (adults aged 18 and over) included 109 people from an arsenic-affected village with high arsenic concentration in drinking well water (>50 μg/liter) and 32 people from a neighboring village with low arsenic concentration in drinking well water (<50μg/liter; control). Subjects were surveyed regarding socio-demographic conditions, residential history, occupation, working conditions, duration of using tube-well and health-related lifestyle habits, such as smoking and alcohol consumption. After the interviews, subjects underwent a skin examination by an expert Chinese physician; skin disorders were diagnosed using established clinical criteria.

The field team collected water samples from all tube wells used by participants for at least six months in the last 20 years. Samples were collected from all tube wells in the villages studied (n=49 in the high arsenic concentration village and n=7 in the low arsenic concentration village). Total inorganic arsenic (iAs) was determined using an atomic absorption spectrophotometer.

Information about tube well usage at each residence and work site and the results of the arsenic measurements were used to construct arsenic exposure histories. Descriptive analyses were conducted by comparing general characteristics, mean arsenic concentrations, and the number of tube wells used by the subjects. Logistic regression was conducted to calculate the odds ratio (OR) of skin lesions associated with arsenic exposure with adjustments for sex, age group, smoking, and duration of exposure. The multilevel of exposure was classified into four groups: ≤50, 51-99, 100-149, and >150 μg/liter. The ORs of exposure duration for arsenic dermatosis were also estimated, dividing the duration of exposure into three levels (less than 6 years, 6 to 15 years, and more than 15 years).

Arsenic-induced skin lesions, including keratosis, pigmentation, and/or depigmentation were diagnosed in 56 and 3 subjects in the arsenic-affected and control villages, respectively. A consistent dose-response relationship between arsenic exposure level and skin lesion risk was observed. Compared to those with iAs concentration <50μg/liter, the adjusted odds ratios of skin lesions for the subjects with 51-99, 100-149 and >150μg/liter were 33.3 percent (OR =15.50, 95 percent CI: 1.53-248.70), 46.7 percent (OR =16.10, 95 percent CI: 3.73-69.63) and 55.7 percent (OR= 25.70, 95 percent CI: 6.43-102.87), respectively. Duration of using well was not associated with increased risk of skin lesions in this population; (OR =1.68, 95 percent CI: 0.40-6.91 for 6-15 years, OR = 2.30, 95 percent CI: 0.58-9.14 for more than 15 years) compared with the duration of less than 5 years. Associations among skin lesions risk and smoking and drinking did not occur.

Guo, X., Y. (2007). Cancer Risks Associated with Arsenic in Drinking Water. Environmental Health Perspectives 115(7):A339-A340.

Guo (2007) commented on the reanalysis of data by Lamm et al. (2006). Lamm et al. (2006) conducted a cancer risk assessment for arsenic in drinking water using data from six townships in southwest Taiwan. Lamm et al. (2006) reported a "township" cofounder, in which three of the six townships studied (2, 4, and 6) showed positive dose-response relationships with arsenic exposure and the three other (1, 3, and 5) showed cancer risks independent of arsenic exposure.

Lamm et al. (2006) were unable to identify the individual townships; however, Guo (2007) was able to identify these townships based on data collected in previous studies. Four of the townships analyzed have the highest prevalence of Blackfoot disease (BFD) in the country. Guo (2007) states that there indeed might be a "township factor" because all three of the townships affected by this factor were in the endemic area. One township that was not affected by the factor was in the endemic area.

Lamm et al. (2006) had indicated that bias may have occurred with water sampling because villages with high BFD prevalence may have been selected intentionally for sampling. Gou (2007) stated that the chance of bias occurring in the selection of the villages sampled within the townships was small, because almost all of the villages in the Kuo (1968) survey were covered.

Guo (2007) agreed with Lamm et al. (2006) in that their findings of a threshold-like model indicates no increase of bladder cancer with exposure <150 µg/L. Guo (2007) noted that this is consistent with other studies covering all of Taiwan and southwest Taiwan only.

Guo (2007) noted that the use of a median arsenic level by Lamm et al. (2006) as the exposure indicator might not generate accurate results because villages with similar median arsenic levels can have very different distributions of exposures.

Lamm, H., M. Feinlab, R. Chen. (2007). Cancer Risks Associated with Arsenic in Drinking Water. Environmental Health Perspectives 115(7):A340-A341.

Lamm et al. (2007) responded to comments from Guo (2007) regarding reanalysis of data in Lamm et al. (2006). Lamm et al. (2006) conducted a cancer risk assessment for arsenic in drinking water using data from six townships in southwest Taiwan. Lamm et al. (2006) reported a "township" cofounder, in which three of the six townships studied (2, 4, and 6) showed positive dose-response relationships with arsenic exposure, and the three other (1, 3, and 5), showed cancer risks independent of arsenic exposure.

With regard to three townships (1, 3, and 5) showing cancer risks independent of arsenic exposure, Lamm et al. (2007) state that they are less inclined to believe that the "township" factor is related to Blackfoot disease.

Lamm et al. (2007) examined whether using alternative exposure indictors would affect the accuracy of the risk results. When using the mean instead of the median arsenic exposure, similar results were obtained.

Lamm et al. (2007) stated that the analytical findings are robust. They best fit a nonlinear or threshold carcinogenic risk model for arsenic with an inflection point of 150 μg/L with the presence of at least one confounding risk factor. Lamm et al. (2007) state that further deciphering of the village code will be conducted and that interpretation of the data would be made with caution as the Wu et al. (1989) data only contains data for about one-third of the villages in the six-township area.

NOTE:

1. A separate summary has been provided for Lamm et al. (2006).

Lamm, S.H., A. Engel, C.A. Penn, R. Chen, M. Feinlab. (2006). Arsenic Cancer Risk Confounder in SW Taiwan Dataset. Environmental Health Perspectives 1-37 (online January 13, 2006).

2. Citation for Wu et al. (1989).

Wu MM, Kuo TL, Hwang YH, Chen CJ. (1989). Dose-response relation between arsenic concentration in well water and mortality from cancer and vascular diseases. Am J Epidemiol 130:1123 -1132.

Hopenhayn, C., H. Bush, A. Bingcang, I. Hertz-Picciott. (2006). Association between arsenic exposure from drinking water and anemia during pregnancy. J Occup Environ Med 48(6):635–643.

Hopenhayn et al. (2006) investigated the association between exposure from arsenic contaminated drinking water and development of anemia during pregnancy. This study examined the rates of anemia over time in a cohort of pregnant women, which included participants from two cities in Chile with contrasting drinking water arsenic levels: Antofagasta with an average of 40 μg/L (range, 33–53 μg/L), and Valparaíso <1 μg/L.

A total of 810 pregnant women, ranging in age from 18 to 45 years, living in the cities of Antofagasta and Valparaíso, were recruited from the Chilean public health care system for maternal and child health. Study subjects completed in-depth interviews, including questions on sociodemographic characteristics, lifestyle and dietary habits, vitamin and fluid consumption, medical and previous pregnancy history, self-perceived stress, employment history, and paternal exposures. Pregnancy and birth information was obtained from medical records. The medical records included information on Hgb and hematocrit (Hct) measurements from routine blood sample analyses conducted during pregnancy, as well as other relevant pregnancy and birth information. A subgroup of women (75 from each city) also participated in a blood biomarker study for which they provided a blood sample, generally during their last trimester of pregnancy. Serum samples were analyzed for various markers, including foliates and transferrin receptor (TfR) concentrations. The participants were women who gave birth to live, singleton infants and had at least one hemoglobin determination during pregnancy. Hgb and Hct measurements were classified into trimesters with 490, 340, and 514 women with measurements in trimester 1, 2, and 3, respectively.

Bivariate analyses were conducted to evaluate the association of the general demographic, lifestyle and pregnancy characteristics of the study group by exposure group, according to city of residence, and the prevalence of anemia by different levels of each covariate were compared. The criteria for determining potential confounding variables to be considered for the multivariable adjusted analysis were based on an association with both the city residence (exposure) and the prevalence of anemia for any trimester (outcome of interest). The multivariable analysis included city as the predictor variable and the following covariates: iron supplementation, BMI, marital status, PNC, parity, and education. Arsenic-exposed women were more likely to be anemic during pregnancy after adjusting for other factors.

In both cities, an upward trend in the percent of anemia was observed as pregnancy progressed; the rate increase in Antofagasta (exposed city) was significantly higher than in Valparaíso. Slightly more women in Antofagasta (11 percent) were anemic than in Valparaíso (7 percent) ($P = 0.12$) during the first trimester. The rates of anemia increased during the second trimester to 29 percent (Antofagasta) and 12 percent (Valparaíso); and by the third trimester, the percent of anemia in Antofagasta (49 percent) was nearly three times that of Valparaíso (17 percent). Hopenhayn et al. (2006) concluded that women who received moderate levels (40μg/L) of arsenic in drinking water were more likely to develop anemia, particularly in the last trimester.

Hopenhayn, C., C. Ferreccio, S. Browning, B. Huang, C. Peralta, H. Gibb, I. Hertz-Picciott. (2003). Arsenic exposure from drinking water and birth weight. Epidemiology 14(55): 593–602.

Hopenhayn et al. (2003) investigated the association between drinking water arsenic exposure and fetal growth, as manifest in birth weight. The primary aim of this study was to compare reproductive outcomes of pregnant women from two large Chilean cities with contrasting arsenic concentrations in their public drinking water.

Pregnant women, 18 to 45 years of age, living in Antofagasta and Valparaíso were recruited from the Chilean public health care system for maternal and child health. The eligible study subjects (n = 844) completed in-depth interviews including questions on sociodemographic characteristics, lifestyle and dietary habits, vitamin and fluid consumption, medical and previous pregnancy history, self-perceived stress, employment history, and paternal exposures. Pregnancy and birth information was obtained from medical records. The birth weight analysis was restricted to live born, singleton infants born between December 1998 and February 2000. The participants had resided in the selected cities for the past 12 months and would continue to reside in the cities for the next 12 months. The final study group consisted of 424 infants from Antofagasta and 420 from Valparaíso.

Water was sampled in 1998, where tap water samples were obtained on two different days from three households per city. Mean inorganic arsenic concentrations in Antofagasta were 42 µg/L (range = 32.9–52.7) and <1 µg/L (0.5–1.1); in Valparaíso (quantitation limit =0.5 µg/L). Urine samples were collected from the study participants and urinary arsenic concentrations were utilized as biologic confirmation of exposure consistent with each city's arsenic water levels. Total urinary inorganic arsenic averaged 54.3 µg/L (SD =33.8) in Antofagasta, and 5.3 µg/L (SD = 3.3) in Valparaíso, confirming the contrast in exposure between the two cities.

Differences in mean birth weights were examined using bivariate analysis for the exposures and covariates potentially related to the exposure of interest. Analysis of covariance was used to estimate the effect of city of residence on infant birth weight, while adjusting for confounding variables. Based on final multivariable analyses, residence in Antofagasta was associated with a reduction in birth weight of -57 g (95 percent CI: -122 to 9). The difference between the crude and adjusted birth weight analysis was due primarily to the following parameters in sequential order: adequacy of prenatal health care, parity, BMI, and income. When these parameters were removed from the multivariable model, the effect of city on birth weight dropped to -6 g.

Using individual arsenic exposure in the analysis, the estimate was -0.26 g birth weight per µg of arsenic (95 percent CI = -0.85, 0.31), which at the mean Antofagasta water intake of 2.3 L (almost 100 µg arsenic/day) would represent -26 g (95 percent CI -85, 31). For birth weight divided into 2 gestational age periods, the effect of city as a measure of exposure is twice as strong among early deliveries (GA ≤38 weeks vs. >38 weeks), but the interaction of gestational age and birth weight in the multivariable model was not statically significant (P =0.74). The authors noted that this interaction needs further investigation with a larger sample size.

Hopenhayn et al. (2003) stated: “this study suggests that moderate arsenic exposures from drinking water (<50 μg/L) during pregnancy are associated with reduction in birth weight, similar in magnitude to that resulting from other environmental exposures such as environmental tobacco smoke and benzene.”

“Although there has been much research on effects of environmental arsenic, most have been in highly exposed populations and few have included reproductive outcomes. Our findings suggest the possibility that arsenic reduces intrauterine growth at moderate exposure levels. The stronger association in those of lower gestational age underscores the potential for adverse consequences of lowered birth weight. However, the clinical importance of these results, or their implications for other possible effects on the fetus, remains to be determined” (Hopenhayn et al., 2003).

Hsieh, F., T. Hwang, Y. Hsieh, H. Lo, C. Su, H. Hsu, H. Chiou, C. Chen. (2008). Risk of erectile dysfunction induced by arsenic exposure through well water consumption in Taiwan. Environ Health Perspect 116:532–536.

Hsieh et al. (2008) investigated whether exposure to arsenic enhances the risk of erectile dysfunction (ED). Many risk factors are associated with ED, such as aging, sex hormone levels, hypertension, cardiovascular diseases, and diabetes mellitus. Arsenic exposure has been reported to damage peripheral vessels and cardiovascular disease, potentially increasing the risk of ED.

Male subjects ≥ 50 years of age, who lived in a confirmed arsenic-endemic area of Lanyang Basin in Taiwan, were recruited, resulting in 66 participants for the first group. A second group, 111 males ≥ 50 years of age, represented a non-arsenic-endemic area were recruited through health examinations in Taipei Wan-Fang Hospital and Taipei Medical University Hospital in 2003.

A self-completed questionnaire was utilized to determine demographic characteristics (age, marital status, occupation, and education); lifestyle factors (cigarette smoking; alcohol, tea, or coffee drinking; and physical activity); disease record, and erectile function IIEF-5 (International Index of Erectile Function-5) scores. The IIEF-5 scores were used to assign the final study participant groups: ED (n = 129) participants and control group (n = 48). Nonfasting blood samples were drawn by venipuncture from each study subject during health screening. Sex hormones, including total testosterone and sex hormone-binding globulin, were determined by radioimmunoassay from these samples. Well water samples were collected during the home interview. Arsenic concentrations in well water ranged from undetectable (< 0.15 ppb) to $3.59x10^3$ ppb.

The prevalence of ED was greater in the arsenic-endemic area (83.3percent) than was in the non-arsenic-endemic area (66.7 percent). No significant difference was found in the distributions of age, hypertension, diabetes mellitus, and cardiovascular disease between study subjects from these two areas. Lower average levels of testosterone and free testosterone were found in subjects with arsenic exposure > 50 ppb, when ED was not considered.. Subjects with arsenic exposure > 50 ppb [odds ratio (OR) = 3.4 (95 percent CI, 1.1–10.3)] or free testosterone < 0.23 nmol/L [OR = 4.8 (95 percent CI, 1.3–18.0)] possessed significant risk of ED. After adjusting for serum free testosterone and traditional risk factors of ED, arsenic exposure still enhanced the risk of developing ED (OR = 3.0; 95 percent CI, 1.0–9.2). A drastically enhanced OR (7.5; 95 percent CI, 1.8–30.9) for severe ED in subjects with arsenic exposure > 50 ppb was observed after adjusting for age, free testosterone, diabetes mellitus, and cardiovascular disease. Hsieh et al. (2008) reported that results of the study suggested that chronic arsenic exposure has a negative impact on erectile function. “The potential pathways of arsenic exposure leading to ED include the inhibition of the sex hormone level, or reduction of NOS activity to impair the functions of penile smooth muscle and blood vessels” (Hsieh et al., 2008).

Kazi, T.G., M.B. Arain, J.A. Baig, M.K. Jamali, H.I. Afridi, N. Jalbani, R.A. Sarfraz, A.Q. Shah, A. Niaz. (2009). The correlation of arsenic levels in drinking water with the biological samples of skin disorders. Science of the Total Environment 407:1019-1026.

Kazi et al., (2009) evaluated the relationship between arsenic levels in drinking water (ground and surface) and biological samples (scalp hair and blood) collected from arsenic-affected villages situated on the banks of Manchar Lake in Pakistan. A previous study showed that the lake water was anthropogenically contaminated with arsenic, and inhabitants of the villages near the lake had been exposed to arsenic via consumption of unsafe drinking water for a long period of time. The study population consisted of 187 adults ranging in age from 20 to 45 years (95 males and 92 females). Scalp hair and blood samples were collected from adults with skin lesions (n=125) and without (n=62) skin lesions. For comparison purposes, biological samples were also collected from 121 referent subjects from areas having low arsenic levels in drinking water (<10 μg/L). Lake surface water, groundwater, and municipal treated water were collected on a monthly basis between 2004 and 2007, and measured for arsenic contamination.

Total arsenic concentration in the lake and groundwater were observed in the range of 35.5 to 157 μg/L and 23.3 to 96.3 μg/L, respectively, while the average arsenic concentration in drinking water supplied by the municipal treatment plant was 8.4±1.9 μg/L. Average arsenic concentrations in hair from male and female referent subjects were 0.43±0.17 μg/g and 0.58±0.16 μg/g, respectively. These concentrations were higher than the range of permissible values (0.08 to 0.25 μg/g). The average arsenic concentrations in hair from exposed male and female subjects with skin disorders (2.68±0.83 μg/g and 2.77±0.60 μg/g, respectively) were significantly higher than exposed male and female subjects without skin disorders (1.74±0.37 μg/g and 1.58±0.54 μg/g, respectively). Average arsenic concentrations in blood from male and female referent subjects were 1.75±0.58 μg/L and 1.39±0.25 μg/L, respectively. The average arsenic concentrations in blood from exposed male and female subjects with skin disorders (8.23±1.68 μg/L and 8.72±1.70 μg/L, respectively) were significantly higher than exposed men and women without skin disorders (5.94±0.94 μg/L and 6.13±1.06 μg/L, respectively). The linear regressions showed good correlation between arsenic concentrations in water versus hair and blood samples of exposed skin-diseased subjects as compared to nondiseased subjects, respectively.

Clinical complications of human arsenic toxicity, including skin disorders, were observed in the affected population of the villages situated on the banks of Manchar Lake in Pakistan. Some additional clinical features, such as weakness and muscle cramps, respiratory problems, anemia, and gastrointestinal problems were also observed. The results from this study indicated a good correlation between arsenic concentrations in biological samples (scalp hair and blood) of skin-diseased subjects and intake of arsenic contaminated drinking water.

Knobeloch, L.M., K.M. Zierold, H.A. Anderson. (2006). Association of Arsenic-contaminated Drinking water with Prevalence of Skin Cancer in Wisconsin's Fox River Valley. J Health Popul Nutr 24(2):206–213.

Knobeloch et al., (2006) provides results from a study conducted by the Wisconsin Division of Public Health between July 2000 and January 2002. Residents within 19 rural townships in Winnebago and Outagamie counties were asked to collect well water samples from their private drinking water wells and complete a questionnaire regarding residential history, consumption of drinking water, and family health. In total, 2,233 families comprising of 6,669 residents, aged less than one year to 100 years, provided well water samples and completed the questionnaire. This represented 78 percent of the 2,849 families that participated in the well water testing program. Of the 6,669 residents, 51 percent were male, and 49 percent were female.

Prior to 2001, the maximum contaminant level (MCL) for arsenic in drinking water was 50 µg/L. The EPA recently lowered this standard to 10 µg/L. The well water arsenic levels ranged from less than 1.0 to 3,100 µg/L, with a median arsenic level of 2.0 µg/L. Overall, 920 (41 percent) of the wells that were sampled contained no detectable arsenic and 866 (39 percent) of the wells contained an arsenic concentration between 1 µg/L and 9.9 µg/L. The remaining 447 wells (20 percent) contained arsenic concentrations equal to or greater than the current EPA standard of 10 µg/L. Data from the surveys were analyzed statistically using SAS. Only those persons who were 35 years or older and were exposed to their well water for 10 years or more were included in the analysis (n=2,131). The majority of these people (57 percent) had a detectable level of arsenic in their well water, and 34 percent had an arsenic level of >5 µg/L. One hundred eighteen (3.47 percent) of these people reported a diagnosis of skin cancer. Adults whose well water samples contained an arsenic concentration of ≥10 µg/L reported the highest rate of skin cancer and were significantly more likely to report skin cancer than those whose water-arsenic levels were <1.0 µg/L. Tobacco use was also associated with higher rates of skin cancer, and arsenic exposure seemed to enhance the effect of smoking on skin cancer risk. Among smokers, the rates of skin cancer increased three-fold as the arsenic level in their drinking water increased from <1.0 to >1.0 µg/L.

Kwok, R.K., P. Mendola, Z.Y. Liu, D.A. Savitz, G. Heiss, H.L. Ling, Y. Xia, D. Lobdell, D. Zeng, J.M. Thorp, J.P. Creason, J.L. Mumford. (2007). Drinking water arsenic exposure and blood pressure in healthy women of reproductive age in Inner Mongolia, China. Toxicology and Applied Pharmacology 222:337–343.

Kwok et al. (2007) conducted a cross-sectional study of 8,790 women who recently had been pregnant in an area of Mongolia, China, known to have a gradient of exposure to arsenic-contaminated drinking water. The study characterized associations between drinking water arsenic concentrations and elevated six-week post partum blood pressure levels in these women. All health data used in this study were collected from a database of routine prenatal and postpartum healthcare visits. All women had a pregnancy outcome between December 1, 1996 and December 31, 1999. Demographic information, such as age and body weight, was collected during the first prenatal visit. Postpartum information was collected six weeks after delivery. Of the 8,790 women (referred to as the overall population), 5,530 were missing age and body weight information, resulting in an adjusted population of 3,260 women.

The drinking water arsenic exposure of each individual was determined based on a total of 14,866 well water measurements in 2,270 sub-villages, measured throughout the study area. Exposure was assessed retrospectively by matching subjects to the arsenic well water database. The exposure categories were as follows: below the limit of detection to 20; 21 to 50; 51 to 100; and >100 μg of As/L. Univariate statistics were calculated for systolic and diastolic blood pressure and assessed for normality. This study observed increased systolic blood pressure levels with increasing drinking water arsenic, at lower exposure levels than previously reported in the literature. As compared to the referent category (below limit of detection to 20 μg of As/L), the overall population (n=8,790 women) mean systolic blood pressure rose 1.29 mm Hg (95% CI 0.82, 1.75), 128 mm Hg (95 percent CI 0.49, 2.07), and 2.22 mm Hg (95 percent CI 1.46, 2.97) as drinking water arsenic concentration increased from 21 to 50, 51 to 100 and >100 μg of As/L, respectively. Controlling for age and body weight (n=3,260 women), the population mean systolic blood pressure rose 1.88 mm Hg (95 percent CI 1.03, 2.73), 3.90 mm Hg (95 percent CI 2.52, 5.29), and 6.83 mm Hg (95 percent CI 5.39, 8.27) as drinking water arsenic concentration increased from 21 to 50, 51 to 100 and >100 μg of As/L, respectively. As for diastolic blood pressure effect, while statistically significant, was not as pronounced as systolic blood pressure. Mean diastolic blood pressure rose 0.78 mm Hg (95 percent CI 0.39, 1.16), 1.57 mm Hg (95 percent CI 0.91, 2.22) and 1.32 mm Hg (95 percent CI 0.70, 1.95), respectively for the overall population, and rose 2.11 mm Hg (95 percent CI 1.38, 2.84), 2.74 mm Hg (95 percent CI 1.55, 3.93), and 3.08 mm Hg (95 percent CI 1.84, 4.31), respectively, for the adjusted population (n=3,260 women) at drinking water arsenic concentrations of 21 to 50, 51 to 100, >100 μg of As/L.

Lamm, S.H., A. Engel, C.A. Penn, R. Chen, M. Feinleib. (2006). Arsenic Cancer Risk Confounder in SW Taiwan Dataset. Environmental Health Perspectives 1-37 (online January 13, 2006).

Lamm et al. (2006) performed a quantitative analysis for the risk of human cancer from the ingestion of inorganic arsenic based on the reported cancer mortality experienced in the Blackfoot Disease (BFD) endemic area of Southwest Taiwan. Southwest Taiwan has been the site for health studies for more than 45 years because of the associations between high arsenic levels in local artesian wells and a variety of diseases, including cancers. This study expanded a dataset from a previous dataset (Wu et al., 1989), by adding township and well arsenic level information published in NRC (1999). The Wu et al. (1989) study examined the 1973 to 1986 cancer mortality for 42 villages. The Lamm et al. (2006) study examined the dose-response relationship for both the low-dose villages and high-dose villages for possible explanatory variables, including township, apart from arsenic level.

Linear regression analyses of the village data was performed with the village standardized mortality ratios (SMRs) as independent observations and with median village well arsenic level as a continuous predictor. Stratified analyses were based on township (individual or grouped), number of wells per village (one vs. multiple), artesian well dependency, and exposure strata (low vs. higher; <0.13 ppm vs. >0.25 ppm). The outcomes analyzed in this study are the 1973 to 1986 bladder and lung cancer mortality (age 20+) experiences (SW Taiwan-based SMRs) of the 42 study villages. This dataset comprises 490,929 person-years of observation (age 20+) and 441 bladder or lung cancer deaths (175 bladder and 266 lung cancer deaths). The primary exposure variable is the median village well water arsenic level, which represents one well for 20 villages and multiple wells (2 to 47) for the other 22 villages. Linear regression analysis shows that arsenic as the sole etiologic factor, accounts for only 21 percent of the variance in the village standardized mortality ratios (SMRs) for bladder and lung cancer. This was a six Township study with Townships labeled as 0, 2, 3, 4, 5, and 6. Only three Townships (2, 4, and 6) showed a significant positive dose-response relationship with arsenic exposure. The other three Townships (0, 3, and 5) demonstrated significant bladder and lung cancer risks that were independent of arsenic exposure.

The data for bladder and lung cancer mortality for Townships 2, 4, and 6 fit an inverse linear regression model ($p<0.001$) with an estimated threshold at 151 µg/L (95 percent CI, 42 and 229 µg/L). Such a model is consistent with epidemiological and toxicological literature for bladder cancer. Exploration of the SW Taiwan cancer mortality dataset has clarified the dose-response relationship with arsenic exposure by separating out township as a confounding factor. Certain townships in the study demonstrated a significantly increased cancer risk at low-dose exposures that is independent of the village arsenic exposure levels. Removal of the data confounded by the "township factor" reveals an underlying dose-response curve for bladder and lung cancer mortality and arsenic level (median village well arsenic level) that displays as a "threshold-like" model.

Liao, C.M., T.L. Lin, S.C. Chen. (2008). A Weibull-PBPK model for assessing risk of arsenic-induced skin lesions in children. Science of the Total Environment 392:203-217.

Liao et al. (2008) quantified the risk of skin lesions on children from arsenic in drinking water and estimated safe drinking water arsenic guidelines based on reported arsenic epidemiological data. The analyses were conducted by linking a physiologically-based pharmacokinetic (PBPK) model and the Weibull dose-response function. The dataset evaluated was from Guha Mazumder et al. (1998). The dataset is a cross-sectional survey conducted between April 1995 and March 1996, for the population in the arseniasis-endemic area of West Bengal, India. Water arsenic levels were obtained for 7,683 people (4,093 females and 3,590 males) in this area. A standard questionnaire was used to collect information, including sources of drinking water, current diet and water intake, medical symptoms, height and weight. Additionally, a general medical examination was given. Based on this analysis of the dataset, Guha Mazumder et al. (1998) reported that the age-adjusted prevalence of keratosis was strongly related to arsenic levels in water, and the findings were similar for hyperpigmentation with strong dose-response relationships. Datasets from other areas, such as Bangladesh and the BFD-endemic area in Taiwan, were also evaluated.

Liao et al. (2008) calculated odds ratios to assess the relative magnitude of the effect of the arsenic exposure on the likelihood of the prevalence of skin lesions in children at a particular setting by calculating proposed Weibull-based prevalence ratios of exposed groups to control groups associated with the age group-specific PBPK model predicted dimethylarsinite (MMA(III)) levels in urine. Positive relationships between arsenic exposures and cumulative prevalence ratios of skin lesions were found using Weibull-dose-response model. By fitting the Weibull model to arsenic epidemiological data, the results indicate that male skin lesions have the highest r^2 values (0.94 to 0.96) than those of female skin lesions (r^2=0.91). The fitted Weibull model was used to further estimate the age-specific safe drinking water arsenic concentrations with excess risk of 10^{-3} and age-specific median daily drinking water uptake rates of 0.65, 1.29, 1.75, and 2.22 L/day, respectively for four age groups (0 to <1, 1 to 6, 7 to 12 and 13 to 18 years of age). Safe drinking water arsenic standards were recommended to be 2.2 and 1 μg/L for male and 6 and 2.8 μg/L for female in 0–6 and 7–18 years age groups, respectively, based on hyperpigmentation with an excess risk of 10^{-3} for a 75-year lifetime exposure.

The overall predicted odds ratio distributions of children skin lesions gave the mean estimates with 95 percent confidence interval (CI) of 2.7 (1.26-4.95), 2.47 (1.29-4.33) and 5.92 (1.97-16.13), 4.92 (2.08-12.57) for male and female, respectively, in West Bengal, India, and Bangladesh; whereas, the risk in southwestern Taiwan was larger and had wider ranges of 95 percent CI for both males (8.05 (3.58-16.24)) and females (6.56 (3.61-12.65)). Findings also suggest that increasing urinary monomethylarsonic acid (MMA) levels are associated with an increase in risks of arsenic-induced children skin lesions.

Liao, C.M., H.H. Shen, C.L., Chen, L.I., Hsu, T.L. Lin, S.C. Chen, C.J. Chen. (2009). Risk assessment of arsenic-induced internal cancer at long-term low dose exposure. Journal of Hazardous Materials 165:652–663.

Liao et al. (2009) provided an integrated approach to assess health effects of long-term low-dose exposures by linking the Weibull dose-response function and a physiologically based pharmacokinetic (PBPK) model to estimate the reference arsenic guideline. The proposed epidemiological data in this study are based on an eight year follow-up study done by the Blackfoot Disease Study Group (BDSG), which provided data on 10,138 residents in arseniasis-endemic areas in southwestern and northeastern Taiwan. The BDSG used a standardized questionnaire interview to collect information including arsenic exposure, cigarette smoking, and alcohol consumption, as well as other risk factors, including sociodemographic characteristics, residential and occupational history, and history of drinking well water. Residents in the southwestern endemic area had consumed artesian well water (100 to 300 m in depth) for more than 50 years before the implementation of the tap water supply system in the early 1960s. The estimated amount of ingested arsenic mainly from drinking water was ≥1 mg/day in this area. Residents in the northeastern endemic area had consumed water from a shallow well (<40 m in depth) since the late 1940s through the early 1990s. Arsenic levels in well water in the northeastern Lanyang Plain ranged from <0.15 to >3,000 μg/L. The incorporation of external exposure concentration to internal exposure concentration was considered in the age-stage PBPK model. The arsenic exposure-response relationship was transformed into internal dose-based response function by incorporating the PBPK model into the Weibull model to account for the variability of risk estimates and reference arsenic guideline based on drinking water uptake rate distribution.

Results indicated that bladder cancer has the highest r^2 values (>0.85) for all genders than those of lung (nearly 0.6) and liver (<0.5) cancers, respectively. For bladder cancer, higher r^2 values reveal a significant association of cumulative incidence ratios with arsenic concentration and age (the duration of water consumption) (male r^2 = 0.86 and female r^2=0.87). The Weibull dose-response surface for bladder cancer and the cumulative incidence ratio was positive in proportion of arsenic concentration in drinking water and age. For lung cancer, the average r^2 value is nearly 0.6 (male r^2=0.67 and female r^2=0.58), indicating that arsenic exposure concentration is not the only influencing factor for lung cancer incidence (Liao, 2009). For liver cancer, the correlation of liver incidence between arsenic exposure concentration and age is not significant (male r^2=0.45 and female r^2=0.41) (Liao, et al, 2009). The 0.01percent and 1percent excess lifetime cancer risk-based point-of-departure analyses were adopted to quantify the internal cancer risks from arsenic in drinking water. According to Liao et al. (2009), the results show that the reference arsenic guideline is recommended to be 3.4 μg/L, based on male bladder cancer with an excess risk of 10^{-4} for a 75-year lifetime exposure. The likelihood of reference arsenic guideline and excess lifetime cancer risk estimates range from 1.9 to 10.2 μg/L and $2.84x10^{-5}$ to $1.96x10,^{-4}$ respectively, based on the drinking water uptake rates of 1.08 to 6.52 L/day. These results imply that the Weibull model-based arsenic epidemiology and the life stage PBPK framework can provide a scientific basis to quantify internal cancer risks from arsenic in drinking water and further, recommend the reference drinking water arsenic guideline (Liao, 2009).

Lin, W. S.L. Wang, H.J. Wu, K.H. Chang, P. Yeh, C.J. Chen, H.R. Guo. (2008). Associations between arsenic in drinking dater and pterygium in Southwestern Taiwan. Environmental Health Perspectives 116(7):952–955.

Lin et al. (2008) evaluated the association between arsenic exposure through drinking water and the occurrence of pterygium in southwestern Taiwan. Pterygium is a fibrovascular growth of the bulbar conjunctiva and underlying subconjunctival tissue that may cause blindness. Participants older than 40 years of age were recruited from three villages in the arseniasis-endemic area in southwestern Taiwan (exposed villages) and four neighboring nonendemic villages (control comparison villages). High arsenic levels were found in the water from artesian wells in the exposed villages, with the median arsenic concentration ranging from 0.70 to 0.93 mg/L. The median arsenic concentrations of well water in the control villages were <0.005 mg/L. There were a total of 223 participants from the exposed villages (128 males and 95 females) and 160 participants from the control villages (85 males and 75 females). The mean age of the participants from the exposed villages and the control villages was 63.3 and 62.7 years, respectively. Each participant received an eye examination and a questionnaire interview in 2005. The residents of both village groups had similar racial origins, socioeconomic status, living environment, lifestyles, dietary patterns, medical facilities, and educational levels. Photographs taken of both eyes were graded by an ophthalmologist to determine pterygium status. Of the 223 participants in the study, 134 were found to be positive for pterygium during the examination. The prevalence of pterygium was higher in the exposed villages across all age groups in both sexes and increased with cumulative arsenic exposure. A significant association between cumulative arsenic exposure and the prevalence of pterygium was observed. After adjusting for age, sex, working under sunlight, and working in sandy environments, it was evident that the cumulative arsenic exposure of 0.1 to 15.0 mg/L-year and ≥15.1 mg/L-year were associated with increased risks of developing pterygium. The adjusted odds ratios were 2.04 [95 percent confidence interval (CI), 1.04-3.99] and 2.88 (95 percent CI, 1.42-5.83), respectively. Results from this study indicate that chronic exposure to arsenic in drinking water was related to the occurrence of pterygium and the association was still observed after adjusting for exposures to sunlight and sandy environments.

McDonald C., R. Hoque, N. Huda, and N. Cherry. (2006). Prevalence of arsenic-related skin lesions in 53 widely-scattered villages of Bangladesh: An Ecological Survey. J Health Popul Nutr 24(2):228-235.

McDonald et al. (2006) conducted a survey to provide a representative assessment of prevalence and risk of arsenic-related skin lesions in relation to geographical distribution of arsenic in wells of rural Bangladesh. Extensive studies by the British Geological Survey (BGS), over a three-year period, 1998 to 2000, reported that levels of arsenic far in excess of 50 µg/L commonly were found in water from tube wells in the southern half of the country and in some areas of the north. A systematic random sample of 53 villages in four divisions of Bangladesh was selected for the survey conducted by McDonald et al. (2006). In January 2004, 16,740 women aged 18 years or older were indentified in the selected villages. Complete examinations were carried out and findings were recorded for 13,705 of the women (mean age of 36.2 years). Trained paramedics recorded the presence of skin thickening and nodules on the palms and soles, together with information on tube well use. A wide prevalence survey of keratotic skin lesions on the hands and feet was needed to determine whether these changes were related significantly to exposure, as provided by published arsenic data from the BGS. Arsenic exposure was taken as the mean concentration reported by the BGS. The women surveyed were distributed fairly evenly among villages in districts with a range of arsenic concentrations reported by the BGS. All but 281 women were still drinking tube well water at the time of the survey, with a mean of 19.5 years of drinking this water at their present address

The prevalence of skin lesions was related to the mean concentration of arsenic and to the date the first well in the village was installed. Overall, 176 (1.3 percent) on 13,705 women were recorded to have nodules or thickening. The skin lesions recorded were more commonly nodules than thickening and were observed more frequently on hands than on feet. The prevalence of skin lesions within the villages ranged from 0 percent (no cases) in 26 villages to 23 percent (48 of 209) in one village. The median was 0.22 percent with only 13 villages having a prevalence of 11.0 percent or greater. The prevalence of skin lesions by mean arsenic concentration in well water was as follows:

- Arsenic concentration of ≤5 µg/L = prevalence of 0.4 percent (10 cases),
- Arsenic concentration of 6 to ≤10 µg/L = prevalence of 0.4 percent (16 cases),
- Arsenic concentration of 11 to ≤50 µg/L = prevalence of 0.7 percent (33 cases), and
- Arsenic concentration of >50 µg/L = prevalence of 6.9 percent (117 cases).

The analysis was also conducted using women (rather than village) as the unit of analysis. In this analysis, both duration of tube well use at the present address and age were related to lesions.

The results from this study suggest that in the absence of other information, priority should be given to areas of the country where the average concentration arsenic is above 50 µg/L.

McDonald C., R. Hoque, N. Huda, and N. Cherry. (2007). Risk of arsenic-related skin lesions in Bangladeshi villages at relatively low exposure: a report from Gonoshasthaya Kendra. Bulletin of the World Health Organization 85(9):668-673.

McDonald et al. (2007) gathered data on arsenic exposure levels and skin lesion prevalence to address the lack of knowledge about risks where the average arsenic concentration was low. The nongovernmental organization called Gonoshasthaya Kendra, did three related studies of keratotic skin lesions since 2004: (1) an ecological prevalence survey among 13,705 women aged ≥18 in a random sample of 53 villages; (2) a case-control study of 176 cases and age- and village-matched referents; and (3) a prevalence survey of the entire population of 11,670 in two additional villages. Prevalence was calculated as a function of average arsenic concentrations as reported in the National Hydrochemical Survey and measured arsenic concentrations in wells used by subjects in the case-control study. These three surveys' essential findings indicate that the prevalence of skin lesions was low (0.37 percent among 6,448 women living 25 villages with an average arsenic concentration of 5 µg/L or less. It was 0.63 percent among 5,547 women in 21 villages, average concentration 16 to 50 µg/L, but much higher (6.84 percent) among 1,710 women in seven villages with an average concentration of 81 µg/L. In the case-control analysis, relative risk of skin lesions increased threefold at concentrations above 50 µg/L. The authors are confident that any excess risk in areas of the country where the average arsenic concentration was below 10 µg/L is most unlikely. There is evidence of a small risk in areas with averages between 11 and 50 µg/L, probably explained by wells in which 50 µg/L was exceeded. Above 50 µg/L, though data at higher concentrations are scanty, the risk appears substantial.

McDonald et al. (2007) found no clear systematic trend in the prevalence of skin lesions and socioeconomic status, as was found in other studies. However, McDonald et al. (2007) was unable to use individual socioeconomic data. Rather, the analysis examined the main geographical groups to which the 53 villages belonged. The proportion of pregnant women in the last two years classified as poor or very poor was selected as the best available index of socioeconomic status.

McDonald et al. (2007) concludes that little serious skin disease is likely to occur if the arsenic concentration in drinking water is kept below 50 µg/L; but ensuring this water quality will require systematic surveillance and reliable testing of all wells, which may be impractical. More research is needed on feasible prevention of toxic effects from arsenic exposure in Bangladesh.

Milton, A., W. Smith, B. Rahman, Z. Hasan, U. Kulsum, K. Dear, M. Rakibuddin, A. Ali. (2005). Chronic arsenic exposure and adverse pregnancy outcomes in Bangladesh. Epidemiology 16:82–86.

Milton et al. (2005) conducted a cross-sectional study to assess the association between arsenic in drinking water and spontaneous abortion, stillbirth, and neonatal death. The study population included 533 women in 30 villages in Bangladesh. Information on sociodemographic characteristics, drinking water use, and adverse pregnancy outcomes were gathered from the women. Water samples were also collected and measured for arsenic in the 233 tube wells reported to be used by the participants. Arsenic concentration ranged from nondetectable (3 to 30 µg/L) to 1,710 µg/L.

An increased risk of spontaneous abortion and stillbirth was observed for arsenic more than 50 µg/L. As compared to water arsenic concentrations under 50 µg/L, the odds ratio was 2.5 (95 percent CI of 1.5 to 4.3) for spontaneous abortion and 2.5 (95 percent CI of 1.3 to 4.9) for still birth. For neonatal death, the association with arsenic exposure was weaker, with an odds ratio of 1.8 (95 percent CI of 0.9 to 3.6). For all three outcomes, risks were generally higher with longer duration of arsenic exposure. Milton et al. (2005) concluded that that chronic arsenic exposure may increase the risk of fetal and infant death. Limitations to this study noted by the authors include the reliance on respondents to obtain information and the lack of directly measured individual exposure data over time. Milton et al. also noted that larger case-control studies are needed for confirmation of the associations suggested in their conclusions.

Myers, S., D.T. Lobdell, Z. Liu, Y. Xia, H. Ren, Y. Li, R.K. Kwok, J.L. Mumford, and P. Mendola. (2009). Maternal drinking water arsenic exposure and perinatal outcomes in Inner Mongolia, China. Journal of Epidemiology and Community Health. Downloaded from jech.bmj.com on November 5, 2009.

Myers et al. (2009) conducted an analysis of singleton deliveries in a defined geographic area of Inner Mongolia from December 1996 to December 1999, to evaluate the relationship between maternal drinking water arsenic exposure and perinatal endpoints (term birth weight, preterm birth, stillbirth, and neonatal death). The study population only included pregnancies that had one or more arsenic well water measurements for their sub-village of residence. Therefore, of the total singleton pregnancies (22,050), only 45 percent (9,890) were included in this analysis. Information on drinking water arsenic exposure was obtained from an existing database of well water arsenic concentrations collected between 1991 and 1997. On average, each subject was assigned an arsenic exposure category based on 4.7 well water measurements in their sub-village. Pregnancy outcome information, as well as health and other covariate data, were also obtained from an existing database.

Overall, only about 3 percent of the deliveries were preterm and less than 1 percent was low birth weight. The majority of the women lived in sub-villages with average arsenic concentrations less than 20 µg/L. Approximately 14 percent of the women lived in sub-villages with average arsenic concentrations higher than 50 µg/L (the Chinese standard). Mean birth weight at term was compared across four arsenic categories using analysis of covariance. In the highest arsenic exposure category (>100 µg/L), the average term birth weight was 0.05 kg higher (95 percent CI of 0.02 to 0.08) as compared to the referent (below the detection limit to 20 µg/L). For the other three endpoints (preterm birth, stillbirth, and neonatal death) odds ratios were estimated by logistic regression with arsenic exposure dichotomized at 50 µg/L. Arsenic >50 µg/L was associated with an increased risk of neonatal death (odds ratio of 2.01, 95 percent CI of 1.12 to 3.59). No relationship was found between maternal arsenic exposure and preterm or stillbirth delivery.

Myers et al. (2009) concluded that maternal arsenic exposure does not appear to contribute to adverse birth outcomes (reduced term birth weight, risk of preterm or stillbirth delivery). The authors did find a twofold increase in the risk of neonatal death for maternal arsenic exposure above the 50 µg/L drinking water standard. The study may suggest that drinking water arsenic exposure during pregnancy may be associated with increased risk of neonatal death; however, neonatal death rates in the population are low, and the association should be researched further.

Rahman, M., M. Vahter, N. Sohel, M. Yunus, M. Wahed, P. Streatfield, E. Ekström, Å. Persson. (2006). Arsenic exposure and age- and sex-specific risk for skin lesions: a population-based case-referent study in Bangladesh. Environmental Health Perspectives 114(12):1847–1852.

Rahman et al. (2006) conducted a study in Bangladesh to assess the susceptibility to arsenic-induced skin lesions by age and sex. The study base included 166,934 people over four years old who had lived and consumed drinking water within a specific area (the Health and Demographic Surveillance System (HDSS) area) for at least six months prior to the study. All participants were examined in the field for arsenic-associated skin lesions according to a structured protocol. Suspected cases were referred to study physicians for further examination. Physicians diagnosed arsenic-related skin lesions in 579 individuals. Dermatologists confirmed the skin lesions in 504 of the 579 cases. All staff members were blinded to the arsenic exposure of the participants. Arsenic concentrations in the tube wells were tested using a field kit for all participants.

Using the HDSS database, two referents per expected case were selected randomly to participate in the study. In total, 1,830 referents were interviewed to gather information on drinking water use and examined in the field. Six persons among the referents had confirmed arsenic-related cases of skin lesions. Arsenic concentrations were determined in the tube wells that had been identified as a water source for each of the referent participants since 1970. Both average and cumulative historical arsenic exposure was calculated for each referent participant. Exposures were based on use of all drinking water sources since 1970 or birth.

For the total number of cases identified with arsenic-related skin lesions (n=504), they all had significantly higher average chronic arsenic exposure than the referents since 1970. Arsenic exposure levels were 200 µg/L in male cases, 211 µg/L in female cases, 143 µg/L in male referents, and 155 µg/L in female referents. Cumulative arsenic exposure followed a similar pattern. A dose-response relationship was identified for both sexes, using the lowest exposure category as a reference. A dose-response relationship was also identified with socioeconomic status, as risks increased with rising socioeconomic status. In the highest average exposure quintile, males had a higher risk of obtaining skin lesions than females (odds ratio of 10.9 versus 5.78). Exposure to arsenic before one year of age was not associated with higher risk of obtaining skin lesions as compared to the start of arsenic exposure later in life. Rahman et al. (2006) concluded that males are more susceptible than females to developing skin lesions when exposed to arsenic from tube wells.

Rahman, A., M. Vahter, E. Ekström, M. Rahman, A. Haider, M.E. Mustafa, M.A. Wahed, M. Yunus, and L. Persson. (2007). Association of arsenic exposure during pregnancy with fetal loss and infant death: A Cohort Study in Bangladesh. Am J Epidemiol 165:1389–1396.

Rahman et al. (2007) evaluated the effect of arsenic exposure on fetal and infant survival in a cohort of 29,134 pregnancies in Matlab, Bangladesh, in 1991 to 2000. Information on pregnancy outcome was collected by a monthly household surveillance run by the International Centre for Diarrheal Disease research, Bangladesh. Information on exposure to arsenic was from data in a separate study on health consequences of arsenic exposure conducted in 2002 to 2003, but was assumed that the arsenic concentrations were similar to the 1991 to 2000 study. Arsenic exposure for individual pregnancies was based on arsenic concentration in tube well water used by the women during pregnancy.

The 29,134 pregnancies resulted in 2,444 fetal losses (1,615 early fetus losses and 829 late fetal losses), 1,096 induced abortions, 850 neonatal deaths, and 523 post neonatal deaths. Arsenic concentration in drinking water varied widely, ranging from <1 µg/L to 513 µg/L, with a mean concentration of 239 µg/L. The arsenic concentration in the drinking water used exceeded 10 µg/L in about 80 percent of the pregnant women. A Cox proportional hazards model was used to calculate risk of fetal loss and infant death in relation to arsenic exposure. Drinking tube well water with an arsenic concentration of more than 50 µg/L significantly increased the risks of fetal loss (relative risk = 1.14, 95 percent CI: 1.04, 1.25) and infant death (relative risk = 1.17, 95 percent CI: 1.03, 1.32). For infant death, there was a significant dose-response pattern. Similar risk estimates were observed for different strata of infants (neonatal and postnatal), calendar year, and mother's location in the service area.

The authors concluded that results of the study show an association between arsenic exposure via tube well water and increased mortality in humans during early development. Arsenic exposure of infants was probably limited, so the observed arsenic associated decrease in infant survival was due most likely to prenatal exposure instead of postnatal exposure (Rahman et al., 2007).

Tseng, C., Y-K Huang, Y-L Huang, C. Chung, M. Yang, C. Chen, and Y. Hsueh. (2005). Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in Blackfoot disease -hyperendemic villages in Taiwan. Toxicology and Applied Pharmacology. 206:209–308.

Tseng et al. (2005) examined the interaction between arsenic exposure and urinary arsenic speciation on the risk of peripheral vascular disease (PVD) in the Blackfoot disease (BFD) hyperendemic area in Taiwan. Blackfoot disease is a unique PVD disease. The study included 479 adults, including 220 males and 259 females living in a BFD area. The subjects were examined in 1993 for PVD using Doppler ultrasound. In 1989, urinary samples were collected from these subjects for determination of total arsenic and arsenic speciation, including inorganic arsenic (As^{III}) and arsenate (As^{V}), monomehtylarsonic acid (MMA^{V}), and dimethylarsenic acid (DMA^{V}). A primary methylation index (PMI) and a secondary methylation index (SMI) were calculated. The participants were interviewed on water consumption and other lifestyle variables. Arsenic exposure was estimated by an index of cumulative arsenic exposure, derived from arsenic concentration in artesian well water and the duration that the well water was consumed (described in a previous study). Associations between PVD and urinary arsenic parameters were evaluated, with consideration of the cumulative arsenic exposure and other factors such as age, gender, body mass index, total cholesterol, triglycerides, cigarette smoking, and alcohol consumption.

The results showed that aging was associated with diminishing capacity to methylate inorganic arsenic and that the arsenic methylation capacity of women was more efficient than of men. Additionally, the risk of PVD increased with higher cumulative arsenic exposure (CAE) as well as a lower capacity to methylate arsenic to $DMA.^{V}$ Subjects who smoked cigarettes had significantly higher levels of total urinary arsenic and percentage $MMA,^{V}$ and lower SMI. The authors noted that smoking could have an effect on the second methylation phase. They also stated that although the highly toxic trivalent forms of methylated arsenic had been found in another study, it was not observed in this study; this may have been attributed to the fact that trivalent methylated arsenic forms were not known to be present, had not been identified from the urine when samples were collected and analyzed, or the concentrations were too low to be detected. Also the subjects had stopped drinking artesian well water for 15 to 20 years when the urine samples were collected, and they were not administered sodium 2,3-dimercapto-1-propane sulfonate (DMPS) before we collected their urine. Therefore, the study authors stated it would not be too surprising for not detecting the trivalent methylated species in the samples. The study authors also stated that because MMA^{V} and DMA^{V} are considered nontoxic generally, they could not exclude the possibility that the higher MMA^{V} in the urine is a marker of higher MMA^{III} in the blood or inside the cells where injuries occur.

The multivariate-adjusted odds ratio for cumulative arsenic exposure of 0 mg/L was 1. For other cumulative arsenic exposure levels, multivariate-adjusted the odds ratios were the following:

- 0.1 to 15.4 mg/L and >15.4 mg/L per year, the odds ratios were 3.41 (0.74 to 15.78) and 4.62 (0.96 to 22.21 mg/L), respectively.
- PMI ≤1.77 and SMI ≥6.93, odds ratio was 1.00.
- PMI>1.77 and SMI>6.93, odds ratio was 2.93 (0.90 to 9.52).

- PMI >1.77 and SMI≤6.93, odds ratio was 2.85 (1.05 to 7.73).
- PMI≤1.77 and SMI≤6.93, odds ratio was 3.60 (1.12 to 11.56).

Tseng et al. (2005) concluded that individuals with higher arsenic exposure and a lower capacity to methylate inorganic arsenic to DMA^V have a higher risk of developing PVD in the BFD-hyperendemic area in Taiwan. Tseng et al. noted that the association between PVD and the undetected trivalent forms of the methylated metabolites needs further clarification.

Tseng, C., Y-K Huang, Y-L Huang, C. Chung, M. Yang, C. Chen, and Y. Hsueh. (2006). Erratum: Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in Blackfoot disease-hyperendemic villages in Taiwan. Toxicology and Applied Pharmacology. 211:175.

This article is an erratum for Tseng et al. (2005). On page 303, in Table 3, Model 7, the second line from the bottom should read ">1.77" instead of "≤1.77" for PMI.

Tseng, C., Y-K Huang, Y-L Huang, C. Chung, M. Yang, C. Chen, and Y. Hsueh. (2005). Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in Blackfoot disease-hyperendemic villages in Taiwan. Toxicology and Applied Pharmacology. 206:209-308.

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Tseng, H., Y. Wang, M. Wu, H. The, H. Chiou, and C. Chen. (2006). Association between Chronic Exposure to Arsenic and Slow Nerve Conduction Velocity among Adolescents in Taiwan. J Health Popul Nutr 24(2):182–189.

Wang et al. (2006) studied the association between chronic exposure to arsenic and peripheral neuropathy by conducting a cross-sectional study in which 130 Taiwanese students aged 12 to 14 years were examined for motor and sensory nerve condition velocity (NCV) of peripheral nerves in their right upper and lower limbs. These students lived in an area where some residents still use well water, though tap water systems do exist. Arsenic concentrations were determined in well water of each household. The arsenic levels ranged from undetectable (<0.015 ppb) to 3.59 ppm. A detailed history of residential water consumption was obtained from each subject. NCV was examined by an NCV machine. Ulnar, peroneal, and tibial compound motor action potentials (CMAPS) and ulnar and sural sensory action potentials (SAPs) of the right upper and lower limbs were recorded for each subject.

Subjects with a cumulative arsenic dosage of greater than 100 mg had significant decreased NCV of sural SAP. After adjustment for gender and height, the odds ratio was 2.9 (95 percent CI of 1.1 to 7.5) as compared to the reference group. Additionally, a borderline statistical significance was observed in subjects who drank water from wells with arsenic concentrations greater than 50 μg/L and had a cumulative arsenic dosage of greater than 100 mg. The odds ratio was 7.8 (95 percent CI of 1.001 to 69.5) for decreased NCV of sural SAP. There was slight, but not significant, decreased NCV among subjects who drank water from wells with arsenic concentrations greater than 50 μg/L, including for median CMAP, ulnar CMAP, peroneal, CMAP, and surreal SAP. After adjustment for gender and height, the odds ratio was 2.4 (95 percent CI of 0.7 to 8.1). For those subjects who had stopped drinking well water for more than one year, a statistically nonsignificant odds ratio of 1.3 (95 percent CI of 0.4 to 4.7) was observed. Wang et al. (2006) concluded that chronic exposure to arsenic might induce peripheral neuropathy, and the NCV of sural SAP might be an early marker of chronic arsenic neuropathy.

T. Wade, Y. Xia, K. Wu, Y. Li, Z. Ning, X.C. Le, X. Lu, Y. Feng, X. He, J.L. Mumford. (2009). Increased mortality associated with well-water arsenic exposure in Inner Mongolia, China. Int. J. Environ. Res. Public Health 6:1107-1123.

Wade et al. (2009) conducted a retrospective study in an Inner Mongolian village (Ba Men) with arsenic contamination in well water since the 1980s. The goals of the study were to determine and classify the underlying cause of death in Ba Men occurring between January 1, 1997 and December 1, 2004, and to assess the relationship between mortality rate and arsenic exposure from the primary water source of each individual. As cause of death is not systematically recorded in Ba Men, therefore Wade et al. gathered information by conducting two interviews with the adult household members and one interview with a medical professional. Additional information was obtained from medical records. A team of medical experts evaluated the data and assigned an underlying cause of death. The medical team was unaware of and blinded to the arsenic exposure levels from the water source. Water samples were collected at the time of the interviews and analyzed for arsenic concentrations.

A total of 572 deaths were identified in the study period (crude mortality rate of 731 deaths per 100,000 person years). Diseases of the heart were the leading causes of mortality, accounting for 36 percent of the deaths. Cerebrovascular diseases accounted for 25 percent of the deaths, followed by malignant neoplasms (13 percent), suicide (4 percent) and accidents (3 percent). Among both living and deceased residents, total arsenic in water samples from current primary sources of water ranged from below the detection limit to 637.7 μg/L, with a mean exposure of 38 μg/L (median 21 μg/L). At least 9 percent of the deceased were exposed to well water with arsenic levels above 100 μg/L.

Among all residents, there was a significant association between heart disease and arsenic exposure. A 50 μg/L increase in arsenic levels was associated with a 12 percent increase in heart disease mortality. Additionally, as the time exposed to the water source increased, the risk of heart disease mortality also increased. There was also some evidence that there was an association in a dose response manner between arsenic exposure and total cancer mortality, especially for those exposed for 10 to 20 years to arsenic levels in well water. The trend was significant in residents exposed since before 1995. A 50 μg/L increase in arsenic exposure was also associated with 12 percent and 15 percent all-cause mortality in residents exposed since before 1990 and 1985, respectively.

Wasserman, G., X. Liu, F. Parvez, H. Ahsan, P. Factor-Litvak, A. van Geen, V. Slavkovich, N. Lolacono, Z. Cheng, I. Hussain, H. Momotaj, and J. Graziano. (2004). Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environmental Health Perspectives 112(13):1329–1333.

Wassermann et al. (2004) reported the results of a cross-sectional investigation on the intellectual function of 201 children (10 years of age) in Bangladesh, who potentially are exposed to high concentrations of naturally occurring arsenic in drinking water supplied by tube wells. The children received a medical examination in which weight, height, and head circumference was measured. Intellectual function was drawn from the Wechsler Intelligence Scale for Children, Version III, and was assessed by summing weighted items across domains to create Verbal, Performance, and Full-Scale raw scores. Urine samples were also collected from the children to measure arsenic and creatinine, as well as blood samples in approximately half of the children to measure blood lead and hemoglobin concentrations.

Arsenic concentrations in the water samples collected from the residential tube wells ranged from 0.094 to 790 µg/L, with a mean of 117.8 µg/dL. Manganese was also tested in some of the samples. The mean manganese concentration was 1,386 µg/L. Eighty-two percent of the children were consuming water in excess of the WHO recommended maximum contamination level (MCL) of 500 µg/L. Exposure to arsenic in drinking water was associated with reduced intellectual function after adjustment for sociodemographic covariates and water manganese concentrations. The effect was observed in a dose-response pattern, with children receiving water arsenic greater than 50 µg/L, achieving significantly lower Performance and Full-Scale scores than children with water As levels less than 5.5 µg/L.

Children in the highest quartile of water arsenic scored approximately 10 points lower in the Performance raw scores than those in the lowest quartile. The relationship between total urinary arsenic concentration and child intellectual function was observed in the same pattern as for water arsenic concentration; however, the associations were not significantly different. No significant differences were seen between child intellectual function and other hemotologic measures, such as log BPb or Hgb. The relationship between manganese and children's intellectual function suggested a possible adverse effect, however, when arsenic was added to the models; manganese made no significant contribution to intellectual function. One limitation identified by the study authors is the lack of intelligence tests specifically designed for the Bangladesh population. Also, the study only evaluated a single age group at a single point in time.

Watanabe, C., T. Matsui, T. Inaoka, K. Takefumi, K. Miyazaki, M. Bae, T. Ono, R. Ohtsuka, A. Bokul. (2007). Dermatological and nutritional/growth effects among children living in arsenic-contaminated communities in rural Bangladesh. Journal of environmental Science and Health Part A 42:1835-1841.

Watanabe et al. (2007) studied the effects of chronic arsenic exposure through the consumption of contaminated groundwater among children living in two rural villages in northern Bangladesh. The participating children consisted of 109 boys and 132 girls between the ages of 4 and 15. The study, which was conducted in 2002, focused on two endpoints: dermatological manifestation and nutritional status. Dermatological manifestation was assessed through inspection for melanosis of body and oral cavity and keratotic lesions of palms and soles. Nutritional status was assessed through height and body mass index (BMI).

Arsenic concentrations in water collected from tube wells (the only source of water for these villages) ranged from less than the detection limit (<1 ng/mL) to 535 ng/mL, with a median of 10 ng/mL. Approximately 30 percent of these children used tube wells with arsenic concentrations exceeding 50 ng/mL (the provisional guideline of Bangladesh) and 63 percent used those exceeding 10 ng/ML (the provisional guideline set by WHO for drinking water). The median arsenic concentration in spot urine samples was 110 ng/mL (not adjusted for creatinine) or 260 ng/mg creatinine. Boys showed significantly higher arsenic concentrations in urine than the girls.

Positive dermatological findings were observed in 141 children (59 percent). The most frequently observed symptom was keratosis on the sole of the foot. Pigmentation change on the trunk rarely was observed. Melanosis was only observed in eight children and leucomelanosis was not observed. Malignant tumors and precancerous changes were also not found. Marginal differences between sexes were observed for palm manifestations, and significant differences were observed for sole manifestations, with boys having higher manifestations than girls in both cases. There was no apparent relationship between creatinine-adjusted arsenic in urine and frequency of positive dermatological manifestations, although one subgroup (boys in the village identified as SV), showed a positive correlation between dermatological manifestations and creatinine-adjusted arsenic in urine.

According to the study authors, the dermatological findings were similar to those in adults in the same community in terms of prevalence and relative frequency of the site-specific symptoms. With respect to nutritional status, a positive relationship between BMI and exposure level was observed. The proportion of children with lower BMI significantly increased with increasing exposure levels. This dose-response relationship was observed in all subgroups. Watanabe et al. (2007) concludes that nutritional status may be a more sensitive endpoint than dermatological manifestations of children in this area.

Yang, C.H. (2006). Does arsenic exposure increase the risk of development of peripheral vascular diseases in humans? Journal of Toxicology and Environmental Health, Part A 69: 1797-1804.

Yang (2006) examined whether mortality rates attributed to peripheral vascular disease (PVD) would decrease among residents living in the Blackfoot disease (BFD) endemic areas in Taiwan. Residents in this area consumed high amounts of arsenic present in artesian well water since 1910. Arsenic well water concentrations in the early 1960s ranged from 0.35 to 1.14 ppm, with a median of 0.78 ppm. A tap water system was implemented in the 1960s, though usage remained low until the 1970s; well water was no longer used for drinking water after mid-1970. The arsenic concentration in the tap
water supply was reported to be less than 0.01 ppm (Chen and Chen, 1975). The number of PVD-related deaths and the mid-year population categorized by age, gender, and calendar year between 1971 and 2003 was obtained from the Taiwan Provincial Department of Health. Standardized mortality ratios (SMR) were calculated for PVD. Moving averages of the three yearly SMRs between 1971 and 2003 were calculated to minimize statistical instability from sudden changes in PVD mortality rates over time. Cumulative sum techniques were used to detect the occurrence of significant changes in the average level of a series of these means.

Data show that mortality due to PVD declined gradually for approximately 25 to 27 years following cessation of arsenic well water. During 1971 to 2003, the means of the three-year SMRs for PVD in the study area were appreciably higher than for Taiwan as a whole. The derived cumulative sums show that a marked change occurred between 1998 and 2000 for males, when the values fell from higher than the 33-year general average to below the 33-year general average. For females, the marked change occurred between 1996 and 1998. Yang (2006) concludes that based on the reversibility criterion, the association between arsenic exposure and PVD-attributed mortality is likely to be causal.

CADMIUM

Diamond, G., W. Thayer, H. Choudhury. (2003). Pharmacokinetics/ Pharmacodynamics (PK/PD) modeling of risks of kidney toxicity from exposure to cadmium: Estimates of dietary risks in the U.S. population. Journal of Toxicology and Environmental Health, Part A 66:2141–2164.

Diamond et al. (2003) conducted an analysis of epidemiological studies of associations between exposure to cadmium and kidney toxicity. Dose-response functions relating low-molecular-weight (LMW) proteinuria to various indices of cadmium dose were obtained from 15 studies of diverse exposures. The individual dose-response functions from each study were implemented to arrive at estimates of the measured dose corresponding to probabilities of LMW proteinuria of 0.1, 0.15, or 0.2. The resulting effective doses (ED) were then transformed from the reported units into estimates of renal cortex cadmium burden (RC, μg Cd/g renal cortex) at age 55 years.

A modified version of the Kjellström and Nordberg cadmium PK model was used to interconvert external and internal cadmium dose estimates from epidemiological studies, allowing direct comparison of dose-response relationships across studies that might otherwise be incomparable. U.S. dietary intakes were derived from an analysis of the U.S. Food and Drug Administration Total Diet Studies (TDS, 1982–1994) and food consumption patterns derived from the Third National Health and Nutrition Examination Survey (NHANES III, 1988–1994).

The study authors reported that a 0.1 risk of LMW proteinuria was associated with a median renal cortex concentration (RC_{10M}) of 153μg Cd/g cortex (95 percent CI, 84–263) at age 55 years. The corresponding cadmium intakes were reported as 2 μg/kg/d (95% CI, 1–3.6) in females and 4.3 μg/kg/d (95 percent CI, 2.2–7.7) in males. The lower confidence limit on the median (RC_{10L}), 84 μg/g cortex, exceeded the 99th percentile of the estimates of renal cortex cadmium concentrations among U.S. females (78 μg/g cortex) and males (36 μg/g cortex), whose exposures were assumed to be entirely from the dietary pathway (i.e., nonsmokers who have no history of occupational exposure).

According to the study authors, the data suggest that dietary-derived risks are negligible. However, they also note that extreme upper percentile dietary cadmium exposures among U.S. females may leave little margin for exposures from nondietary sources without incurring significant risk of nephrotoxicity. Tobacco smoking can contribute significantly to renal cadmium burdens. People who smoke heavily (e.g., one to two packs of cigarettes per day) were found to have a renal burden twice that of nonsmokers. However, even among smokers, the U.S. dietary intake of cadmium was predicted to result in renal cortex cadmium concentrations that are less than the RC_{10L}, although the margin for additional sources of exposure that would not exceed the RC_{10L} was predicted to be appreciably less for most of the population of smokers compared to nonsmokers.

Gallagher, C., J. Kovach, J. Meliker. (2008). Urinary cadmium and osteoporosis in U.S. women ≥ 50 years of age: NHANES 1988–1994 and 1999–2004. Environmental Health Perspectives 116(10):1338-1343.

Gallagher *et al.* (2008) investigated the statistical association between urinary cadmium (U-Cd), at levels ≤ 1 μg/g creatinine, and osteoporosis, as indicated by hip bone mineral density (BMD) and self-report in a population-based sample of U.S. women ≥ 50 years of age.

Data sets reviewed in this study were obtained from the Third U.S. National Health and Nutrition Examination Survey (NHANES III) 1988–1994 survey data (n = 3,207), for the primary analysis of hip BMD, and from NHANES 1999–2004 data (n = 1,051), for the secondary analysis of survey-respondent-reported physician diagnosis of osteoporosis. Osteoporosis was indicated by hip BMD cutoffs, based on the international standard and on self-report of physician diagnosis. U-Cd levels were analyzed for association with osteoporosis using multiple logistic regressions. For both primary and secondary analyses, U-Cd was obtained from a single urine specimen collected and measured by the CDC laboratory using atomic absorption spectrometry, and urinary creatinine was obtained from the same urine specimen. Logistic regression was performed to calculate the urinary creatinine-adjusted Cd odds ratio (OR) for osteoporosis, both unadjusted and adjusted for age, race (white compared with nonwhite), family income category (lowest to highest), underweight (< 127 lb), and smoking status.

The study authors reported that for a 1-μg/g creatinine increase in U-Cd, women ≥ 50 years of age had 15 percent greater odds for osteoporosis (OR = 1.15; 95 percent CI, 1.00–1.33; p = 0.05), as defined by hip BMD and adjusted for age, white race, income, ever-smoker, and underweight. Women ≥ 50 years of age with U-Cd levels between 0.50 and 1.0 μg/g had 43 percent greater odds for osteoporosis (OR = 1.43; 95 percent CI,1.02–2.00; p = 0.04), compared to the reference group, as measured by hip BMD and adjusted for age, white race, income, ever smoker, and underweight. Women who reported a diagnosis of osteoporosis had similar corresponding OR (OR = 1.46).

According to the study authors, the study results suggest that U.S. women are at risk for osteoporosis at U-Cd levels below the U.S. Occupational Safety and Health Administration's 3-μg/g safety standard. Seventy-three percent of U.S. women ≥ 50 years of age were estimated to have Cd body burdens that are associated with excess risk (> 0.50 μg/g creatinine), as estimated from the NHANES III data set, suggesting that 21 percent of osteoporosis prevalence among women ≥ 50 years of age may be attributable to Cd. Given null findings among smokers, the study authors concluded that dietary Cd, rather than tobacco, was the likely source of Cd-related osteoporosis risk for the U.S. female population ≥ 50 years of age.

Gamo, M., K. Ono, J. Nakanishi. (2006). Meta-analysis for deriving age- and gender-specific dose–response relationships between urinary cadmium concentration and β_2-microglobulinuria under environmental exposure. Environmental Research 101: 104–112.

Gamo *et al.* (2006) conducted a meta-analysis to derive age- and gender-specific dose-response relationships between urinary cadmium (Cd) concentration and β_2-microglobulinuria (β2MG-uria) under environmental exposure. The maximum permissible level of urinary Cd concentration was also derived by using the obtained dose-response relationships.

β2MG-uria was defined by a cutoff point of 1,000 mg/g creatinine. This cutoff point was determined based on the observation that the renal dysfunction progressed at a urinary β2MG concentration of more than 1,000 mg/g creatinine even after the high exposure had ceased. This progression of β2MG-uria was considered evidence of the toxicological significance of the cutoff point of 1,000 mg/g creatinine.

Data from epidemiological studies on populations environmentally exposed to Cd were collected. A model was developed to describe the relationships among the inter-individual variabilities in urinary Cd concentration, the ratio of Cd concentrations in the target organ and in urine, and the threshold Cd concentration in the target organ for age- and gender-specific subpopulations. The parameters in the model were determined so that good agreement was achieved between the prevalence rates of β2MG-uria reported in the literature and those estimated by the model.

The maximum permissible level of urinary Cd concentration was defined as the maximum geometric mean of the urinary Cd concentration that would not result in a statistically significant increase in the prevalence rate of β2MG-uria. This was estimated to be approximately 3 mg/g creatinine for a population in a small geographical area and approximately 2 mg/g creatinine for a nationwide population. The study authors noted that, although there was a tendency whereby the maximum permissible level decreases with an increase in age, the differences in the maximum permissible level in different age and gender classes appeared to be small.

Ikeda, M., T. Ezaki, T. Tsukahara, J. Moriguchi, K. Furuki, Y. Fukui, H. Ukai, S. Okamoto, H. Sakuri. (2003). Threshold levels of cadmium in relation to increases in urinary β_2-microglobin among general Japanese populations. Toxicology Letters 137:135–141.

Ikeda *et al.* (2003) investigated the possibility that there could be a urinary cadmium (Cd-U) level above which β_2-microglobin (β_2MG) and α_1-microglobin **(**α_1MG) might increase with steeper slopes than had been reported previously.

Paired data on cadmium and β_2MG levels in urine, corrected for creatinine concentration, were obtained from published literature for 32 groups of men and 58 groups of women among Japanese populations. For the purposes of this study, data from previous studies on populations in areas without environmental cadmium pollution were combined with data from populations in polluted areas. The number of subjects in a group varied, from a small group of three exposed women, to a group of more than 1,000 nonexposed women. More groups were available for women than men; thus, priority was placed on women when evaluating the results. Data for men were utilized to determine if the observations in women were reproducible in men.

Exposed groups with a substantial increase in urinary β_2MG levels were selected and placed into two subsets: those with >400 µg/g creatinine (25 groups) and those with >1000 µg/g creatinine (19 groups). Regression analysis gave a slope of 6194 µg β_2MG/µg Cd-U for groups with >400 µg/g creatinine and 6642 µg β_2MG/µg Cd-U for groups with >1000 µg/g creatinine. The regression line for the control group overlapped with the horizontal axis. The intercept between the control and exposed regression lines, the point of flexion, was 11.0 µg/g creatinine and 11.7µg/g creatinine for the exposed groups with > 400 µg/g creatinine and >1000 µg/g creatinine, respectively. These observations were reproducible in the analysis of data for men. According to the study authors, the results of this study suggest that the relationship of β_2MG and Cd-U is not liner, but rather in the shape of a 'J.' β_2MG sharply increases when Cd-U exceeds levels of 10-12 µg/g creatinine.

Järup, L., L. Hellström, T. Alfvén, M. Carlsson, A. Grubb, B. Persson, C. Pettersson, G. Spång, A. Schütz, C. Elinder. (2000). Low level exposure to cadmium and early kidney damage: the OSCAR study. Occupational and Environmental Medicine 57:668–672.

Järup et al. (2000) studied the dose-response relation between cadmium dose and renal tubular damage in a population of workers and people environmentally or occupationally exposed to low concentrations of cadmium.

Early kidney damage in people, occupationally or environmentally exposed to cadmium, was assessed from cadmium in urine to estimate dose, and protein HC (α_1-microglobulin) in urine to assess tubular proteinuria**.** The study population included people between 16 and 80 years of age, who had lived for at least five years in the area close to a nickel cadmium battery plant, and people from a nearby area (n=904). A group of selected battery workers (n=117) was also included in the study population. Each study subject filled out a questionnaire to provide information about employment, residences, smoking, and food, as well as medical history, especially kidney diseases, and diseases related to osteoporosis. Morning urine was collected, stored frozen (-20°C) until transfer to the analytical laboratory, and measured by inductively coupled plasma mass spectrometry. Data were analyzed with standard statistical methods. Odds ratios (ORs) and 95 percent confidence intervals (95 percent CIs) were computed with stratified analyses and multiple logistic regression by EGRET software.

The study authors reported a positive, highly significant, linear relation between dose (cadmium in urine) and effect (urinary protein HC) after adjustment for age for both sexes. A total of 171 people had increased protein HC concentrations in urine with a clear dose-response relation between urinary cadmium and the prevalence of increased protein HC in urine. The prevalence of tubular proteinuria ranged from 5 percent among unexposed people to 50 percent in the most exposed group. The corresponding prevalence odds ratio was 6.0 (95 percent CI: 1.6 to 22) for the highest exposure group, adjusted for age and sex. Multiple logistic regression analysis showed an increasing prevalence of tubular proteinuria with urinary cadmium as well as with age. After adjustment to the mean age of the study population (53 years), the results show an increased prevalence of 10 percent tubular proteinuria (taking into account a background prevalence of 5 percent) at a urinary cadmium concentration of 1.0 nmol/mmol (µg/g) creatinine.

Jin, T., X. Wu, Y. Tang, M. Nordberg, A. Bernard, T. Ye, Q. Kong, N. Lundström, G. Nordberg. (2004). Environmental epidemiological study and estimation of benchmark dose for renal dysfunction in a cadmium-polluted area in China. BioMetals 17: 525–530.

Jin *et al.* (2004) investigated the critical concentration of urinary cadmium (UCd) required for the development of renal dysfunction. Population groups living in three areas in South East China were studied: a highly exposed area with a cadmium content of rice of 3.70 mg/kg (n=294), a moderately exposed area with a content of 0.51 mg/kg (n=243), and a control area with a content of 0.05 mg/kg (n=253).

UCd was used as an indicator of cadmium exposure and accumulation, while the concentrations of *N*-acetyl-*β*-D-glucosaminidase (NAG), its iso-form B (NAG-B), *β*2- microglobulin (B2M), retinol binding protein (RBP) were measured as indicators of renal tubular dysfunction caused by cadmium. Albumin (ALB) in urine was measured as an indicator of cadmium-related renal glomerular dysfunction. The benchmark dose (BMD) procedure was used to estimate the critical concentration of UCd in the population (PCCUCd).

The study authors reported a significantly increased prevalence of hyperNAGuria, hyperNAG-Buria, hyperB2Muria, hyperRBPuria and hyperALBuria with increasing levels of Cd excretion in urine. The study authors also found that approximately 38.4 percent renal tubular dysfunction and 10.6 percent glomerular damage occurred in residents of the highly polluted area; in comparison, there was a 5 percent prevalence of renal dysfunction in the control area.

The lower confidence limit of the BMD (LBMD-05) of urinary cadmium for a 5 percent level of risk above the background level was estimated for each of the renal effect indicators. The BMD-05/LBMD-05 were estimated to be 4.46/3.99, 6.70/5.87, 8.36/7.31, 7.98/6.98 and 15.06/12.18 µg/g creatinine for urinary NAG-B, NAG, B2M, RBP and ALB, respectively. According to the study authors, the data suggest that the lower confidence limit of the PCCUCd of tubular dysfunction for 5 percent excess risk level above the background may be approximately 3–4 µg/g creatinine, and that cadmium concentration in urine should be kept below this level to prevent renal tubular damage.

Kobayashi, E., Y. Suwazono, M. Dochi, R. Honda, M. Nishijo, T. Kido, H. Nakagawa. (2008). Estimation of benchmark does as threshold levels of urinary cadmium, based on excretion of β_2-microglobulin in cadmium-polluted and non-polluted regions in Japan. Toxicology Letters 179: 108-112.

Kobayashi et al. (2008), using a benchmark dose (BMD) approach, calculated the threshold level of urinary cadmium for β_2-microglobulin (MG)-urea for people living in cadmium-polluted and nonpolluted areas of Japan. A multiple logistic model was used to take into account the effects of other possible covariates, such as age in the estimation of the threshold level of urinary cadmium. In this study, the population for the cadmium-polluted area was selected from a 1981 and 1982 health survey conducted among persons (>50 years of age) residing in the Kakehashi River basin. Subjects known to ingest household rice were selected as the target population for Kobayashi et al. (2008). The total study population was 3,103 subjects. The study group consisted of 3,103 participants (1,397 male and 1,706 female) from cadmium-polluted areas. There were 289 participants (130 male and 159 female from nonpolluted areas. An additional 2,640 participants, 50 years and older, from three different cadmium nonpolluted areas from a previous study were also included, totaling 2,929 (1,181 male and 1,748 female) participants from nonpolluted areas in Japan.

Cut-off values for urinary β_2-MG were defined as those corresponding to the 84^{th} and 97.5^{th} percentile of β_2-MG levels in the controls, and 1,000 μg/g creatinine (Cr). The BMD low (BMDL) was calculated using the profile likelihood method. When the benchmark response was 5 percent, the BMD/BMDL of cadmium for the 84^{th} percentile of β_2-MG for mean age, 55, 65, and 75 years was 3.0/2.7, 4.6/4.2, 2.8/2.6, and 1.8/1.6 μg/g Cr in male and 3.4/3.2, 5.8/5.5, 3.2/3.1, and 1.8/1.7 μg/g Cr in female subjects, respectively. The value for the 97.5^{th} percentile for each age was 4.9/4.5, 7.6/7.0, 4.6/4.3, and 2.6/2.4 μg/g Cr in males and 5.9/5.6, 9.7/9.2, 5.6/5.3, and 2.8/2.6 μg/g Cr in females. The authors noted that the prevalence of β_2-MG-uria increased with age using any of the cut-off values.

The threshold level of urinary cadmium for the 84^{th} percentile of β_2-MG among the controls was estimated at 2.7 μg/g Cr in males and 3.2 μg/g Cr in females for the mean age (about 64 years in both sexes). Kobayashi et al. concluded that the study suggests that subjects with a urinary Cd of greater than 3 μg/g will have renal dysfunction and an increased risk of mortality. The authors reported that results from this study indicated that the margin between the threshold level and average excretion level of urinary cadmium was small in the older population in Japan.

Kobayashi, E, Y. Suwazono, M. Uetani, T. Inaba, M. Oishi, T. Kido, M. Nishijo, H. Nakagawa, K. Nogawa. (2006). Estimation of benchmark dose as the threshold levels of urinary cadmium, based on excretion of total protein, β_2-microglobulin, and N-acetyl-b-D-glucosaminidase in cadmium nonpolluted regions in Japan. Environmental Research 101: 401–406.

Kobayashi *et al.* (2006) investigated the association between urinary cadmium (Cd) concentration and indicators of renal dysfunction and estimated the threshold levels of urinary Cd in the general environment without any known Cd pollution. The study population consisted of the inhabitants of the three different Cd nonpolluted areas (1,114 men and 1,664 women). All were >50 years of age. The areas were selected on the basis of available environmental data to give a sufficient range of Cd body burden in the study population. All three areas were rural, and their socioeconomic environments were similar. The subjects were asked to complete a detailed questionnaire and to provide blood and urine for biological measurements. Samples were analyzed for Cd, total protein, β_2-microglobulin (β_2-MG), and N-acetyl-b-D-glucosaminidase (NAG).

The threshold levels of urinary Cd as the benchmark dose low (BMDL) was estimated using the benchmark dose (BMD) approach. Urinary Cd excretion was divided into 10 categories, and an abnormality rate of urinary total protein, β_2-MG, and NAG in each subgroup was calculated. Cut-off values for urinary substances were defined as corresponding to the 84 percent and 95 percent upper limit values of the target population who have not smoked. BMD and BMDL were then calculated for all urinary substances using a log-logistic model.

The study authors reported that the prevalence calculated for each cut-off value (84 percent and 95 percent upper limit values) increased with increasing urinary Cd concentration in both men and women. The BMDL for the 84 percent and 95 percent cut-off value of total protein, setting an abnormal value at 5 percent, was 3.1 and 6.8 mg/g creatinine in men and 4.2 and 7.3 mg/g creatinine in women, respectively. The BMDL for the 84 percent and 95 percent cut-off value of β2-MG, setting an abnormal value at 5 percent, was 2.4 and 4.5 mg/g creatinine in men and 3.3 and 7.3 mg/g creatinine in women, respectively. The study authors concluded that the results demonstrated that the threshold level of urinary Cd could be estimated in people living in the general environment without any known Cd-pollution in Japan, and the value was inferred to be almost the same as that in Belgium, Sweden, and China.

Omarova A., C.J.C. Phillips. (2007). A meta-analysis of literature data relating to the relationships between cadmium intake and toxicity indicators in humans. Environmental Research 103:432–440.

Omarova and Phillips (2007) performed a meta-analysis of literature data to determine the relationship between cadmium intake and cadmium toxicity indicators, in particular β_2-microglobulin, and compared the results with the current Provisional Tolerable Weekly Intake (PTWI) set by FAO/WHO. The Joint FAO/WHO Expert Committee on Food Additives and Contaminants (JECFA) established a PTWI for cadmium of 0.007 mg/kg body weight (BW) per week.

Scientific literature from the past 30 years was examined for relevant studies from which to relate cadmium intake to β_2-microglobulin in urine and cadmium in blood and urine. A meta-analysis of the data was conducted to determine critical intake. From the initial database of several thousand articles on various aspects of cadmium intake and cadmium accumulation in humans, 79 reports of exposure to cadmium in food involving 27,537 people were identified for inclusion in the meta-regression. A total of 59.2 percent of the studies involved women only; 35.1 percent involved men only; and 5.7 percent included both genders. Most of the data (68.9 percent) originated in Asia. The parameters extracted from the studies, and their relevant units, were the independent variable, mean daily cadmium intake in food (µg/day) and the dependent variables, duration of exposure (years), age (years) and gender of the subject, cadmium and β_2-microglobulin in urinary creatinine (µg/g creatinine) and cadmium in blood (µg/L). A nonparametric Cochran's Q test for multiple regression determined that the frequencies in all of the dependent variables were not identical. Ultimately, a multiple Ridge regression model was used to analyze the data.

The study authors state that the meta-analysis indicated that the concentration of β_2-microglobulin in urinary creatinine increased rapidly above a cadmium intake of 302 µg/day. This equates to a PTWI of 3.02 µg/kg BW weight (when a safety margin of 10 is included), or less than one-half of the current value of 7 µg/kg BW approved by the JECFA. Cadmium in blood and urine were positively related to cadmium intake and participant's age. The meta-analysis also determined that the duration of exposure and participant's age were related to the β_2-microglobulin concentration.

Shimizu A., E. Kobayashi, Y. Suwazono, M. Uetani, M. Oishi, T. Inaba, T. Kido, K. Nogawa. (2006). Estimation of benchmark doses for urinary cadmium based on β2-microglobulin excretion in cadmium-polluted regions of the Kakehashi River basin, Japan. International Journal of Environmental Health Research 16(5):329–337.

Shimizu et al. (2006) estimated the threshold levels of urinary cadmium as a benchmark dose low (BMDL) using data from the Kakehashi River basin in Japan. BMDL was defined as the value corresponding to the lower 95 percent confidence interval of the BMD and can be used in the evaluation of dose-response relationships as a replacement for the no-observed-adverse-effect level (NOAEL) or the lowest-observed-adverse-effect level (LOAEL). Three cut-off values for β2-microglobulin excretion were used in this study. These cut-off values included the 84 percent and 95 percent upper limit values which were calculated from the control subjects, and 1,000 μg/L or 1,000 μg/g creatinine (corrected value of urinary β2-microglobulin concentration, normalized for creatinine excretion). The study population was drawn from the population evaluated in a previous 1981 and 1982 health survey conducted among the entire population (over 50 years of age) residing in the Kakehashi River basin. A target population of 3,103 participants from cadmium-polluted areas (1,397 men and 1,706 women) and 289 participants from nonpolluted areas (130 men and 159 women) were selected for this study based on the availability of age and urinary analysis data. Another population living in three cadmium nonpolluted areas was used to calculate the cut-off values for β2-microglobulin-uria corresponding to the 84 percent and 95 percent upper limit values of a control group. The selected target population from this group consisted of 2,035 inhabitants (424 men and 1,611 women), all of whom were 50 years or older and had never smoked. Morning urine samples were collected from all participants and analyzed for urinary cadmium and urinary β2-microglobulin.

A statistical analysis was performed to determine the abnormality rates for urinary β2-microglobulin excretion in males and females. The BMD and BMDL values were calculated based on the distribution of rates of abnormality fitted to a quantal linear model or log-logistic model. The BMDL values at which the excess risk is 0.05 (0.10) were calculated as 2.9 to 4.0 (4.2–5.5) μg/g creatinine for men and 1.5–3.6 (2.7–5.7) μg/g creatinine for women by performing the BMD procedure using the three cut-off values. BMDL values were 1.3 to 2.4 times lower using the 84 percent upper limit value than using the other two cut-off values. The study authors state that the results from this study indicate that using the BMD approach is useful when estimating the threshold level of urinary cadmium in cadmium-exposed subjects. The study authors further state that the study has also shown that a BMD approach could be applied to estimate the threshold level of urinary cadmium in people living in the general environment without any known cadmium pollution since the BMD approach does not need abnormality rates of urinary findings in the control subjects.

Suwazono Y., S. Sand, M. Vahter, A.F. Filipsson, S. Skerfving, J. Lidfeldt, A. Akesson. (2006). Benchmark Dose for Cadmium-Induced Renal Effects in Humans. Environmental Health Perspectives 114(7):1072–1076.

The benchmark dose (BMD) can be defined as the exposure that corresponds to a certain change in response compared with the background. The lower 95 percent confidence bound of the BMD (BMDL) has been suggested to replace the no-observed-adverse-effect-level (NOAEL). Suwazono et al. (2006) calculated BMDs and their 95 percent lower confidence bounds (BMDLs) for renal effects of cadmium in a population with low environmental exposure using a hybrid approach. This hybrid approach uses the concept of risk for a continuous outcome (effect variable), avoiding the limitations associated with categorization of data. The analysis used urinary cadmium (U-Cd) levels as a biomarker of long-term cadmium exposure and urinary *N*-acetyl-β-D-glucosaminidase (NAG) and human complex-forming protein (pHC) as markers of tubular effects, and estimated glomerular filtration rate (GFR), based on serum cystatin C, as a marker of glomerular effects.

Morning urine and blood samples were collected from a total target population of 820 Swedish women ranging in age from 53 to 64 years. The U-Cd, NAG, and pHC were measured in 790 of the 820 women and the GFR was estimated for 700 of the women. Age, body mass index, use of nonsteroidal anti-inflammatory drugs, and blood lead levels were used as covariates for the estimated GFR. The maximum likelihood approach was used to fit the dose-response curve to the data. The BMDs were calculated using the hybrid approach and the BMDLs were calculated using the profile likelihood method. BMDs/BMDLs corresponding to an additional risk (benchmark response, BMR) of 5 to 10 percent were calculated (the background risk at zero exposure was set to 5 percent). The results were compared with the estimated critical concentrations obtained by applying logistic models used in previous studies.

For tubular effects (both NAG and pHC), the BMDs of U-Cd were 0.5 - 1.1 μg/L (adjusted for specific gravity of 1.015 g/mL) and 0.6 - 1.1 μg/g creatinine. For glomerular effects (estimated GFR), the BMDs were 0.8–1.3 μg/L (adjusted for specific gravity) and 1.1–1.8 μg/g creatinine. The BMDLs for tubular effects, using a cutoff $P(0)$ of 5 percent, were 0.4 μg/L (0.5 μg/g creatinine) at a BMR of 5 percent and 0.7 μg/L (0.8 μg/g creatinine) using a BMR of 10 percent. The corresponding BMDLs for glomerular effects were 0.5 μg/L (0.7 μg/g creatinine) and 0.9 μg/L (1.2 μg/g creatinine) for BMRs of 5 and 10 percent, respectively. The study authors found that this critical U-Cd level for glomerular effects was lower and closer to the critical levels for tubular effects than expected from previous studies. Generally, the critical concentrations obtained by the hybrid method approach were lower than those previously reported. The lowest observed effect levels based on the same data were on average 10 percent higher than the BMDs from this study.

Trzcinka-Ochocka M., M. Jakubowski, G. Razniewska, T. Halatek, A. Gazewski. (2004). The effects of environmental cadmium exposure on kidney function: the possible influence of age. Environmental Research 95:143–150.

Trzcinka-Ochocka (2004) assessed the possible influence of long-term environmental exposure to cadmium and age, at the time of exposure, on renal function. This is a follow-up study, conducted in 2000, involving a select group of people from a 1991 to 1994 study of inhabitants of a cadmium-contaminated area in the vicinity of a zinc smelter in Poland. The target population consisted of 308 people, who in 1993, presented with urinary cadmium levels ≥0.5 µg/L, adjusted for a specific gravity of 1.020. Of these 308 people, 136 were exposed to cadmium during their childhood (referred to as former children), and 172 were exposed only as adults having no such childhood exposure (referred to as unexposed adults). The former children group consisted of 72 males and 64 females, and the unexposed adults group consisted of 44 males and 128 females. Blood and urine cadmium concentrations (Cd-B and Cd-U) and blood lead concentrations (Pb-B) were measured. The markers of renal tubular dysfunction (β_2-microglobuline in urine (β_2M-U), retinol binding protein in urine (RBP-U), *N*-acetyl-β-D-glucosaminidase in urine (NAG), along with two isoforms of NAG in urine (NAG-A, NAG-B)), were measured. Markers of glomerular dysfunction (albumin in urine (Alb-U), and β_2-microglobuline in serum (β_2M-S)), were also measured. Persons with kidney disease, diabetes, or a history of occupational exposure to cadmium were excluded from the study based on questionnaire data. All biologic test data were log-transformed for statistical analysis to approximate normality. All of the statistical tests were applied at the significance level of α=0.05.

Cd-B and Cd-U levels for exposed children and unexposed adults indicated that between 1993 and 2000, the geometric mean concentrations increased in both the unexposed adults and the former children groups. However, the increase from 1.28 to 2.23 µg/g creatinine, was statistically significant only for Cd-U in unexposed adults. Pb-B concentrations were significantly lower in 2000 than in 1993 in both the unexposed adults and former children groups. Based on a previous study, the study authors state that the levels (below 339 µg/g creatinine) would not have a statistically significant effect on increased excretion of the biomarkers of renal tubular dysfunction. Based on a cut-off level of 2.4 mg/L, the β_2M-S concentrations were within the range of values reported for the control group from the 1991-1994 study. A significant correlation was found between Cd-U and β_2M-U, RBP-U, Alb-U, NAG, NAG-A, and NAG-B in the group of unexposed adults. In the former children group, a correlation could be noted only between Cd-U and β_2M-U, RBP-U, and Alb-U. In 2000, the geometric mean Cd-U level in unexposed adults was twice as high as it was in 1993. Within the same period, the rise in the Cd-U concentration in the group of former children was rather low and not statistically significant. The study authors stated that these data suggest that adults exposed to cadmium as children may be more susceptible to the renal toxicity of cadmium than persons only exposed as adults.

Uno T., E. Kobayashi, Y. Suwazono, Y. Okubo, K. Miura, K. Sakata, A. Okayama, H. Ueshima, H. Nakagawa, K. Nogawa. (2005). Health effects of cadmium exposure in the general environment in Japan with special reference to the lower limit of the benchmark dose as the threshold level of urinary cadmium. Scand J Work Environ Health 31(4):307–315.

Uno et al. (2005) conducted a basic epidemiologic study on renal dysfunction in areas without known environmental cadmium pollution in 1997 to 1998 and calculated the threshold level of urinary cadmium. The presence of renal effects induced by cadmium exposure was determined using 24-hour urine samples from men and women between the ages of 40 and 59 years of age. The study was conducted on subjects living in three areas of Japan without any known environmental cadmium pollution. The total target population was 828 participants, consisting of 410 males and 418 females.

The samples were analyzed for urinary indicators of renal dysfunction (total protein, β_2-microglobulin (β_2-MG), and *N*-acetyl- β -D-glucosaminidase (NAG)) at detection limits of 1 mg/L for protein, 1 µg/L for β_2-MG, 0.05 U/L for NAG, 0.05 µg/L for cadmium, and 0.005 g/L for creatinine. The association between indicators of cadmium exposure and indicators of renal dysfunction were evaluated using multiple regression and logistic regression analyses. The lower limit of a benchmark dose (BMDL) was calculated from a benchmark dose (BMD) as the threshold level for urinary cadmium. Therefore, the lower 95 percent confidence limit of the dose (BMD) corresponding to a 5 percent ($BMDL_5$) or 10 percent ($BMDL_{10}$) level of each indicator of renal dysfunction above the background level was calculated as the threshold level of urinary cadmium. With all the expressed units (g creatinine^{-1} and day^{-1}) in the multiple regression analysis, the partial regression coefficients showed a significant association between urinary cadmium concentration and total protein, β_2-MG, and NAG for both genders, except for total protein for women (g creatinine^{-1} and day^{-1}). The same results were obtained for both genders in the logistic regression analysis. The $BMDL_{10}$ of the urinary cadmium concentration for NAG was 0.6 µg/g Cr and 1.2 µg/g Cr for men and women, respectively, when corrected for creatinine, and 0.8 µg/day and 0.5 µg/day for men and women, respectively, for 24-hour excretion.

Uno et al. stated that the results from this study indicated that cadmium exposure and the level of the indicators of renal dysfunction were associated among the men and women aged 40 to 59 years of age in areas of Japan without any known environmental cadmium pollution. Further, the threshold level of urinary cadmium in Japan was about the same as those generated in previous Belgium and Sweden studies. Uno et al. noted that in studies of health effects caused by exposure to cadmium, the urinary cadmium is often used as an indicator of internal dose and considers urinary cadmium to be useful as an indicator of cadmium exposure to the general population. According to Uno et al., the significance of the slight kidney effect observed in the study was not clear, but it should be noted that even a slight urinary excretion of β_2-MG is associated with increased mortality.

Wu X., T. Jin, Z. Wang, T. Ye, Q. Kong, G. Nordberg. (2001). Urinary calcium as a biomarker of renal dysfunction in a general population exposed to cadmium. J Occup Environ Med 43:898–904.

Some studies of humans and animals have shown that the increased excretion of urinary calcium may reflect early renal dysfunction induced by cadmium. Therefore, Wu X. et al. (2001) examined urinary calcium excretion in groups of individuals with various levels of environmental cadmium exposure to determine whether a dose-response relationship existed. The total target population of (499) consisted of 252 subjects in the control group and 247 subjects from a cadmium polluted area in southern China. In 1995, a village known to be polluted with cadmium had an average cadmium concentration of 3.71 mg/kg in rice grown in that area. A control location with no known cadmium pollution had an average cadmium concentration in rice of 0.07 mg/kg. The samples were collected, analyzed, creatinine measured, and the values of the urinary indices were adjusted for urinary creatinine.

A statistical analysis of the results using the X^2 test, X^2 trend test, regression analyses, curve estimations and logarithmic transformation were performed. The data were expressed in terms of the geometric mean. The levels of urinary cadmium and urinary calcium in person from the exposed area were significantly higher ($P<0.05$) than those in the control area for both men and women. There was no significant difference regarding urinary zinc between the two study areas. The urinary cadmium concentration in the women was significantly higher than that in the men. Most of the urinary cadmium values were less than 2 μg/g creatinine in the control area but greater than 5 μg/g creatinine in the exposed area. Hypercalciuria and excess urinary secretion of zinc, *N*-acetyl-β-D-glucosaminidase (NAG), and Urinary β_2-microglobulin (β_2-MG) were defined according to the 5 percent prevalence in the control area. The cutoff points for urinary calcium, zinc, and NAG were 220 mg/g creatinine, 0.450 mg/g creatinine, and 120 μmol/g creatinine hour, respectively. The cutoff point for urinary β_2-MG was 0.8 mg/g creatinine for men and 0.9 mg/g creatinine for women.

The authors reported that a significant dose-response relationship between the prevalence of hypercalciuria and the excretion of urinary cadmium was observed, and a significantly increased prevalence of calciuria was found when excretion of urinary cadmium exceeded 2 μg/g creatinine. The findings were similar to those for excess urinary secretion of β2-MG and NAG.

It was concluded by Wu et al. that cadmium exposure can result in increased excretion of urinary calcium in a general population and that there is a significant dose-response relationship. Wu et al. further concluded that urinary calcium can be used as a biomarker of renal dysfunction induced by cadmium.

CHROMIUM

Acharya, S., K. Mehta, S. Krishnan, C. V. Rao. (2001). A subtoxic interactive toxicity study of ethanol and chromium in male Wistar rats. Alcohol 23:99–108.

Acharya et al. (2001) evaluated the interactive toxicity of ethanol with potassium dichromate ($K_2Cr_2O_7$-chromium). The purpose of the study was to determine whether chronic ethanol consumption leads to enhanced toxicity with chromium and whether Wistar rats show sex-specific toxicity to the administration of subtoxic doses of alcohol and chromium combined.

Young, male Wistar rats (100-120 g, 1.5 months old) were divided into four groups of five or six animals. The three groups of treated animals were dosed for 22 weeks, through drinking water, with 10 percent (v/v) ethanol (commercial alcohol), 25 ppm potassium dichromate, or a combination of ethanol plus potassium dichromate at the same concentrations, ad libitum. A control group of animals received untreated drinking water during the same period. All of the animals received a normal diet, ad libitum. The consumption of food and water was monitored every day, and the animals were weighed once a week. After the completion of 22 weeks of treatment, the rats were anesthetized and blood was collected from the dorsal aorta. The serum obtained from the blood was used for estimating the activity of enzymes: succinate dehydrogenase (SDH), alkaline phosphatase (ALP), acid phosphatase (ACP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Portions of liver and kidney were fixed in buffered formaldehyde for histological examination. The remaining part of the liver was homogenized and used for the estimation of total triglycerides, total cholesterol, glycogen, and total glutathione. Statistical analysis of the data was conducted using the Newman-Keuls test and Duncan multiple range test. One-way analysis of variance was used. The significance criterion was $p \leq 0.05$.

Results indicated that the serum SDH and liver total triglyceride levels were reduced significantly in the three treated groups. The ALP levels were reduced significantly in ethanol-treated rats, and there was no significant change in the ACP activity. AST and ALT levels in the three treated groups were increased significantly. The liver glycogen significantly decreased in both the ethanol-treated and the chromium-treated rats. There was a significant increase in liver total cholesterol levels in chromium-treated rats. Total glutathione levels were decreased significantly in the livers of ethanol-treated and ethanol plus chromium-treated rats. The livers of alcohol-treated animals showed altered hepatic architecture in the centrilobular and periportal areas. Similar changes were observed in a histological examination of the livers of chromium-treated rats, except that the damage was more confined to the periportal areas. Moreover, histological examination of the livers of ethanol plus chromium-treated rats revealed uniform damage in the centrilobular and periportal areas, as was observed in the groups treated either with ethanol or chromium. The histological examination of the kidneys in the three treated groups revealed significant damage to the renal tubules and Bowman's capsule. The findings correlate with the serum enzyme levels found in the treated groups. The study authors concluded that chronic ethanol consumption sensitizes the liver to the toxic action of agents such as chromium, leading to impairment of the biochemical functions in the liver, and causing liver and kidney damage. Thus, long-term simultaneous exposure to ethanol and chromium may cause severe health problems in people who are alcoholics and occupationally exposed to chromium.

Aruldhas, M. M., S. Subramanian, P. Sekhar, G. Chandra Hasan, P. Govindarajulu, M. A. Akbarsha. (2004). Microcanalization in the epididymis to overcome ductal obstruction caused by chronic exposure to chromium–a study in the mature bonnet monkey (*Macaca radiata* Geoffroy). Reproduction 128:127–137.

Aruldhas et al. (2004) investigated the toxic effects of chromium on the epididymis in male bonnet monkeys (*Macaca radiate*). Twelve adult male bonnet monkeys were divided into four groups, each consisting of three animals. Groups I to III were provided with drinking water containing Cr (VI) (potassium dichromate) at a concentration of 100, 200, or 400 ppm, respectively, for 180 days. Group IV consisted of control animals, which were provided with chromium-free drinking water. All of the animals were housed individually and fed a standard monkey diet, ad libitum. At the end of the experimental period, the animals were sacrificed and the testicles and epididymides were removed. The testicles and segments of epididymis from the control and treated monkeys were subjected to light microscopic (resin-embedded semithin sections) and transmission electron microscopic analyses.

The study authors reported the appearance of two types of microcanals in the cauda epididymidal epithelium of monkeys in all three treatment groups. The predominant type of microcanal consisted of irregular oval- or oblong-shaped lumina, which contained few spermatozoa, but cell debris was seen occasionally. The second type of microcanal was rare, narrow and more circular in outline than the earlier one and invariably contained sperm and/or cell debris. The study authors state that the results indicated that the obstruction of the distal portion of the cauda epididymidis may be the cause of these microcanals in the chromium-treated monkeys. Obstruction may be due to: (1) hypertrophy and hyperplasia of the epithelium to such an extent that it formed into villositis almost obliterating the lumen; (2) accumulation of abnormal and/or immature germ cells arriving from the testis and macrophages arriving from the epididymal epithelium; and (3) cell debris and nuclei of disintegrating principal cells of the proximal segments of the epididymis filling up the lumen. The microcanals appear to allow passage for spermatozoa to bypass obstruction in the main duct (Type 1) and to entrap spermatozoa to prevent extravasation of sperm and avoid an autoimmune response of spermatic granuloma formation (Type 2).

The study authors state that the histopathological changes in the testis and epididymis, as well as the incidence of microcanalization in the cauda epididymidal epithelium, were dependent on the concentration of chromium in the drinking water. The most acute impacts occurred in the animals exposed to drinking water containing 400 ppm of chromium, followed by the groups exposed to 200 ppm and 100 ppm. The study authors concluded that chronic exposure to drinking water can produce pathological manifestations in the epididymal epithelium, but the epididymis is capable of overcoming such adverse occurrences.

Aruldhas, M., S. Subramanian, P. Sekhar, G.Vengatesh, G. Chandrahasan, P. Govindarajulu and M. Akbarsha. (2005). Chronic chromium exposure-induced changes in testicular histoarchitecture are associated with oxidative stress: study in a non-human primate (*Macaca radiata* Geoffroy). Human Reproduction 20(10):2801–2813.

Aruldhas *et al.* (2005) reported testicular toxicity of hexavalent chromium (CrVI) in bonnet monkeys.

Adult bonnet monkeys were divided into four groups, each consisting of six animals. Groups I to III were provided with drinking water containing CrVI (potassium dichromate) at a concentration of 100, 200, and 400 ppm, respectively, for 180 days. Group IV consisted of control animals, which were provided with chromium-free drinking water. At the end of the experimental period, testicles from three of each of the control and treated monkeys were removed for ultrastructural and biochemical analyses. The testes of the remaining three monkeys in each group were removed after withdrawing CrVI treatment for a period of 180 days.

The study authors reported that the plasma concentration of chromium increased up to tenfold in the monkeys that were provided with drinking water containing chromium, and it returned to the normal level in the recovery group. The absolute weights of the testis did not show any appreciable change due to CrVI treatment. However, there was a statistically significant decrease in the relative weight of the testes of monkeys that were exposed to CrVI. The weight of the testes in monkeys belonging to the withdrawal group showed a trend of recovery to control level. CrVI treatment disrupted spermatogenesis, leading to accumulation of prematurely released spermatocytes, spermatids and uni- and multinucleate giant cells in the lumen of seminiferous tubules. The seminiferous tubules of the chromium-treated monkeys were disorganized, with decreased diameter, depletion of germ cells, and hyperplasia of Leydig cells relative to the control group. Transmission electron microscopy revealed granulation of chromatin and vacuolation between acrosomal cap and manchette microtubules of elongated spermatids and in the Golgi area of round spermatids. Pachytene spermatocytes had fragmented chromatin and swollen mitochondria with collapsed cristae. Macrophages containing phagocytosed sperm and dense inclusions in Sertoli cells were seen. Specific activities of the antioxidant enzymes and concentrations of the nonenzymatic antioxidants decreased, while concentrations of H_2O_2 and hydroxyl radicals increased in the testis of chromium-treated monkeys. Withdrawal of chromium treatment for six months normalized spermatogenesis and the status of pro- and antioxidants in the testis.

The authors concluded that CrVI disrupts spermatogenesis by inducing free radical toxicity, and supplementation of antioxidant vitamins may be beneficial to the affected subjects.

Aruldhas, M., S. Subramanian, P. Sekhar, G.Vengatesh, P. Govindarajulu, M. Akbarsha. (2006). In vivo spermatotoxic effect of chromium as reflected in the epididymal epithelial principal cells, basal cells, and intraepithelial macrophages of a nonhuman primate (*Macaca radiata* Geoffroy). Fertility and Sterility 86(Suppl 3): 1097–1105.

Aruldhas *et al.* (2006) investigated the potential in vivo spermatotoxic toxic effect of hexavalent chromium (CrVI) in men who are occupationally or environmentally exposed to it, through a simulation experiment in a nonhuman primate model.

Adult bonnet monkeys were divided into four groups, each consisting of three animals. Groups I to III were provided with drinking water containing CrVI (potassium dichromate) at a concentration of 100, 200, and 400 ppm, respectively, for 180 days. Group IV consisted of control animals, which were provided with chromium-free drinking water. At the end of the experimental period, testicles and epididymides of each of the control and treated monkeys were removed and subjected to transmission electron microscopic analyses.

The study authors reported that the abundance of basal cells (BC) and intraepithelial macrophages (IEM) and the content of lipofuscin (LF) material in these cell types increased. The principal cells phagocytosed from the lumen the dead sperm resulting from CrVI exposure and processed them partially into LF material, which was acquired by the BCs and IEMs and processed further. The LF material–laden BCs and IEMs appeared to leave the epithelium, accompanied by recruitment of fresh BCs and IEMs.

The study indicated that CrVI exposure leads to increase in abundance of BCs and IEMs in the monkey epididymal epithelium and that the cytoplasm of both cell types in the initial segment, caput, and corpus were laden with LF material. Both cell types appeared to acquire the LF material from the PCs, produced as a result of partial digestion of spermatozoa, which were phagocytosed through the apical plasma membrane. The spermatozoa thus phagocytosed are to be considered dead because live sperm are not phagocytosed, excepting in the case of autoimmune response to sperm antigens.

According to the study authors, the results supported earlier findings that the exposure of monkeys to CrVI through the oral route for a chronic period of 180 days can bring about toxic effects on the testis and epididymis, including spermatotoxicity.

Beaumont, J. , R. Sedman, S. Reynolds, C. Sherman, L. Li, R. Howd, M. Sandy, L. Zeise, G. (2008). Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. Epidemiology 19 (1):12–23.

Beaumont et al. (2008) evaluated data on Cr^{+6}contamination of groundwater and cancer mortality in nine study regions near a ferrochromium factory. Data were obtained from reports and other communications from investigators at the local Jinzhou Health and Anti-Epidemic Station, spanning from 1965 to 1986. Information was used to: (1) describe the chronology, hydrogeology, and level of Cr+6 contamination of drinking water in the study regions; (2) estimate population at risk and numbers of cancer deaths in the 1970 to 1978 mortality observation period; and (3) calculate rate ratios (RRs) for comparison of cancer mortality rates in the Cr^{+6}-exposed study regions (combined) to rates in the Cr^{+6}-unexposed study regions (combined) and Liaoning Province.

Jinzhou investigators identified three principal sources of Cr^{+6}: (1) wastewater containing water-soluble Cr^{+6} discharged from the factory into a ditch that flowed into a dry riverbed and percolated into groundwater prior to reaching Yangxing village; (2) leaking parts in the equipment releasing Cr^{+6}-containing liquid waste onto the ground at the factory work sites; and (3) chromium ore residue stored on open ground, allowing water soluble Cr^{+6} to percolate into groundwater during rains. In 1965, 397 drinking water wells were sampled in the mortality study regions by Jinzhou investigators, and reported detectable $Cr.^{+6}$ (The detection limit was 0.001 mg/L, but information for Cr^{+6} analytic methods was not provided.) Pollution control measures were implemented and rapid decline in groundwater Cr^{+6} concentrations occurred after 1967.

Jinzhou investigators used an ecological epidemiologic study design to observe cancer mortality in nine geographic regions near the ferrochromium factory over a nine-year period, 1970 to 1978. Jinzhou investigators calculated cancer mortality rates by dividing the number of cancer deaths by estimates of person-years-at-risk in the 1970 to 1978 observation period. Jinzhou reports did not present details of the person-years estimation methods). Rates by sex, age, and time period within the 1970 to 1978 observation period were not reported and were not recoverable. The investigators reported both crude and age-adjusted rates for total cancer. Beaumont et al. (2008) estimated the numbers of deaths in the study regions for all cancer, stomach cancer, lung cancer, and other cancers by multiplying the estimated person years by the estimated age-adjusted rates and rounding to the nearest whole number. The nine study regions were divided into two groups, exposed (five regions) and unexposed (four regions), based on whether Cr^{+6} was or was not reported in drinking water. The rates of cancer mortality in the combined exposed regions were compared to the rates in the combined unexposed study regions and to the rates in Liaoning Province as a whole by calculating RRs.

The all-cancer mortality rate in the combined five study regions with Cr^{+6}-contaminated water was elevated negligibly in comparison with the rate in the four combined study regions without contaminated water (rate ratio = 1.13; 95 percent confidence interval = 0.86 –1.46), but was somewhat more elevated in comparison with the whole province (1.23; 0.97–1.53). Stomach cancer mortality in the regions with contaminated water was more substantially elevated in comparison with the regions without contaminated water (1.82; 1.11–2.91) and the whole province (1.69; 1.12–2.44). Lung cancer mortality was elevated slightly in comparison with the

unexposed study regions (1.15; 0.62–2.07), and more strongly elevated in comparison with the whole province (1.78; 1.03–2.87). Mortality from other cancers combined was not elevated in comparison with either the unexposed study regions (0.86; 0.53–1.36) or the whole province (0.92; 0.58 –1.38).

McCarroll, N., N. Keshava, J. Chen, G. Akerman, A. Kligerman, and E. Rinde. (2009). An Evaluation of the Mode of Action Framework for Mutagenic Carcinogens Case Study II: Chromium (VI). Environmental and Molecular Mutagenesis. 2009 Aug 25. [Epub ahead of print].

In response to the 2005 revised U.S Environmental Protection Agency's (EPA) Cancer Guidelines, McCarroll et al. (2009) developed a strategy/framework in which mutagenicity and genotoxicity data combined with additional information relevant to mutagenicity are assessed to determine whether a carcinogen operates through a mutagenic mode of action (MOA). This information is necessary for proper implementation of the 2005 revised U.S. EPA Cancer Guidelines and deciding whether age-dependent adjustment factors (ADAFs) should be applied to the cancer risk assessment.

A decision tree for the framework developed is shown as Figure 1 in the article. The first step in the process is to gather and organize genotoxicity data and determine if the criteria established provide sufficient evidence of mutagenicity could be satisfied. The second step is to determine whether a mutagenic MOA for carcinogenesis can be demonstrated in animals and, if so, to determine whether a mutagenic MOA is plausible and/or supported in humans. McCarroll et al. (2009) used chromium (VI) as a case study to test the framework. In vitro and in vivo genetic toxicology data for chromium (VI) were obtained from open literature (as previously described). The genetic activity profile (GAP) used was developed jointly with the International Agency for Research on Cancer (IARC). The GAP graphically displays genetic toxicology data as a function of concentration or dose. Carcinogenicity data were extracted from the NTP two-year rat and mouse drinking water studies and the open literature.

In their study, NTP concluded that the results with Cr (VI) showed clear evidence of carcinogenicity in male and female mice and rats. Cr (VI) is also mutagenic, in numerous in vitro assays, in animals (mice and rats) and in humans. Accordingly, Cr (VI) was processed through the MOA framework; postulated key steps in tumor formation were interaction of DNA with Cr (VI) and reduction to Cr (III), mutagenesis, cell proliferation, and tumor formation. Within the timeframe and tumorigenic dose range for early events, genetic changes in mice (single/double-stranded DNA breaks) commence within 24 hours. Mechanistic evidence was also found for oxidative damage and DNA adduct formation contributing to the tumor response. The weight of evidence supports the plausibility that Cr (VI) may act through a mutagenic MOA. Therefore, the Cancer Guidelines recommend a linear extrapolation for the oral risk assessment.

Cr (VI) also induces germ cell mutagenicity and causes DNA deletions in developing embryos. Therefore, the authors recommended that the ADAFs be applied because data showed DNA deletions in mouse offspring following transplacental exposure to Cr (VI). Similarly, there is evidence of dominant lethal mutations in mice as well as micronucleus induction in the peripheral blood and liver of mouse fetuses. Although no data were found for children in the two to <16 years of age group, it is likely, given the ability of Cr (VI) to penetrate cellular membranes through a nonspecific anion channel and the intracellular mechanisms leading to mutations that children in this age group would be at risk.

National Toxicology Program (NTP). (2007). NTP Technical Report On the toxicity studies of sodium dichromate dihydrate administered in drinking water to male and female F344/N rats and B6C3F$_1$ mice and male BALB/c and *am3*-C57BL/6 mice. NTP Toxicity Report Series 72:1–144.

The NTP (2007) performed two studies to determine doses and the most appropriate test species for future long-term studies of sodium dichromate dehydrate. These studies were short-term (three-month) toxicology tests in rats and three strains of mice.

In the first study, groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice were given drinking water containing 0 (control), 62.5, 125, 250, 500, or 1,000 mg sodium dichromate dehydrate/L for three months. Additional groups of 10 rats per sex were exposed to the same concentrations of sodium dichromate dehydrate for 4 weeks. The sodium dichromate dehydrate was greater than 99 percent pure. These doses were equivalent to average daily doses of approximately 5, 10, 17, 32, or 60 mg sodium dichromate dehydrate/kg body weight to rats and 9, 15, 26, 45, or 80 mg/kg body weight to mice. All of the rats and mice survived to the end of the study. Reduced body weights occurred in 500 and 1,000 mg/L male rats, 1,000 mg/L female rats, and in male and female mice exposed to 125 mg/L or greater. Tissues from 35 sites were examined for each animal. Exposure to sodium dichromate dehydrate caused a microcytic hypochromic anemia in rats and mice, but the severity was less in mice. Male and female rats exposed to 1,000 mg/L had focal ulceration, hyperplasia, and metaplasia of the forestomach. Additionally, increased histiocytic infiltration in the liver (female), duodenum of the small intestine, and/or pancreatic lymph nodes at concentrations as low as 62.5 mg/L. Mice exposed to sodium dichromate dehydrate significantly had increased hyperplasia of the small intestine at all exposure levels. Histiocytic infiltration was also observed in the mesenteric lymph nodes at 125 mg/L or greater in the B6C3F1 strain.

In the second study, sodium dichromate dehydrate was administered in drinking water to groups of 10 male B6C3F$_1$ mice, 10 male BALB/c mice, and five *am3*-C57BL/6 mice at concentrations of 0 (control), 62.5, 125, or 250 mg/L for three months. These doses were equivalent to average daily doses of approximately 8, 15, or 25 mg sodium dichromate dehydrate/kg body weight. All of the mice survived to the end of the study. Mean body weights of 125 and 250 mg/L B6C3F$_1$ and BALB/c mice and all exposed groups of *am3*-C57BL/6 mice were less than those of the control groups. Tissues from the liver and stomach of each animal were examined microscopically. Exposure concentration-related decreases in mean red cell volumes and mean red cell hemoglobin values were observed in all three mouse strains. Erythrocyte counts were increased in exposed B6C3F$_1$ and BALB/c mice but not in *am3*-C57BL/6 mice. Changes in organ weights were generally consistent with reduced body weights in exposed groups in all mouse strains. No biologically significant differences in reproductive parameters were observed in any strain. Histiocytic cellular infiltration and epithelial hyperplasia of the duodenum occurred in most mice exposed to 125 or 250 mg/L, and the incidences of these lesions were increased in the 62.5 mg/L group compared to controls. The incidence of histiocytic cellular infiltration in the mesenteric lymph node was significantly increased in the 250 mg/L group of male *am3*-C57BL/6 mice. Also the incidences of glycogen depletion of the liver were significantly increased in male B6C3F1 mice exposed to 125 or 250 mg/L and in all exposed groups of male *am3*-C57BL/6 mice.

The study concluded that exposure to sodium dichromate dihydrate caused hyperplasia and ulceration of the stomach in rats and an anemia and lesions of the small intestine in rats and mice.

National Toxicology Program (NTP). (2008). NTP Technical Report On the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR 546. NIH Publication No. 08-5887:1–200.

NTP (2008) studied the effects of sodium dichromate dihydrate in drinking water on male and female rats and mice to identify potential toxic or cancer-related hazards. Groups of 50 male and 50 female F344/N rats were given drinking water containing 14.3, 57.3, 172, or 516 mg of sodium dichromate dihydrate per liter of water for two years (equivalent to average daily doses of approximately 0.6, 2.2, 6, or 17 mg sodium dichromate dihydrate/kg body weight for males and 0.7, 2.7, 7, or 20 mg/kg for females). Groups of 50 male $B6C3F_1$ mice were given 14.3, 28.6, 85.7, and 257.4 mg of sodium dichromate dihydrate per liter of water for two years (equivalent to average daily doses of approximately 1.1, 2.6, 7, or 17 mg sodium dichromate dihydrate/kg body weight). Groups of 50 female $B6C3F_1$ mice were given 14.3, 57.3, 172, or 516 mg sodium dichromate dihydrate per liter of water (equivalent to average daily doses of approximately 1.1, 3.9, 9, or 25 mg sodium dichromate dihydrate /kg). Control animals received the same tap water with no chemical added. Survival of the exposed groups was similar to that of the control groups. Mean body weights of 516 mg/L male and female rats were less than control groups. Mean body weights of the exposed male mice were similar to control group by the end of the study. Mean body weights of the exposed female mice for the 172 and 516 mg/L groups were less than the control group. The lower body weights were attributed partly to poor palatability of the dosed water and consequent reductions in water consumption. At the end of the study, tissues from more than 40 sites were examined for every animal. The results from both of these tests (rats and mice) indicate that there was clear evidence of carcinogenic activity of sodium dichromate dihydrate in male and female F344/N rats, based on incidences of cell neoplasms of the oral cavity. There was also clear evidence of carcinogenic activity of sodium dichromate dihydrate in male and female B6C3F1 mice, based on increased incidences of neoplasms of the small intestine (duodenum, jejunum, or ileum). Exposure to sodium dichromate dihydrate resulted in histiocytic cellular infiltration in the liver, small intestine, and the pancreatic and mesenteric lymph nodes of rats and mice. The incidences of diffuse epithelial hyperplasia were increased significantly in the duodenum of all exposed groups of male and female mice.

The study concluded that sodium dichromate dihydrate in the drinking water caused oral cancers in rats and cancer of the small intestine in mice.

Oliveira, H., M. Spano, M. Guevara, T. Santos, C. Santos, M. Pereira. (2009). Evaluation of *in vivo* reproductive toxicity of potassium chromate in male mice. Experimental and Toxicol Pathology. 14 pages.

Oliveira et al. (2009) evaluated the effects of potassium chromate on mice sperm cells after a short-term exposure. Three groups of 10 male ICR-CD1 mice (eight-weeks-old) were subcutaneously injected with 5 or 10 mg K_2CrO_4/kg body weight (bw) for four consecutive days. Control groups of 10 mice each were injected with saline. One group of mice was sacrificed five days after the first dose was administered, and a second group of mice was sacrificed 35 days after the first dose was administered. Chromium contents in mice testes were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Testes and epididymis histology was evaluated by light microscopy, and testicular cells populations were evaluated by flow cytometry (FCM). Spermatozoa were collected from the epididymis and their morphology and several functional parameters (density, motility, mitochondrial function, acrosome integrity) were evaluated. DNA fragmentation and chromatin status of sperm cells were assessed at both Day 5 and Day 35. The control mice presented chromium levels of 0.15 µg Cr/g fresh weight. Chromate-injected mice presented higher levels of chromium ($p<0.001$) on their testis (445 percent increase for mice injected with 5 mg K_2CrO_4/kg bw and 689 percent increase for mice injected with 10 mg K_2CrO_4/kg bw). Testis and epididymis histology were not affected by exposure to 5 and 10 mg K_2CrO_4/kg bw for four consecutive days. There was a reduction in seminiferous tubules diameter. The concentration of sperm cells was not affected by exposure to chromium, suggesting that only higher doses and/or longer periods of chromium exposure may induce changes in the number of sperm cells. An increase in the percentage of multiple abnormalities of spermatozoa after five days was observed in both doses of chromium, and a decrease in the percentage of normal spermatozoa was found at Day 35. Although spermatozoa mitochondrial function or viability was not affected, its motility was reduced significantly by potassium chromate exposure at both Day 5 and Day 35. A decrease in acrosome integrity was found in mice injected with 10 mg K_2CrO_4/bw after 35 days. Exposure to potassium chromate did not affect either DNA fragmentation or chromatin susceptibility to acid denaturation of sperm cells.

According to the study authors, this study was able to show the effects of potassium chromate on spermatozoa morphological and physiological parameters and demonstrated that the doses did not induce DNA damage to sperm cells after one spermatogenic cycle (35 days).

Shara, M., T. Yasmin, A. Kincaid, A. Limpach, J. Bartz, K. Brenneman, A. Chatterjee, M. Bagchi, S. Stohs, D. Bagchi. (2005). Safety and toxicological evaluation of a novel niacin-bound chromium (III) complex. Journal of Inorganic Biochemistry 99:2161–2183.

Shara et al. (2005) determined the broad spectrum safety profile of niacin-bound chromium (NBC). Acute oral, acute dermal, primary dermal irritation and primary eye irritation toxicities of NBC were evaluated. Ames bacterial reverse mutation assay, mouse lymphoma test, and a dose-dependent 90-day subchronic toxicity were also conducted.

Acute oral toxicity of NBC was assessed after administering a single oral dose of 5,000 mg/kg to three Sprague-Dawley male rats, 10 to 11 weeks of age (242.3–251.9 g body weight (bw)) and three nulliparous and nonpregnant female rats, 10–11 weeks of age (198.6–212.3 g bw). The animals were observed for mortality and signs of intoxication for 14 days. All animals were sacrificed at the end of the observation period. The acute oral LD_{50} of NBC was found to be greater than the limit dose level of 5,000 mg/kg bw in both male and female Sprague-Dawley rats. No changes in body weight or adverse effects were observed at necropsy.

An acute dermal toxicity test was conducted on five male and five nulliparous and nonpregnant female Sprague-Dawley rats 9 to 10 weeks old (298-310 g bw and 199–205 g bw, respectively). Individual doses of NBC were calculated based on the initial body weights obtained prior to dosing with a 2,000 mg/kg bw, which was placed on a shaved area of the animal (approximately 10 percent of the body surface) for 24 hours. The animals were observed for mortality, signs of gross toxicity, and behavioral changes. Individual body weights were recorded on Days 7 and 14 after the application. All animals were sacrificed at the end of the observation period. The acute dermal LD_{50} of NBC was found to be greater than 2,000 mg/kg bw in male and female rats. There were no signs of gross toxicity, dermal irritation, adverse pharmacologic effects, or abnormal behavior.

A primary skin irritation test was conducted on one male and two nulliparous and nonpregnant female New Zealand albino rabbits to determine the potential for NBC to produce an irritation after a single topical application. The route of NBC administration was direct application to shaved intact skin. Five-tenths of a gram of the NBC test mixture (NBC and corn oil) was applied to a 6 cm^2 intact dose site on each animal. The NBC pads were removed after four hours of exposure. Individual evaluation of test dose sites was scored according to Draize Scoring System at approximately 1, 24, 48, and 72 hours after removal of the pad. The animals were observed for signs of gross toxicity and behavioral changes once a day during the test period. Results from this test showed that at one hour after the application, very slight erythema was observed at all three treated sites. The overall incidence and severity of irritation decreased with time.

The primary eye irritation test was conducted with NBC on two male and one nulliparous and nonpregnant New Zealand albino rabbits. The route of NBC administration was direct conjunctival installation, standard for assessment of local ocular irritative potential. One-tenth of a milliliter (0.05 g) of NBC in water was instilled into the conjunctival sac of the right eye of each rabbit. Ocular irritation was evaluated macroscopically using a high-intensity white light. The animals were observed for signs of gross toxicity and behavioral changes once a day during

the test period. Under the conditions of this study, the maximum mean total score of NBC powder is 2.0, classifying NBC to be practically nonirritating to the eye. There were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behavior.

The *Salmonella typhimurium* reverse mutation test was conducted to determine the ability of NBC to induce reverse mutation. Test results indicate that NBC did not induce mutagenic effects in the bacterial reverse mutation test in five *S. typhimurium* strains (TA1535, TA98, TA100, TA97a, and TA102), either with or without metabolic activation. Similarly, NBC did not induce mutagenic effects in the mammalian cell gene mutation test in L5178Y mouse lymphoma cells TK (+/-), either with or without metabolic activation.

A dose-dependent 90-day subchronic toxicity study was conducted on six male and female Sprague-Dawley rats, five to six weeks old (140.4–157.9 g and 137.3–153 g bw, respectively). The animals were given either 0 ppm (controls), 5 ppm, 50 ppm, or 125 ppm doses of NBC per day for 90 consecutive days in rodent chow. Mortality/morbidity was evaluated once daily. Groups of animals were sacrificed on 30, 60, and 90 days of treatment. Organ weights as such and as percentages of body and brain weight, hematology, clinical chemistry, hepatic lipid peroxidation, and DNA fragmentation were performed on 30, 60, and 90 days of treatment. Results from the study demonstrated no significant changes in selected organ weights, individually and as percentages of body and brain weight. NBC supplementation did not cause changes in hepatic lipid peroxidation or DNA fragmentation after 30, 60, or 90 days of treatment. Hematology, clinical chemistry and histopathological evaluations did not show any adverse effects in all organs tested.

Shara M., A.E. Kincaid, A.L. Limpach, R. Sandstrom, L. Barrett, N. Norton, J.D. Bramble, T. Yasmin, J. Tran, A. Chatterjee, M. Bagchi, D. Bagchi. (2007). Long-term safety evaluation of a novel oxygen-coordinated niacin-bound chromium (III) complex. Journal of Inorganic Biochemistry 101:1059–1069.

Shara et al., (2007) studied the long-term safety of niacin-bound chromium (III) (NBC) by orally administering either 0 (control) or 25 ppm, which is the human equivalency dose of 1,000 µg elemental chromium (III) as NBC per day for 52 consecutive weeks, to Sprague-Dawley rats. Six male (142-160 g body weight) and six female (137-155 g bw) rats aging from 5 to 6 weeks were used in each group and time point. Food and water consumption were measured twice or thrice weekly. Mortality/morbidity was evaluated once daily. Body weights and ocular health were taken on day one, weekly thereafter, and before necropsy. Animals of each group and each gender were sacrificed on 26, 39, or 52 weeks of treatment. Organs including adrenal glands, brain, epididymides, esophagus, eyes, heart, intestine, kidney, liver, lymph nodes, lungs, mammary glands, ovaries or testes, pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, thymus, thyroid glands, trachea and urinary bladder were collected at necropsy on 26, 39, and 52 weeks of treatment. Selected organ weights as such and as a percentage of liver and brain weight, hepatic lipid peroxidation and DNA fragmentation, hematology and clinical chemistry, and histopathological evaluations were conducted on each group at each time point.

Routine observations did not reveal any physical or ocular abnormalities attributable to NBC supplementation at anytime during the study. There were no statistically significant differences in the food consumption patterns of the NBC supplemented animals relative to their corresponding controls. At 26, 39, or 52 weeks of treatment, body weight gain was significantly reduced by 7.7 percent, 8.1 percent, and 14.9 percent in male rats, and 5.5 percent, 11.4 percent, and 9.6 percent in female rats, respectively, in the NBC treatment groups. There were no significant differences observed in the weights of select organs as such and as a percentage of liver and brain weight at 52 weeks of treatment. No significant changes were observed in hepatic lipid peroxidation and DNA fragmentation, hematology and clinical chemistry, and histopathological evaluation between control and NBC groups at these time points. According to the authors, the extensive animal toxicology studies from this long-term study in conjunction with previously conducted subchronic safety studies (Shara et al., 2005) combined with previous human clinical studies demonstrate the broad spectrum safety of NBC.

Staniek H., Z. Krejpcio. (2009). The effects of tricentric chromium (III) propionate complex supplementation on pregnancy outcome and maternal and foetal mineral status in rat. Food and Chemical Toxicology 47:2673-2678.

Staniek et al., (2009) in a repeated dosage study investigated the effects of tricentric chromium (III) propionate complex (CrProp) supplementation on pregnancy outcome and maternal and fetal mineral status in the rat. Twenty male and 20 female Wistar Albino rats (14 weeks old) were mated. After successful conception, they were fed either AIN-93G diet supplemented with CrProp (100 mg Cr/kg diet, equivalent to 7.2 mg Cr/kg body mass/day) or non-supplemented diet (0.27 mg Cr/kg diet, equivalent to 0.02 mg Cr/kg body mass/day) as the control for 21 days. Feed intake was monitored daily, while body mass gain was measured every second day of the gestation period. The Dams were sacrificed on day 21 of gestation, blood and tissue samples collected and live fetuses were examined for adverse effects. In addition, organs of the dams and live fetuses were removed and examined. Special attention was paid to the influence of CrProp on the mineral status of maternal and fetal organs because Cr may affect adsorption of some microelements, especially iron. Therefore, the maternal and fetal organs were analyzed for Fe, Cu, Zn, and Cr content. Neither histological analyses nor genotoxicity tests were performed, since in previous unpublished work using tenfold higher dosages (72 mg Cr/kg body mass/day) did not produce any indication of deleterious effects in tissues, organs and lymphocyte DNA of rats.

Supplemental Cr given at repeated dosages of 100 mg Cr/kg diet did not affect feed intake, body mass, or internal organ masses in the dams. Also, maternal blood biochemical indices were not different in the dams fed the supplemental CrProp; the exception was that serum total protein concentration was 9% lower than in the control group. However, this concentration was still within normal limits.

The dams receiving the repeated supplemental Cr dosages showed significant changes in tissue mineral levels, in comparison to the control group. Liver and kidney Cr levels increased by 177 percent and 455 percent, respectively, while Cu and Zn liver concentrations decreased by 9 percent and 12 percent, respectively. Supplemental Cr given did not affect pregnancy outcome, litter size, fetal masses or fetal internal organ masses. No abnormalities in gross organ morphology of fetuses were detected.

CrProp did not affect Fe levels in the dams. In addition, CrProp did not affect fetal Cr and Fe levels; however it did significantly increase fetal liver Zn by 181 percent and decreased kidney Cu level by 34 percent, in comparison to the control group. The authors noted that the results presented in this study indicate that repeated dosages of 100 mg Cr/kg diet does not produce deleterious effects on pregnancy outcomes or the maternal and fetal organism, but can affect maternal and fetal tissue Zn and Cu levels in rats.

Stout, M., R. Herbert, G. Kissling, B. Collins, G. Travlos, K. Witt, R. Melnick, K. Abdo, D. Malarkey, M. Hooth. (2009). Hexavalent chromium is carcinogenic to F344/N rats and B6C3F1 mice after chronic oral exposure. **Environmental Health Perspectives** 117(5):716-722.

Stout et al. (2009) characterized the chronic oral toxicity and carcinogenicity of hexavalent chromium [Cr(VI)] in rodents. Due to concerns over Cr(VI) presence in drinking water source supplies, its potential health effects, including carcinogenicity, and the lack of adequate carcinogenicity studies by the oral route, the California Congressional Delegation, California Environmental Protection Agency, and California Department of Health Services nominated Cr(VI) to the National Toxicology Program (NTP) for toxicity and carcinogenicity testing. Sodium dichromate dihydrate (SDD) was selected for testing because it is the primary base material for the production of chromium compounds, is widely used in industrial applications, and is the most water-soluble chromate. NTP conducted two-year drinking water studies of SDD in male and female F344/N rats and B6C3F1 mice.

Exposure concentrations for the two-year studies of SDD were selected after review of a previous NTP three-month toxicity study. The highest exposure concentration for the two-year studies was limited by the toxicity observed in the three-month studies, and a wider spacing of exposure concentrations was selected to extend the dose–response curve. An additional low-exposure group [5 mg Cr(VI)/L] was added to the two-year studies to provide a concentration closer to human exposure through contaminated drinking water.

In the two-year studies, groups of 50 male and 50 female F344/N rats and B6C3F1mice, six to seven weeks of age, were randomly distributed into groups of approximately equal initial mean body weights. Feed and tap water were available ad libitum. The animals were exposed to SDD in drinking water at concentrations of 0, 14.3, 57.3, 172, or 516 mg/L (male and female rats and female mice) or 0, 14.3, 28.6, 85.7, or 257.4 mg/L (male mice) for 105–106 weeks (see Table 1 below). Water consumption was recorded weekly for the first 13 weeks and every four weeks thereafter. Animals were weighed initially, weekly for the first 13 weeks, at four-week intervals thereafter, and at the end of the studies. Animals were observed twice daily and clinical findings were recorded at 4-week intervals beginning at week 5. Animals were sacrificed by carbon dioxide asphyxiation. Complete necropsies and microscopic examinations were performed on all core study rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all protocol-required tissues were fixed and preserved, processed, sectioned, and stained for microscopic examination. The entire gastrointestinal tract was opened and potential lesions were collected for microscopic evaluation. Oral mucosa and tongue are not protocol-required tissues; however, because gross lesions in these tissues were diagnosed as neoplasms, the oral mucosa and tongue of all animals were evaluated histologically. All of the data were analyzed statistically.

Results showed that Cr(VI) exposure resulted in significantly increased incidences of rare neoplasms of the squamous epithelium that lines the oral cavity (oral mucosa and tongue) in male and female rats at 516 mg/L. Both male and female mice showed a clear exposure concentration response for increased incidences of adenoma or carcinoma (combined) at all sites (combined) of the small intestine (duodenum, jejunum, or ileum). These increases were significant at 85.7 and 257.4 mg/L in males and at 172 and 516 mg/L females, the two highest

exposure concentrations in each sex. In addition, the incidence in 57.3 mg/L females exceeded the historical control ranges for drinking water studies and for all routes of administration. Cr(VI) exposure resulted in transient microcytic hypochromic anemia in rats and microcytosis in mice. Cr(VI) exposure did not affect survival but resulted in reduced mean body weights and water consumption, due at least in part to poor palatability of the dosed water. The study authors concluded that Cr(VI) was carcinogenic after administration in drinking water to male and female rats and mice.

Table 1. Concentrations in drinking water and average daily ingested doses of SDD and CR(VI) after exposure for two years.

	Concentration in drinking water (mg/L)		**Average daily ingested dose (mg/kg)**	
Animals	**SDD**	**Cr(VI)[a]**	**SDD[b]**	**CR(VI)[c]**
Male rats	0	0	0	0
	14.3	5	0.6	0.2
	57.3	20	2.2	0.8
	172	60	6	2.1
	516	180	17	5.9
Female rats	0	0	0	0
	14.3	5	0.7	0.2
	57.3	20	2.7	0.9
	172	60	7	2.4
	516	180	20	7.0
Male mice	0	0	0	0
	14.3	5	1.1	0.4
	28.6	10	2.6	0.9
	85.7	30	7	2.4
	257.4	90	17	5.9
Female mice	0	0	0	0
	14.3	5	1.1	0.4
	57.3	20	3.9	1.4
	172	60	9	3.1
	516	180	25	8.7

[a] Calculated using the drinking water concentration of SDD and the percent mass of Cr(VI) in SDD.
[b] Calculated using body weight and water consumption data.
[c] Calculated using the average daily dose of SDD and the percent mass of CR(VI) in SDD.

Subramanian S., G. Rajendiran, P. Sekhar, C. Gowri, P. Govindarajulu, M.M. Aruldhas. (2006). Reproductive toxicity of chromium in adult bonnet monkeys (*Macaca radiate* Geoffrey). Reversible oxidative stress in the semen. Toxicology and Applied Pharmacology 215:237-249.

Subramanian et al. (2006) studied the effect of continuous exposure of chromium to monkeys on reproductive toxicity. Adult male bonnet monkeys (*Macaca radiate* Geoffrey) ranging in weight from 7 to 9 kg were chosen as the test species in order to closely represent the probable adverse effects of chromium on human fertility. Monthly semen samples were collected from the monkeys exposed to varying doses (50, 100, 200, and 400 ppm) of chromium (as potassium dichromate) in drinking water for 6 months. All animals were fed *ad libitum* with standard pellet diet, cooked rice with lentils, vegetables and seasonal fruit. One study group, exposed to 400 ppm chromium, was given simultaneous vitamin C supplementation (0.5 g/L; 1.0 g/L; 2.0 g/L) for six months. A second study group, exposed to 400 ppm chromium for six months, was left free of chromium treatment for an additional six month period to study the reversibility of chromium toxicity. The 400 ppm level was the maximum dosage employed because concentrations >400 ppm led to reduced consumption of water and food and death within three months of treatment. Blood samples were collected at one-month intervals and the concentration of plasma chromium was estimated. Other parameters examined included sperm concentration, superoxide dismutase (SOD) and catalase activity (CAT), reduced glutathione (GSH) concentration in sperm and seminal plasma and Hydrogen peroxide (H_2O_2) production.

Except at the 50 ppm dosage level, chromium treatment decreased sperm count, sperm motility and the specific activities of antioxidant enzymes SOD and CAT in a dose- and duration-dependent manner. The GSH concentrations at the 50 and 100 ppm chromium level remained unchanged, but the 200+ ppm levels of chromium decreased GSH in both seminal plasma and sperm in a dose- and duration-dependent manner. However, the quantum of H_2O_2 in the seminal plasma/sperm from monkeys exposed to chromium increased with an increase in dose >50ppm and with duration of exposure. Chromium in plasma increased in the first month of exposure at 50 ppm. Simultaneous supplementation of vitamin C (0.5 g/L; 1.0 g/L; 2.0 g/L) prevented the noted adverse effects. All three doses levels of the vitamin C were equally effective in masking the effect of chromium. The study authors note that the data provided supports their hypothesis that chronic chromium exposure induces a reversible oxidative stress in the seminal plasma and sperm by creating an imbalance between reactive oxygen species and antioxidant system, leading to sperm death and reduced motility of live sperm. The study also shows that the adverse effect of exposure to chromium containing water on semen quality is reversible after removing the chromium (Subramanian et al., (2006). The authors also noted that the results show that it is possible to pre-empt reproductive toxicity of chromium by simultaneous supplementation of Vitamin C. Overall, the study provides evidence for the reproductive toxicity of chronic chromium exposure, with varying intensity depending on the dose and duration of exposure Subramanian et al., (2006).

NOTE: The number of monkeys used in this study was not discussed. However, each time a mean value was reported in a table, the footnote stated that n=3. Therefore, it may be possible that there were three male monkeys in each group tested.

Yousef, M., F. El-Demerdash, K. Kamil, F. Elaswad. (2006). Ameliorating effect of folic acid on chromium(VI)-induced changes in reproductive performance and seminal plasma biochemistry in male rabbits. Reproductive Toxicology 21:322-328.

Yousef et al. (2006) examined possible protective effects of folic acid (FA) on the reproductive toxicity of potassium dichromate ($K_2Cr_2O_7$) in male New Zealand white rabbits at seven months of age and with an initial weight of 2.87±0.06 kg. Twenty-four mature male rabbits were randomly divided into four treatment groups. Group 1 served as control. Groups 2, 3, and 4 were given folic acid (8.3 µg/kg body weight (bw)), potassium dichromate (5 mg/kg bw; containing 3.6 mg chromium (VI)), and potassium dichromate (5 mg/kg bw) plus folic acid (8.3 µg/kg bw), respectively. Dosing with folic acid and/or potassium dichromate was given orally with the aid of a plastic tube directly inserted into the oropharyngeal region. The rabbits were dosed daily for 10 weeks. Feed and water were provided *ad libitum* and the rabbits were monitored closely during the treatment period and showed no adverse clinical signs. Feed intake and body weight was recorded weekly throughout the 10-week treatment period. Semen was collected weekly from each test group. All rabbits were euthanized after the 10-week treatment period. Reproductive performance, lipid peroxidation, enzyme activities and biochemical parameters in seminal plasma were monitored. Body weight, relative weights of testes and epididymis, and plasma testosterone concentration were significantly ($P<0.05$) decreased in rabbits treated with potassium dichromate as compared to control and folic acid-treated rabbits; however, treatment with potassium dichromate did not significantly alter feed intake. Levels of thiobarbituric acid-reactive substances increased, whereas the activities of glutathione *S*-transferase, transaminases and phosphatases decreased in the seminal plasma following potassium dichromate exposure. Folic acid alone caused an increase ($P<0.05$) in body weight, relative weight of testes and epididymis, semen characteristics and seminal plasma enzymes, as compared to control animals. In addition, co-treatment with folic acid alleviated the toxicity of potassium dichromate with respect to various test parameters. The study authors concluded that current administration of folic acid to potassium dichromate-treated animals ameliorated the induced sperm quality damage, significantly improved the sperm parameters and reduced the induction of seminal plasma free radicals.

MERCURY

Atkinson, A., S.J Thompson, A. T Khan, T.C. Graham, S. Ali, C. Shannon, O. Clarke, L. Upchurch. (2001). Assessment of a two-generation reproductive and fertility study of mercuric chloride in rats. Food and Chemical Toxicology 39:73-84.

Atkinison et al. (2001) examined the reproductive performance of mercuric chloride on two successive generations of Sprague Dawley rats. The F_0 generation was divided into four treatment groups for males and females separately (20 rats each group). The F_0 dose levels (male:female) were 0:0 mg/kg/day (control), 0.50:0.75 mg/kg/day (low dose), 1.00:1.50 mg/kg/day (mid dose), and 2.00:3.00 (high dose). Due to toxicity, the high doses were reduced to 1.50 mg/kg/day for males after 43 days and 2.50 mg/kg/day for females after 27 days. Doses were administered by oral gavage, with males receiving daily doses throughout pre-cohabitation (60 days or one complete cycle of spermatogenesis) and cohabitation (21 days) and females receiving daily dose throughout pre-cohabitation (16 days or one complete cycle of oogenesis), cohabitation (21 days), gestation (21 days) and lactation (21 days).

Selected parental F_1 males and females were exposed at the same doses received by their parents for the same periods and mated to produce F_2 offspring. None of the F_1 offspring from the high-dose group were selected to continue with the F_1 generation due to insufficient number of offspring in this group. At the end of cohabitation, the F_0 males were sacrificed and weights of the kidneys, liver, brain, pituitary, adrenals, tested, epididymides, prostate and seminal vesicles were recorded. For F_0 females, they were allowed to deliver offspring naturally and were sacrificed after weaning. At necropsy, the uterus was inspected for implantation sites and weights of the kidneys, liver, brain, pituitary, adrenals, ovaries, and uterus were weighed. The F_1 offspring were examined at birth with respect to total liter size, number of stillborn pups, sex distribution, pup body weight and external congenital abnormalities. At the end of cohabitation for F_1 males and lactation for F_1 females, the animals were sacrificed, necropsied and their organ weights recorded in the same manner as their F_0 parents. The F_2 litters were evaluated in the same manner as their preceding F_1 litters.

Significant differences ($p \leq 0.05$) were observed in the F_0 generation exposed to mercuric chloride as compared to the controls, including implantation efficiency, fertility, live births and day 4 survival indices, litter size, and body weight of F_1 pups. The reproductive and fertility effects in the F_1 generation decreased. In the F_1 generation, fertility index and litter size were not affected, though mercuric chloride exposure did significantly affect implantation efficiency, live birth weights, and day 4 survival indices. According to the study authors, the reason for the decline in reproductive effects seen in the second generation may be because this generation developed a tolerance after prolonged exposure to mercuric chloride, which included embryonic stages of their life. For males specifically, the F_0 generation showed significant differences in body weight and weight of kidney, testes, epididymides, prostate and seminal vesicles, while the F_1 generation showed significant differences in body weight and weights of kidney, brain, liver, testes, prostate and seminal vesicles. For females specifically, the F_0 generation showed significant differences in weights of kidneys, brain and liver, while the F_1 generation showed significant differences in body weight, as well as the weights of the kidneys, liver, adrenals, uterus and ovaries.

Atkinson et al. noted that a limitation with their study is the inability to determine which of the sexes was responsible for the adverse effects on reproduction and a crossover mating trial would be needed. Atkinson et al. reported that "In summary, MC administered to rats at low doses over two successive generations resulted in adverse effects on the number of implants, fertility and reproductive indices, and sex ratios in the F_0 generation. However, the effects on reproduction and fertility were decreased in the succeeding F_1 generation. Finally live birth and four-day survival indices were also significantly reduced in the succeeding F_1 generation."

Boujbiha, M.A., K. Hamden, F. Guermazi, A. Bouslama, A. Omezzine, A. Kammoun, A. El Feki. (2009). Testicular toxicity in mercuric chloride treated rats: Association with oxidative stress. Reproductive Toxicology 28:81-89.

Boujbiha et al. (2009) conducted a study to determine if oxidative stress is involved in the deterioration of testicular functions induced by mercury compounds. Adult sexually mature male Wistar rats were used in the study. One hundred thirty-two albino rats (ages three months old, weighing ~190g) were randomly divided into three treatment groups (44 rats each) and orally dosed with mercuric chloride through drinking water at concentrations of 0 ppm (control), 50 ppm (HG1), or 100 ppm (HG2) for 90 days. The average daily doses of treated water were four mg/kg/day for group HG1 and 8 mg/kg/day for group HG2. Body weight, daily intake of food and water were determined three times per week. At the intervals of 3, 7, 15, 30, 60 and 90 days, six rats from each group were sacrificed, at which time blood samples were taken and organs (testes, epididymis, prostate glands, and seminal vesicles) were removed, dried, weighed, and evaluated microscopically.

The following analyses were conducted: total mercury concentrations in testes and whole blood, epididymal sperm counts, sperm motility, serum testosterone levels, malondialdehyde (MDA) concentrations and the activities of two representative anti-oxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) in testicular tissues, and lipid peroxidation (LPO) production in testes. Additionally, reproductive performance was measured by caging the males with cyclic females and counting sperm in vaginal spear samples. The dams were sacrificed on day 10 post conception to determine the implantation efficiency.

After 90 days of exposure, an increase in absolute and relative wet weight of the testis and a decrease in absolute and relative wet weight the accessory sex glands were observed. Histological examination showed fewer mature luminal spermatozoa in the treatment groups than the control groups. Significant variation in testosterone serum levels were observed over the exposure period, with decreases in the beginning, followed by significant increases. By the end of exposure period, serum testosterone levels in the treated groups were statistically equal to the controls. Epididymal sperm count increased in a time dependant manner, except at the end when the counts decreased in the treatment groups. As compared to the controls, epididymal sperm count was reduced in a dose related manner, with the maximum effect after 7 days of exposure (24percent reduction for HG1 and 47 percent reduction for HG2). Sperm motility followed a similar pattern, with maximum reductions as compared to the control on day seven after exposure (55 percent reduction for HG1 and 58 percent reduction for HG2). Mercury exposure also resulted in a decline of the reproductive performance of treated males. The mating index (number of females showing evidence of mating/number of females placed with males * 100) was affected in the HG2 group (50 percent mating index), but not the HG1 treatment group (100percent mating index). In both treatment groups, however, there was a significant decrease in the number of viable embryos/litter (36 percent for HG1 group and 76 percent for HG2 group). The above effects were attributed to a significant increase in the mercury content of testes and blood, which were observed in a time and dose dependant manner, respectively. According to the study authors, evidence of oxidative stress was observed by the significant dose dependant increase in lipid peroxidation in testicular tissues and through alterations in the activity of SOD and CAT. The study authors suggest that an increase in free radical formation

relative to loss of antioxidant defense system after mercury exposure may cause testis to be more susceptible to oxidate damage, leading to functional inactivation.

Heath, J.C., Y. Abdelmageed, T.D. Braden, A.C. Nichols, D.A. Steffy. (2009). The effects of chronic mercuric chloride in female Sprague-Dawley rats on fertility and reproduction. Food and Chemistry Toxicology 47:1600-1605.

Heath et al. (2009) studied the effects of inorganic mercury on fertility from chronic exposure at low concentrations which do not cause any physical signs. The study was conducted on 60 female Sprague-Dawley rats (30 days old) that were assigned to one of three experimental groups (control, low and high dose) and chronically exposed to mercuric chloride by oral gavage for 60 days. The experimental groups, 20 rats each, were given mercuric chloride or an equivalent volume water as follows: control group (0 mg/kg/day), low dose group (1 mg/kg/day), and high dose group (2 mg/kg/day). After 60 days, ten rats in each group were mated with adult unexposed males. The female rats were sacrificed at approximately gestation day 13, calculated from the day the presence of plug/sperm was confirmed. The subjects were weighed, blood was collected for progesterone determination in the plasma, ovaries were removed and examined for number of corpora lutea, and the pituitary glands were collected for analysis of luteinizing hormone (LH) and follicle stimulating hormones (FSH).

A significant decrease in weight gain was observed in the exposed groups as compared to the controls, which was attributed by the authors to mercury intoxication. Weight gain was dose- and time-dependent. The main finding of the study was that the high dose group, as compared to the low dose and control groups, had a significantly lower number of implantations and significantly higher number of non-viable implantations. The count of corpora lutea, a measure of ovulation rate, showed no difference between any of the groups. The plasma progesterone levels followed a dose related pattern of lower progesterone to high mercury exposure. Plasma progesterone levels were significantly lower in the high dose group (74.2 ng/ml) as compared to the control group (90.6 ng/ml) and were also significantly different between the two exposure groups. Lutenizing hormone was found to be significantly higher in the high dose group (2.3 µg/pituitary gland) as compared to the low dose group (1.9 µg/pituitary glands) and control group (1.93 µg/pituitary glands). For FSH, there was no difference between the control and two exposure groups, however, distribution of FSH showed an upward trend in the levels with increasing exposure. The study authors concluded that low level chronic ingestion of mercuric chloride in female rats does not affect ovulation, but does produce disruption of implantation and fetal viability. Also, they state that mercuric chloride may have a disruptive effect in the corpora lutea which manifests itself after ovulation, as indicated by the lower progesterone levels, higher LH levels, and possibly higher FSH levels.

Khan, A.T., A. Atkinson, T. Graham, S. Thompson, S. Ali, K.F. Shireen. (2004). Effects of inorganic mercury on reproductive performance of mice. Food and Chemical Toxicology 42:571-577.

Khan et al. (2004) examined the effects of mercuric chloride on the reproductive performance of mice. In this study, C57BL/6 mice were randomized into four treatment groups (25 males and 25 females per group) and exposed to 0 (control), 0.25, 0.50, and 1.0 mg/kg/day of mercuric chloride by oral gavage. The mice were exposed daily (7 days/week) throughout all phases of the study. For males, the phases included pre-mating (40 days) and mating (21 days). For females, the phases included pre-mating (16 days), mating (21 days), gestation (21 days), and lactation (21 days). At the end of the pre-mating stage, the mice were paired within the treatment groups for mating. At the end of mating for males and lactation for females, the mice were sacrificed. Blood samples were collected and the mice were necropsied. At termination, organ weights were recorded for the adrenal glands, brain, kidneys, liver, testes, epididymides, seminal vesicles, prostate, ovaries, and uterus. Histopathologic evaluation was also conducted on all of these organs, in addition to analysis of the organs (except adrenal glands) for mercury content. At parturition, the dams were weighed and their litter counted and weighed. Reproductive parameters of parental mice and their offspring included: fertility index, duration of gestation, number of implants, number of pups delivered, sex ratio, and pup viability indices.

Fertility and pup survival indices were significantly reduced in all or some treatment groups when compared to the control. A multiple generation study of the reproductive performance could not be conducted due to the lack of pups, in addition to a smaller than anticipated fertility index in the control. The fertility indices (number of females delivering/number of females cohabitated) were 44percent for the control and 16 percent for all treatment groups. A statistically significant reduction ($p < 0.05$) was observed in all treatment groups as compared to the control. The live birth indices for F_1 pups were 96 percent, 93percent, 93 percent, and 15 percent for the 0, 0.25, 0.50, and 1.0 mg/kg/groups, with a significant reduction ($p < 0.05$) only at the 1.0 mg/kg/day dose level. Exposure of mice to mercuric chloride did not affect litter size, or other reproductive parameters such as number of implants, implant efficiencies, and postpartum dam weights. There was also no evidence of mercury induced target organ toxicity as seen from the clinical pathology parameters or histomorphologic evaluations. However, ovary weights in exposed females were significantly reduced in the 0.50 mg/kg/day group. Mercury accumulation in the females was statistically higher than in the males. As no ill effects were observed in the females, the study authors suggest that females are less sensitive to the adverse effects of mercuric chloride in the kidneys. There were no significant differences in the clinical chemistry parameters except that mean values for cholesterol were lower in the 0.25 and 0.50 mg/kg/day male groups. The study authors conclude that the results suggest that oral exposure to 0.25 to 1.0 mg/kg/day of mercuric chloride produced adverse effects on the reproductive performance of mice in the absence of overt mercury toxicity.

Orisakwe, O. E., O. Afonne, E. Nwobodo, L. Asomugha, C. Dioka. (2001). Low-dose mercury induces testicular damage protected by zinc in mice. European Journal of Obstetrics and Gynecology and Reproductive Biology 95:92-96.

Orisakwe et al. (2001) investigated whether low doses of mercury induce testicular damage on murine testis and if observed toxic effects could be prevented by zinc. In this study CD-1 male mice (about four months old) were distributed into four groups of five animals each. One group was the control and the three other groups received either one of the following: 4 ppm mercury, 800 ppm zinc, or 4 ppm mercury + 800 ppm zinc. The doses were administered via drinking water for 12 weeks. The animals were allowed free access to the drinking water. At the end of the exposure period, the animals were sacrificed, testes excised and weighed, and epididymal sperm number taken. The testes were also processed for histological examination.

Both zinc and mercury significantly decreased ($p < 0.05$) the absolute and relative testicular weights as compared to the controls. The greatest reduction was observed in the mercury group. A significant reduction in testes weight was also observed in the mercury and zinc groups as compared to the mercury/zinc group (see Table 1). The epididymal sperm count was significantly reduced ($p < 0.05$) in the mercury group ($28\pm7.87 \times 10^8$ cells/ml) as compared to the control group ($62\pm1.0 \times 10^8$ cells/ml) and the mercury/zinc group ($51.4\pm4.72 \times 10^8$ cells/ml). The epididymal sperm count in the zinc group ($47.5\pm5.82 \times 10^8$ cells/ml) and the mercury/zinc group showed no statistical differences from the control. Histological examination of the testes showed that mercury produced remarkable degenerative lesions on the testes, whereas the zinc-treated group showed a normal morphology. In the mercury/zinc treatment group, most of the animals showed complete or partial protection as evidenced by the morphology of the seminiferous tubules. The quantities of fluid and food consumed were significantly reduced in the different treatment groups when compared to the control group (13.12 m/mice.35.96 percent efficiency, respectively for controls). But, the volume of fluid ingested increased significantly in the mercury/zinc treatment group (10.30 ml/mouse) when compared with the mercury treatment group (6.90 ml/mouse) alone. The study author concluded that mercury at a low concentration (4 ppm) produced testicular damage in mice and that the majority of the animals in the mercury/zinc treatment group showed complete or partial protection as evidenced by the morphology of the seminiferous tubules. Overall, the authors concluded that zinc prevents mercury-induced testicular damage in the mouse.

Table 1. Mean±SD for Body Weight Gain and Testis Weight of Metal-exposed Mice

Treatment Group	Body Weight Gain (g)	Absolute Testis Weight (g)	Relative Testis Weight (g)[a]
Control (n=5)	10.82±4.25	0.43±0.01	1.15±0.08
Zn (n=5)	7.73±1.85[b]	0.27±0.06[b]	0.74±0.15[b]
Hg (n=5)	7.80±3.52[b]	0.20±0.05[b]	0.62±0.15[b]
Hg/Zn (n=5)	7.88±3.40[b]	0.34±0.05[b, c]	1.99±0.23[c]

a. Relative weight = absolute weight/body weight x 100
b. Significantly decreased compared to control at 5 percent significant level
c. Significantly increased compared to Hg at 5 percent significant level

Penna, S., M. Pocino, M. Marval, J. Lloreta, L. Gallardo, J. Vila. (2009). Modifications in rat testicular morphology and increases in IFN-γ serum levels by the oral administration of subtoxic doses of mercuric chloride. Systems Biology in Reproductive Medicine 55:69-84.

Penna et al. (2009) investigated the effects of oral administration of subtoxic doses of mercuric chloride on the reproductive system of male Sprague-Dawley rats. Twenty rats (approximately two months old) were distributed into four groups with five rats in each group. One group was the control and the three other groups received either one of the following dosages: 0.01, 0.05, or 0.1 μg/ml of mercuric chloride through free access to treated drinking water. The rats were sacrificed after either one, two, or three months of mercury administration. Some animal were sacrificed after seven months of mercury exposure. The testes and epididymides were dissected for histological processing and blood samples were collected for analysis of mercury, Interferon-gamma (IFN-γ) and Interleukin-4 (IL-4) levels.

In general, mercury administration did not influence the body weight or testis and epididymis weights. Additionally, no significant modifications were observed in daily food ingestion. The doses did, however, induce morphological alterations in the reproductive organs, even at the low level. All animals that received mercury showed lesions on either the testis or epididymis that progressed with time. In the testis, progressive degenerative lesions were detected on the seminiferous epithelium; they were observed independent of dose after one month of treatment. The lesions consisted of lack of germ cell cohesion and desquamation, arrest at the spermatocyte stage and hypospermatogenesis, presence of multinucleated giant cells and cytoplasmic vacuolation in Sertoli cells.

After two months of treatment, Leydig cells also showed cytoplasmic vacuolation and nuclear signs of death. In the epididymis, peritubular cell dissociation and giant cells were observed after one month of mercury exposure. Ultrastructural changes were also observed in the testis of the treated animals, including increase in lysosome number and degeneration in mitrochondria, rough endoplasmic reticulum and Golgi. The changes depended on dose and time of treatment. In the epididymis, multivascular bodies and lysosomal electron-dense accumulations were observed. Mercury accumulation was detected in both organs and serum. The IFN-γ levels were higher in the treated rats as compared to the controls, with a significant increase only after seven months of mercury exposure. Results from analysis of pooled blood samples showed that there was a decrease in the level of IL-4 when compared to the control, however change was not significant. The authors concluded that the results showed that under the experimental conditions, mercury increases Th1-type cytokines in the Sprague-Dawley rat. They noted that the morphological modifications detected in the study indicate that oral intake of subtoxic doses of mercuric chloride induces changes in either seminiferous or epididymal tubules. They also noted that mercury induces alterations in the Leydig cells suggesting association with impaired testicular steroidogenesis and thereafter contributing to spermatogenic arrest in the Sprague-Dawley rat.

SELENIUM

Bleys, J., A. Navas-Acien, E. Guallar. (2007). Serum selenium and diabetes in U.S. adults. Diabetes Care 30:829-834.

Bleys et al. (2007) examined the relationship between serum selenium levels and the prevalence of diabetes among U.S. adults by conducting a cross sectional analysis of 8,876 males and females ≥ 20 years of age who participated in the Third National Health and Nutrition Examination Survey (NHANES III). The survey was conducted from 1988 to 1994. The participants examined in the present study had fasted for ≥ eight hours prior to venipuncture or had reported a physician diagnosis of diabetes or current use of insulin or oral hypoglycemic medication. Reported mean serum selenium levels were 126.5 ng/ml for participants with diabetes and 125.6 ng/ml for participants without diabetes. Age-, sex-, race- and BMI-adjusted mean selenium levels were 126.8 ng/ml in participants with diabetes and 124.7 ng/ml in participants without diabetes.

To statistically asses the data, the participants were divided into five quintiles of serum selenium concentrations using the weighted population as a basis. Odds ratios were developed by comparing the four highest quintiles of serum selenium concentration to the lowest quintile using multivariable logistic regression. The multivariable-adjusted odds ratio for diabetes when comparing the highest quintile of serum selenium (≥137.66 ng/ml) to the lowest quintile (<111.62 ng/ml) was 1.57 (1.16-2.13). The spline regression model showed an increase in the odds for diabetes at >130 ng/ml serum selenium. At >150 ng/ml serum selenium, the odds for diabetes reached a plateau.

The increase in prevalence of diabetes between the highest and lowest quintiles of serum selenium was statistically significant. There was no clear dose-response pattern in the middle three quintiles. The positive association between high serum selenium levels and the prevalence of diabetes was consistent for all subgroups, except for participants with a BMI <25 kg/m^2.

The authors noted that limitations of the study included the inability to determine the direction of the observed association, as increased serum selenium may be a consequence rather than a cause of diabetes. Also, the association between selenium and diabetes could have been underestimated because subjects with diabetes and high serum levels could have died and would not have been included in the study.

Bleys, J., A. Navas-Acien, S. Stranges, A. Menke, E. Miller, E. Guallar. (2008). Serum selenium and serum lipids in U.S. adults. Am J Clin Nutr 88:416-423.

Bleys et al. (2008) examined the relationship between serum selenium levels and serum lipid levels in a representative sample of U.S. adults by conducting a cross sectional analysis of 5,452 males and females ≥ 20 years of age who participated in the Third National Health and Nutrition Examination Survey (NHANES III). The survey was conducted from 1988 to 1994. The participants examined in the present study had fasted for ≥ nine hours prior to venipuncture and had participated in morning medical examinations. Excluded were subjects that were pregnant, had a history of cardiovascular disease or cancer, or were missing data for variables of interest. In this study, higher serum selenium concentrations were positively associated with serum concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, apo B, apo A-I, and triacylglycerols. The associations were moderately strong and relatively linear. The associations also were seen after adjustment for age, sex, race, thyroid hormone concentrations, supplement use, or other traditional cardiovascular risk factors.

Participants were divided into four quartiles of serum selenium concentration. When comparing the highest and lowest quartiles of serum selenium (≥134.7 and <113.7 ng/mL, respectively) the multivariable-adjusted differences were:

- 16.6 mg/dL (95% CI: 11.6 – 21.4 mg/dL) for total cholesterol,
- 10.9 mg/dL (95% CI: 6.4-15.4 mg/dL) for LDL cholesterol,
- 3.2 mg/dL (95% CI: 1.6-5.0 mg/dL) for HDL cholesterol,
- 8.9 mg/dL (95% CI: 5.6-12.2 mg/dL) for apo B, and
- 6.9 mg/dL (95% CI: 1.7-12.1 mg/dL) for apo A-I.

After multivariable adjustment, participants in the highest quartile of serum selenium had 10 percent higher concentrations of triacyglycerols than did participants in the lowest quartile (ratio of triacyglycerol concentrations: 1.10 with a 95 percent CI of 1.05-1.17). The difference in the ratios of LDL cholesterol:HDL cholesterol and apo A:apo A-I when comparing the highest and lowest serum selenium quartiles were 0.11 (95 percent CI: -0.02 to 0.25) and 0.03 (95 percent CI: 0 to 0.06), respectively.

The study authors concluded that high serum selenium concentrations were associated with high serum concentration of: total cholesterol, LDL and HDL cholesterol, apo B, apo A-I and triacylglycerol among the adults in the U.S. The author also noted that because of the cross-sectional design of the study, they could not determine the cause and effect relation of the association between selenium and lipid concentrations.

Laclaustra, M., A. Navas-Acien, S. Stranges, J. Ordovas, E. Guallar.(2009). Serum selenium concentrations and diabetes in U.S. Adults: National Health and Nutrition Examination Survey (NHANES) 2003-2004. Environmental Health Perspectives 117:1409-1416.

Laclaustra (2009) examined the relationship of serum selenium levels with fasting plasma glucose, glycosylated hemoglobin levels, and diabetes in a representative sample of U.S. adults A cross sectional analysis was conducted on 917 males and females ≥ 40 years of age who participated in the 2003 to 2004 National Health and Nutrition Examination Survey (NHANES). A fasting morning blood sample was collected from all participants included in this study. Excluded were subjects with self-reported coronary heart disease, stroke, or cancer, and also subjects with missing data for variables of interest. Diabetes was defined as a self-report of current use of hypoglycemic agents or insulin or as a fasting plasma glucose ≥ 126 mg/dL.

Mean serum selenium was 137.1 µg/L and the overall presence of diabetes was 10 percent. Mean serum selenium concentrations were higher in participants with diabetes than without diabetes (143.7 and 136.4 µg/L, respectively). When comparing the highest quartile serum selenium concentration (≥147 µg/L) to the lowest quartile (<124 µg/L) the multivariable adjusted odds ratio for diabetes was 7.64 (95 percent CI: 3.34 to 17.46). The corresponding average differences in fasting plasma glucose and glycosylated hemoglobin were 9.5 mg/dL (95 percent CI: 3.4 to 15.6 mg/dL) and 0.30 percent (0.14 to 0.46 percent), respectively. In spline regression models, diabetes, glucose and glycosylated hemoglobin increased with increasing selenium concentrations up to160 µg/L. The authors concluded that high serum selenium concentrations were positively associated with higher prevalence of diabetes in the survey conducted on 2003-2004 on the U.S. adults, but that more analyses are needed to better understand the relationship.

Laclaustra, M., A. Navas-Acien, S. Stranges, J. Ordovas, E. Guallar. (2009). Serum selenium concentrations and hypertension in the US population. Circ Cardiovasc Qual Outcomes 2:369-376.

Laclaustra (2009) examined the relationship of serum selenium levels with blood pressure and hypertension in a representative sample of U.S. adults by conducting a cross sectional analysis of 2,683 men and women ≥ 40 years of age who participated in the 2003 to 2004 National Health and Nutrition Examination Survey (NHANES). Excluded were subjects that were pregnant or were missing data for variables of interest. Hypertension was defined as blood pressure ≥ 140/190 mm Hg or current use if antihypertensive medication.

Mean serum selenium was 137.1µg/L and the overall presence of hypertension was 45.2 percent. Mean serum selenium concentrations were higher in participants with hypertension than without hypertension (138.7 and 136.1 µg/L, respectively). When comparing the highest quintile serum selenium concentration (≥150 µg/L) to the lowest quintile (<122 µg/L) the average multivariable adjusted differences (95% CIs) were 4.3 mm Hg (1.3 to 7.4 mm Hg) for systolic pressure, 1.6 mm Hg (-0.5 to 3.7 mm Hg) for diastolic pressure, and 2.8 mm Hg (0.8 to 4.7 mm Hg) for pulse pressure. For hypertension, the corresponding odds ratio was 1.73 (1.18 to 2.53). In spline regression models, blood pressure and hypertension increased with increasing selenium concentrations up to160 µg/L. The authors concluded that based on the study, high serum selenium concentrations were associated with a higher prevalence of hypertension in a representative sample of the adult population in the U.S.

Lippman, S., E. Klein, J. Goodman, M. Lucia, I. Thompson et al. (2009). Effect of selenium and Vitamin E on risk of prostate cancer and other cancers. JAMA 301:39-51.

Lippman et al. (2009) investigated whether selenium, vitamin E, or both could prevent prostate cancer and other diseases with little or no toxicity in relatively healthy males using data from the Selenium and Vitamin E Cancer Prevention Trial (SELECT). SELECT is a randomized, placebo-controlled trial in which 35,533 male subjects from the United States, Canada and Puerto were assigned to one of four groups: selenium, vitamin E, selenium + vitamin E, and placebo. The selenium dose was 200 µg/day in the form L-selenomethionine and the vitamin E dose was 400 IU/day in the form of rac-α-tocopheryl acetate. All doses were given orally to the participants. The participants selected for the trial were 50 years or older (African American males) or 55 years or older (all other males), had a serum prostate-specific antigen level of 4 ng/mL or less, and had a digital examination not suspicious for prostate cancer. The trial study was activated in 2001 and ended in 2008. The median follow-up was 5.46 years.

There were no statistically significant differences in the rates of prostate cancer between the four groups described above. Prostate cancer was diagnosed during the trial in 4.43 percent of the placebo trial participants, 4.56 percent of the selenium trial participants, 4.93 percent of the vitamin E trial participants, and 4.56 percent in the selenium + vitamin E participants. Hazard ratios (99 percent confidence intervals) for prostate cancer for these groups as compared to the placebo were 1.04 (0.87-1.24) for selenium only, 1.13 (0.05-1.35) for vitamin E only, and 1.05 (0.88-1.25) for selenium + vitamin E.

As compared to the placebo group, there was a non-significant increase in prostate cancer in the vitamin E only group and a non-significant increase in type 2 diabetes mellitus in the selenium only group (10 percent in selenium group and 9.3 percent in control group). Type 2 diabetes mellitus did not increase in the selenium + vitamin E group (9.1 percent). The only statistically significant differences ($P<0.01$) were for selenium versus placebo for alopecia and grades 1 to 2 dermatitis.

A potential limitation of the study identified by Lippman et al. (2009) is that different formulations or doses of selenium and vitamin E were not tested and results using different subgroups of males were not assessed.

Mueller, A.S., K. Mueller, N.M. Wolf, J. Pallauf. (2009). Selenium and diabetes: an enigma? Free Radical Research 43(11):1029-1059.

Anti-oxidant supplements, such as selenium, have been considered favorable for the therapy of diabetes, as untreated diabetes produces oxidative stress that is responsible for secondary complications of diabetes. However, several current trials have revealed a positive relationship between high selenium levels and diabetes in humans, thus providing inconsistent relation between selenium and diabetes.

Therefore, Mueller et al. (2009) reviewed 226 studies to evaluate the role of selenium in conjunction with the role of oxidative stress in diabetes. The review also specifically discusses possible mechanisms of how selenium influences diabetes in both directions. Based on the review of these studies, Mueller et al. (2009) provided the following three key pieces of information. First, selenium supplements cannot be recommended for prevention of diabetes in populations with high selenium status. Second, antidiabetic effects of selenium occur at high and nearly toxic levels of selenium in humans. Third, future investigations should consider the stage of the disease.

The authors overall opinion from their extensive literature review is that selenium "supplementation above the officially recommended amounts (up to 70 µg/day, depending on age and physiological status) is not indicated for the prevention of insulin resistance and diabetes, since the currently recommended amounts are adequate for optimum activities of functional selenoproteins. On the contrary the permanent intake of Se supplements may even accelerate the development of obesity, insulin resistance and diabetes. Genuine anti-diabetic effects of Se can only be obtained with nearly toxic doses and out of the questions for humans."

Stranges, S., J. Marshall, R. Natarajan, R. Donahue, M. Trevisan, G. Combs, F. Cappuccio, A. Ceriello, M. Reid. (2007). Effects of long-term selenium supplementation on the incidence of type 2 diabetes. Annals of Internal Medicine 147(4):217-224.

Stranges et al. (2007) investigated the effect of long-term selenium supplementation on the incidence of type 2 diabetes by performing a secondary analysis of the Nutritional Prevention of Cancer (NPC) trial, a randomized, double-blind, placebo-controlled clinical trial designed primarily to evaluate the efficacy of selenium supplementation for prevention of cancer. For this study, Stranges et al. (2007) focused on 1,202 participants who did not have type 2 diabetes at baseline (600 selenium recipients and 602 placebo recipients). The participants were recruited in 1983 to 1991, from seven dermatology clinics in areas of low selenium consumption of the eastern United States. The treatment group received 200 μg of selenium daily, supplied in a 0.5 g high-selenium baker's yeast tablet. The placebo group received a tablet containing yeast only. Participants included both males (74 percent 75 percent) and females. They had a mean age of 63 years at baseline and were mostly white. Initial reports of diabetes came from three sources: self-report during the clinical interview; reported use of drugs for diabetes; and medical record documents. The initial report was corroborated in 92 percent of the cases through evaluation of medical documentation. During an average follow-up of 7.7 years, 97 new cases of type 2 diabetes were diagnosed (i.e. incidence of 10.5 cases per 1,000 person-years). Of these cases, 58 developed in selenium recipients and 39 developed in placebo recipients (incidence of 12.6 cases per 1000 person-years vs. 8.4 cases per 1000-person years, respectively). The hazard ratio was 1.55 (95 percent CI, 1.03 to 2.33). A significant difference in the incidence of type 2 diabetes was found between the treatment group and the placebo group.

The lack of benefit of selenium supplementation persisted in analysis of subgroups by age, sex, smoking status, and body mass index. An exposure-response gradient was found across tertiles of baseline plasma selenium levels, with the top tertile showing a statistically significant increase in risk for type 2 diabetes (hazard ratio of 2.70 with a CI of 1.30 to 5.61). The study authors concluded that selenium supplementation does not seem to prevent type 2 diabetes, and it may increase the risk for the disease.

Limitations of the study noted by the study authors included: (1) the incidence of diabetes was not the primary endpoint in the NPC trial; (2) the diagnosis of type 2 diabetes was self reported; (3) detailed information on unmeasured risk factors at baseline were not collected (i.e., history of diabetes, body fat distribution, physical activity); and (4) the NPC samples consisted of elderly individuals from low-selenium areas in the eastern United States with a history of non-melanoma cancer. In addition, the study authors stated that the role of chance in the findings could not be ruled out.

TAB B: Staff Documents on Antimony, Barium, and Cadmium

TAB B

UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
BETHESDA, MD 20814

Memorandum

Date: XXXXX

TO : Mary Ann Danello, Ph.D., Associate Executive Director, Directorate for Health Sciences

THROUGH: Lori E. Saltzman, M.S., Director, Division of Health Sciences

FROM : Dominique W. Johnson, MPH, Toxicologist, Division of Health Sciences

SUBJECT : Staff Documents on Antimony, Barium, and Cadmium

Toxicity Assessment for Antimony (Sb)

Summary

The existing ASTM F 963 standard for antimony is based on a permissible intake of 0.2 µg Sb/day from toys. This corresponds to a daily dose of 0.02 µg Sb/kg/day from toys for a 12kg child (0.2 µg/day ÷ 12kg = 0.02 µg Sb/kg/day). Based on a no observed adverse effect level (NOAEL) of 6 mg Sb/kg/day in a subchronic rat drinking water study (NTP, 1992), an ADI of 6 µg Sb/kg/day can be derived using an uncertainty factor of 1000 for inter- and intra-species variation and use of a subchronic study (6 mg ÷ 1000 = 6 µg SB/day). Adjusting the ADI for allowable percentage of antimony intake from toys, 10 percent, and a child's weight of 12 kg allows for direct comparison to the permissible daily intake the existing standard is based on. CPSC staff finds an updated permissible intake for antimony of 7.2 µg Sb/day. This corresponds to approximately a thirty-fivefold increase in allowable intake when compared to the current ASTM F 963 standard.

Existing Standard for Antimony

Existing standards for children's intake of metals from toys, originally published in ED 12964 EN and EN 71-3, are based on estimated levels of metals in the diet and allowable relative source contributions from toys, ranging from 0.1–10 percent, based on the chemical's toxicity (RIVM, 2006). For antimony, the existing standard is 0.2 µg/day from toys, based on an assumed children's dietary intake of 15 µg Sb/week (calculated as 50 percent of the measured adult intake of 30 µg Sb/week), and an allowable contribution from toys of 10 percent (15 µg Sb/week ÷ 7 days/week x 10% = 0.2 µg Sb/day)(RIVM, 2006). The allowable contribution for antimony from toys remained at 10 percent, which was the maximum percentage contribution from toys allowed by the European Union. Assuming a body weight of 12 kg (RIVM, 2006), the permissible intake of 0.2 µg Sb/day corresponds to a daily dose of 0.02 µg Sb/kg/day from toys.

Review of Selected Studies for Antimony

In preparation for CPSC staff's assessment of antimony, Versar provided a literature search of relevant data within the past 10 years. It was determined that there was only one

key study that may affect the derivation of an allowable daily intake for antimony for use in updating the current toy safety standard, ASTM F 963. Lynch et al. (1999) reviewed the toxicity of antimony potassium tartrate. It was determined that, although Poon et al. (1998) presented a low NOAEL of 0.5 ppm from a 90-day drinking study in rats, it would be more appropriate to use a NOAEL of 50 ppm (6 mg/kg/d) set by NTP (1992) after their 90-day drinking water study in rats. The Poon et al. (1998) study used histopathological changes to develop their conclusions. However, Lynch felt that these changes did not show a dose response, as the NTP (1992) study did, and were mild and reversible, and not indicative of an overt toxic effect. The NTP (1992) study conclusions were based on reduced body weight and decreased intake of food and water. Other agencies have chosen to use the NTP (1992) NOAEL as a basis for their limits (WHO, 1998). Comments between the two laboratories can be found in Valli (2000).

Toxicity Assessment for Antimony

An ADI for antimony can be derived from the subchronic NOAEL of 6 mg/kg/day for reduced body weight in rats (NTP, 1992). By applying an uncertainty factor of 1000 (10 each for inter- and intra-species variation and 10 for the use of a subchronic study), an ADI of 6 µg /kg/day derived.

Comparison of ADI to Existing Toy Standard for Antimony

The ADI of 6 µg/kg/day applies to a daily intake of antimony from all sources. The existing permissible intake for antimony in the European Union's Toy Safety Directive is 0.2 µg Sb/day, which refers specifically to intake from toys. To compare these intake levels (6 µg/kg/day, versus 0.2 µg/day), we applied the same modifying factors that were used to derive the toy standard. A source allocation of 10 percent is applied to the ADI, and a body weight of 12 kg is used to calculate a permissible intake level that is directly comparable to the existing EN 71-3 value (6 µg Sb/kg-day × 10% x 12 kg = 7.2 µg Sb/day from toys). This provides a newly derived permissible intake level of 7.2 µg Sb/day. Comparison of the permissible intake of antimony from toys based on the ADI derived here by CPSC staff, (7.2 µg Sb/kg/day) and the existing permissible intake from toys (0.2 µg Sb/day)(RIVM, 2006), suggests that the revised ADI would allow for an approximate thirty-fivefold increase in allowable intake.

Works Cited

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National Institute for Public Health and the Environment (RIVM). (2006). *Chemicals in Toys RIVM/SIR Revised Advisory Report 0010278A02.* The Netherlands: RIVM.

R. Poon, I. C. (1998). Effects of Antimony on Rats Following 90-day Exposure via Drinking Water. *Food and Chemical Toxicology*, 21–35.

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World Health Organization. (1998). *Water Sanitation Health*. Retrieved September 2010, from http://ww.who.int/water_sanitation_health/dwq/chemicals/0304_74/en/index7.html.

Toxicity Assessment for Barium (Ba)

Summary

The existing toy safety standard, ASTM F-963, gives a permissible intake limit for barium of 25 µg Ba/day. This corresponds to a daily dose of 2 µg Ba/kg/day from toys for a 12 kg child (25 µg Ba/day ÷ 12 kg = 2 µg Ba/kg/day). The Agency for Toxic Substances and Disease Registry (ATSDR) derived an intermediate and chronic minimal risk level (MRL) of 0.2 mg Ba/kg/day, based on a no observed adverse effect level (NOAEL) of 65 mg/kg/day for increased kidney weights in female rats and a lower confidence interval benchmark dose (BMDL) of 60 mg Ba/kg/day for nephropathy in male rats, respectively (60 mg Ba/kg/day ÷ 100 ÷ 3 = 0.2 mg Ba/kg/day) (ATSDR, 2007). Applying the point of departure used by ATSDR, an uncertainty factor of 100 is applied to both intermediate and chronic departure points for human extrapolation and variability, providing a proposed ADI of 0.6 mg Ba/kg/day. Adjusting the proposed ADI for allowable percentage of barium intake from toys, 5 percent, and a child's weight of 12 kg, allows for direct comparison to the permissible daily intake on which the existing standard is based. CPSC staff finds an updated permissible daily intake for barium of 0.36 mg/day (0.6 mg Ba/kg/day x 5% x 12kg = 0.36 mg/day), or 360 µg/day. This would allow for a greater than fourteenfold increase in allowable intake when compared to the current ASTM F 963 standard.

Existing Standard for Barium

Existing standards for children's intake of metals from toys, originally published in ED 12964 EN and EN 71-3, are based on estimated levels of metals in the diet and allowable relative source contributions from toys, ranging from 0.1–10 percent based on the chemical's toxicity (RIVM, 2006). For barium, the existing standard is 25 µg Ba/day from toys, based on an assumed children's dietary intake of 3500 µg Ba/week (calculated as 50 percent of the measured adult intake of 7000 µg Ba/week), and an allowable contribution from toys of 5 percent (3500 µg Ba/week ÷ 7 days/week x 5% = 25 µg Ba/day) (RIVM, 2006). The allowable contribution for barium from toys was reduced to 5 percent from the starting value of 10 percent because of the high normal dietary intake for barium. Assuming a body weight of 12 kg (RIVM, 2006), the permissible intake of 25 µg Ba/day corresponds to a daily dose of 2 µg Ba/kg-day or 0.002 mg Ba/kg-day from toys.

Review of Selected Studies for Barium

In preparation for CPSC staff's assessment of barium, Versar provided a literature search of relevant data for the past 10 years. It was determined that there were no new key studies that may affect the derivation of an allowable daily intake for barium for use in updating the current toy safety standard ASTM F 963. It was found that ATSDR had developed a profile for barium in 2007. The intermediate and chronic MRL was determined to be 0.2 mg Ba/kg/day based on increased kidney weights in female rats and nephropathy in male mice found in a 1994 NTP study (ATSDR, 2007).

Using barium chloride, NTP (1994*[6]) exposed Fischer-344 rats for 90 days through their drinking water. They found significant increases in absolute and relative kidney

[6] *Reviewed by ATSDR (2007) Barium and Barium Compounds

weights in female rats at 115 mg/kg/day. In both male and female rats, at 180 mg/kg/day, the authors found minimal to mild dilation of the proximal convoluted tubules of the outer medulla and renal cortex. A NOAEL of 65 mg/kg/day was found for this study (ATSDR, 2007).

In another study by NTP (1994[*1]), mice were exposed to barium chloride for two years via drinking water. They found that exposures above 160 mg Ba/kg/day resulted in moderate to marked nephropathy in male mice. The nephropathy was characterized by extensive regeneration of cortical and medullary tubule epithelium, tubule dilation, hyaline cast formation, interstitial fibrosis, and glomerulosclerosis. An NOAEL was found at 60 mg Ba/kg/day (ATSDR, 2007).

Toxicity Assessment for Barium

For both the intermediate and chronic MRLs developed by ATSDR, an uncertainty factor of 100 was applied to the NOAEL for animal-to-human extrapolation and human variability, and a modifying factor of 3 was used to account for inadequate developmental toxicity data. These uncertainty factors are consistent with the uncertainty factors used by other federal regulatory agencies, including the CPSC. Therefore, the allowable daily intake (ADI) used in this assessment will be 0.2 mg Ba/kg/day, which was derived by ATSDR as their intermediate and chronic MRLs.

Comparison of ADI to Existing Toy Standard for Barium

The ADI of 0.6 mg Ba/kg/day derived here applies to total daily intake of inorganic barium from all sources. The existing permissible intake for barium in the European toy safety standard is 25 µg Ba/kg/day, which refers specifically to intake from toys. In order to compare these, we applied the same modifying factors that were used to derive the toy standard. Therefore, a source allocation of 5 percent was applied to the ADI, and a body weight of 12 kg was used to calculate a permissible intake level that is directly comparable to the existing value (0.6 mg Ba/kg/day x 5% x 12 kg = 0.36 mg Ba/day from toys). Comparison of the permissible intake of barium from toys based on the ADI derived here (0.36 mg Ba/kg/day or 360 µg Ba/day) and the existing permissible intake from toys (25 µg Ba/day) suggests that the ADI would allow for a greater than fourteenfold increase in allowable intake.

Works Cited

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Toxicity Assessment for Cadmium (Cd)

Summary

The existing standard for cadmium is 0.6 µg Cd/day from toys. This corresponds to a daily dose of 0.05 µg Cd/kg/day from toys for a 12 kg child. Staff has derived an ADI of 0.1 µg Cd/kg/day, based on a lower confidence limit benchmark dose (BMDL) of 0.5 µg Cd/g creatinine[7] after chronic exposure to cadmium in Swedish women (Suwazono et al., 2006). The BMDL is based on the endpoint of kidney toxicity. This corresponds to an intake level of 0.33µg/kg/day, to which an uncertainty factor of 3 was applied for sensitive populations. Adjusting the ADI for allowable percentage of cadmium intake from toys, 5 percent, and a child's weight of 12 kg, allows for direct comparison to the permissible daily intake upon which the existing standard is based. CPSC staff finds an updated permissible daily intake for cadmium of 0.06 µg/day. This is a tenfold decrease in the allowable daily intake when compared to the current ASTM F 963 Standard.

Existing Standard for Cadmium

Existing standards for children's intake of metals from toys, originally published in EU 12964 EN and EN 71-3, are based on estimated levels of metals in the diet and allowable relative source contributions from toys, ranging from 0.1–10 percent, based on the chemical's toxicity (RIVM, 2006). For cadmium, the existing standard is 0.6 µg Cd/day from toys, based on an assumed children's dietary intake of 87.5 µg Cd/week, or 12.5 µg Cd/day, and an allowable contribution from toys of 5 percent (12.5 µg Cd/day x 5% = 0.6 µg/day)(RIVM, 2006). The allowable contribution for cadmium from toys was reduced from the starting value of 10 percent because the normal dietary intake already approached the WHO Provisional Tolerable Weekly Intake for cadmium (RIVM, 2006). Assuming a body weight of 12 kg (RIVM, 2006), the permissible intake of 0.6 µg Cd/day corresponds to a daily dose of 0.05µg Cd/kg/day from toys.

Toxicity Assessment for Cadmium

CPSC staff reviewed available data and developed a toxicity review document of cadmium where an ADI of 0.1 µg Cd/kg/ day was derived based on renal toxicity in Swedish women studied by Suwazono et al. (2006). The analysis used urinary cadmium levels as a biomarker of cadmium exposure and the prevalence of abnormal levels of β2M, pHC, protein, N-acetyl-β-glucosaminidase (NAG), retinol binding protein, ALB, or glomarular filtration rate as biomarkers of renal effects. The $BMD_{0.05}$ (dose associated with a 5 percent extra risk) values for NAG were 0.64, 12.0–10.8, and 6.36–7.74 µg/g creatinine. The results of these BMD analyses suggest that chronic exposure to cadmium resulting in urinary cadmium levels of 0.3 µg/g creatinine would be associated with a 5 or 10 percent additional risk of renal dysfunction.

Comparison of ADI to Existing Toy Standard for Cadmium

The ADI of 0.1 µg Cd/kg/day derived by CPSC staff applies to total daily intake of inorganic cadmium from all sources. The existing permissible intake for cadmium in the

[7] Creatinine is a protein marker of kidney disease commonly found in urine.

European toy safety standard is 0.6 µg/day, which refers specifically to intake from toys. In order to compare these, we applied the same modifying factors that were used to derive the toy standard. Therefore, a source allocation of 5 percent was applied to the ADI and a bodyweight of 12 kg was used to calculate a permissible intake level that is directly comparable to the existing value (0.1 µg Cd/kg/day x 5% x 12 kg = 0.06 µg Cd/day from toys). Comparison of the permissible intake of cadmium from toys based on the ADI derived (0.06 µg/day) and the existing permissible intake (0.6 µg Cd/day) provides a tenfold decrease in allowable daily intake of cadmium.

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Appendix A

Tables of Toy Standards and Agency Standards for Metals

Table 1: Other Heavy Metals in Toys Standards		
Jurisdiction	Scope	Standard
European Union Directive 88/378/EEC on the safety of toys and EN 71-3 Migration of certain elements	All toy material (excluding modeling clay and finger paint) intended for children up to 6 years of age	Migration testing 60 mg/kg (Sb); 25 mg/kg (As); 1000 mg/kg (Ba); 75 mg/kg (Cd); 60 mg/kg (Cr); 90 mg/kg (Pb); 60 mg/kg (Hg); 500 mg/kg (Se)
European Union Directive 88/378/EEC on the safety of toys and EN 71-3 Migration of certain elements	Modeling clay and finger paint	Migration testing 60 mg/kg (Sb); 25 mg/kg (As); 250 mg/kg (Ba); 50 mg/kg (Cd); 25 mg/kg (Cr); 90 mg/kg (Pb); 25 mg/kg (Hg); 500 mg/kg (Se)
United States CPSIA/ASTM F-963	Paints and coatings of toys intended for children under the age of 14 years	Migration testing 60 mg/kg (Sb); 25 mg/kg (As); 1000 mg/kg (Ba); 75 mg/kg (Cd); 60 mg/kg (Cr); 60 mg/kg (Hg); 500 mg/kg (Se)
United States CPSIA	Total lead content by weight for children's products intended for children 12 years of age or younger	Content testing 100 mg/kg (Pb)

Table 2: U.S. Federal Agency Standards – Antimony		
Agency	Scope	Standard
US EPA[8]	Chronic Oral Exposure (RfD) - 1991	4E-4 mg/kg/day
US EPA[9]	Drinking water standard (MCL) - 2000	6 ppb
NIOSH[10]	Air quality as time-weighted average (8hr) (REL) - 2005	0.5 mg/m^3
OSHA[11]	Air quality as time-weighted average (8hr) (PEL) - 1989	0.5mg/m^3

[8] IRIS (1991) Integrated Risk Information System for Antimony. U. S. Environmental Protection Agency. Available at http://www.epa.gov/iris/subst/0006.htm

[9] US EPA (2010) Consumer Factsheet on Antimony. U. S. Environmental Protection Agency. Available at http://www.epa.gov/ogwdw000/pdfs/factsheets/ioc/antimony.pdf

[10] NIOSH (2010) NIOSH Pocket Guide to Chemical Hazards: Antimony. Centers for Disease Control and Prevention. Available at http://www.cdc.gov/niosh/npg/npgd0036.html

[11] ATSDR (1992) Toxicological Profile for Antimony. Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services. Available at http://www.atsdr.cdc.gov/toxprofiles/tp23-c7.pdf

Table 3: U.S. Federal Agency Standards – Arsenic		
US EPA[12]	Noncancer Oral exposure (RfD) – 1993	3E-4mg/kg/day
US EPA[13]	Drinking water standard (MCL) – 2006	10 ppb
US EPA[12]	Cancer Oral Exposure (RSD) – 1997	6.7E-6 mg/kg/d
US EPA[12]	Cancer Inhalation Exposure (RSC) – 1997	2.3E-6 mg/m^3
OSHA[14]	Air quality as TWA (8 hrs) - 2005	10 ug/m^3
NIOSH[15]	Air quality with 15 min. ceiling limit – 2005	2 ug/m^3
FDA[14]	Food	0.5-2 ppm

Table 4: U.S. Federal Agency Standards - Barium		
US EPA[16]	Noncancer oral exposure (RfD) – 2005	2E-1 mg/kg.day
US EPA[17]	Drinking water standard (MCL) – 1999	2 ppm
NIOSH[18]	Air quality as TWA (8hrs) – 2005	0.5 mg/m^3
OSHA[17]	Air quality as TWA (8hrs) – 2005	0.5 mg/m^3
FDA[17]	Bottled drinking water standard – 2004	2.0 mg/L

Table 5: U.S. Federal Agency Standards - Cadmium		
US EPA[18]	Food standard (RfD) – 2008	$1x10^{-3}$ mg/kg/day
US EPA[19]	Drinking water standard	0.005 mg/L

[12] IRIS (1998) Integrated Risk Information System for Arsenic. U. S. Environmental Protection Agency. Available at http://www.epa.gov/iris/subst/0278.htm
[13] US EPA (2011) Arsenic in Drinking Water. U. S. Environmental Protection Agency. Available at http://water.epa.gov/lawsregs/rulesregs/sdwa/arsenic/index.cfm
[14] OSHA (Unidentified) Occupational Safety and Health Standards for Inorganic Arsenic. U. S. Department of Labor. Available at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10023
[15] ATSDR (2007) Toxicological Profile for Arsenic. Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services. Available at http://www.atsdr.cdc.gov/csem/csem.asp?csem=1&po=8
[16] IRIS (2005) Integrated Risk Information System for Barium. U. S. Environmental Protection Agency. Available at http://www.epa.gov/iris/subst/0010.htm
[17] US EPA (2011) Basic Information About Barium in Drinking Water. U. S. Environmental Protection Agency. Available at http://water.epa.gov/drink/contaminants/basicinformation/basicinformation_barium.cfm
[18] ATSDR (2007) Toxicological Profile for Barium. Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services. Available at http://www.atsdr.cdc.gov/toxprofiles/tp24-c8.pdf

	(MCL) – 2003	
OSHA[18]	Air Quality as TWA (8hrs) (PEL)–2007	5ug/m^3
FDA[18]	Bottled drinking water standard–2007	0.005 mg/L

Table 6: U.S. Federal Agency Standards - Chromium		
US EPA[19]	Chronic oral exposure (RfD)–2008	1.5 mg/kg/day Cr III $3x10^{-3}$ mg/kg/day Cr IV
NIOSH[20]	Air Quality as TWA (8hrs) (REL)–2005	0.5 mg/m^3 Chromium 0.001 mg/m^3 Chromium IV
OSHA[19]	Air Quality as TWA (8hrs) (PEL)–2007	0.5-1.0 mg/m^3 5 ug/m^3
US EPA[19]	Drinking water standard (MCL)–2003	0.1 mg/L
FDA[19]	Bottled drinking water standard–2007	0.1 mg/L
FDA[19]	Recommended daily intake – 2007	120 ug

Table 7: U.S. Federal Agency Standards - Mercury		
OSHA[21]	Air Contaminant Standard as TWA (8hrs) (PEL) – 1974	0.1 mg/m^3
US EPA[20]	Drinking water standard (MCL)–1992	0.002 mg/L
FDA[20]	Action level for poisonous or deleterious substances – 1994 and 1998	1 ppm – fish, shellfish, crustaceans, other aquatic animals 1 ppm - wheat
FDA[20]	Bottled water standard–1995	0.002 ug/L
FDA [20]	Cosmetics – 1974	<65 ppm

Table 8: U.S. Federal Agency Standards - Selenium		
NIOSH[22]	Air quality as TWA (8hrs) (REL) – 2001	0.2 mg/m^3

[19] ATSDR (2008) Toxicological Profile for Cadmium (Draft). Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services. available at http://www.atsdr.cdc.gov/toxprofiles/tp5-c8.pdf
[20] ATSDR (2008) Toxicological Profile for Chromium (Draft). Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services. Available at http://www.atsdr.cdc.gov/toxprofiles/tp7-c8.pdf
[21] ATSDR (1999) Toxicological Profile for Mercury. Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services. Available at http://www.atsdr.cdc.gov/toxprofiles/tp46-c7.pdf
[22] ATSDR (2003) Toxicological Profile for Selenium. Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services. Available at http://www.atsdr.cdc.gov/toxprofiles/tp92-c8.pdf

OSHA[21]	General industry as TWA (8hrs) (PEL)–2001	0.2 mg/m^3
EPA[21]	Drinking water standard (MCL)–2001	0.05 mg/L
FDA[21]	Food additive in animal feeds–2001	≤0.3 ppm
FDA[21]	Bottle water standard–2000	0.05 mg/L
FDA[21]	RDA–2000	Men 0.055 mg/day Women 0.055 Pregnant women 0.060 Lactating women 0.070 Infants (0–6 months) 0.015 Infants (7–12 months) 0.020 Children (1–3 years) 0.020 Children (4–8 years) 0.030 Children (9–18 years) 0.040

Table 8: Current ASTM F 963-07 Values and Related European Union's Toy Safety Directive (2009) Values*

Metals	**Current ASTM F 963 Intake Limit (µg/day)**	**European Union Intake Limits ((µg/day); Effective 2013#**
Antimony	0.2	0.37
Arsenic	0.1	0.03
Barium	25	37
Cadmium	0.6	0.02
Chromium	0.3	0.31 (III); 0.0001 (VI)
Mercury	0.5	0.06
Selenium	5	0.31

*An excerpt of updated values related to the metals specified in the ASTM F 963. The full extent of changes includes expansion of metals and scope.
Converted to intake limits from the migration limits specified in the toy safety directive, assuming 8 mg toy ingested per day and 12 kg body weight. Directive available at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:170:0001:0037:en:PDF

TAB C: Response to Reviewer Comments

TAB C

UNITED STATES
CONSUMER PRODUCT SAFETY COMMISSION
BETHESDA, MD 20814

Memorandum

Date:

TO : Mary Ann Danello, Ph.D., Associate Executive Director, Directorate for Health Sciences

THROUGH: Lori E. Saltzman, M.S., Director, Division of Health Sciences

FROM : Dominique W. Johnson, MPH, Toxicologist, Division of Health Sciences

SUBJECT : Response to Comments: Metals in the Toy Safety Standard ASTM F 963[23]

I. Background

On August 14, 2008, the President signed into law the Consumer Product Safety Improvement Act (CPSIA) (PL-110-314). Section 106(d)(1) of the CPSIA requires the U.S. Consumer Product Safety Commission (CPSC or the Commission) to ". . . examine and assess the effectiveness of ASTM F 963 . . . and shall assess the adequacy of such standards in protecting children from safety hazards. . . ."

The toy safety standard, ASTM F 963-07, was made a mandatory CPSC standard by the CPSIA. Under ASTM F 963-07, section 4.3 provides migration limits for eight heavy metals that may be in toy materials. These metals are: Antimony (Sb), Arsenic (As), Barium (Ba), Cadmium (Cd), Chromium (Cr), Lead (Pb), Mercury (Hg), and Selenium (Se). There is now a 2001 version of the ASTM F 963 standard. The 2007 version of the F 963 standard was the current version at the time of initiation of this project. Since the ASTM work and staff's activities related to this Status Report were done concurrently, the analyses presented here could not be considered by the ASTM subcommittee in development of the ASTM F 963-11.

In an effort to begin assessing the effectiveness of the requirements for heavy metals in the toy safety standard, CPSC staff contracted with Versar, Inc., to review the toxicity literature from the years 2000 to 2010, on all the metals listed in the standard, except lead,[24] and to evaluate any toxicity data that may influence the determination of the effectiveness of the current safety limits. In addition, Versar was asked to develop an acceptable daily intake (ADI) value for each metal. The ADI is the amount of a chemical that a person may be exposed to on a daily basis without the chemical posing a significant risk of adverse health effects.

[23] These comments are those of the CPSC staff and have not been reviewed or approved by, and may not necessarily reflect the views of, the Commission.
[24] Lead was excluded because it is addressed separately in section 101 of the CPSIA.

II. Derivation of ASTM F 963-07 Values

CPSC staff believes that the migration limits in ASTM F 963-07 came directly from the European Standard, EN 71-3, based on the following information reviewed by staff. A report by the Netherlands National Institute for Public Health and the Environment (RIVM, 2006) indicates that permissible levels of bioavailable elements from toys were derived by the Science Advisory Committee in the European Union (EU) in 1985. These permissible levels then were specified in the EU's Toy Safety Directive (Council Directive 88/378/EEC, 1988), and subsequently were incorporated into European Standard EN 71-3 (European Standard EN 71-3, 1994). As documented in the RIVM (2006) report, the permissible levels were derived from estimated adult weekly dietary intake levels for each element. The adult dietary intake level was then reduced by half, to estimate a child's dietary intake. An additional adjustment was made to derive the allowable daily intake level that may be contributed by toys.[25] This adjustment was between 0.1 and 10 percent of the estimated children's dietary intake levels. These permissible intake levels were converted to migration levels in milligrams per kilogram (mg/kg) of toy material, with the assumption of daily intake of 8 mg of toy material (8 mg/day), and assuming an average weight of a child of 12 kg.[26]

CPSC staff believes that the values for the migration limits in ASTM F 963-07 come directly from the European Standard, EN 71-3, based in part on the fact that the limits specified in ASTM F 963-07 and EN71-3 are identical. It should be noted that the scope of the migration limits of the two standards differ in that ASTM F 963-07 currently applies to paints and surface coatings only, while EN 71-3 applies to toy substrate materials, such as plastic and metal, in addition to paints and surface coatings.

III. Steps Taken by CPSC Staff

In order to compare the existing values in ASTM F 963-07 and those derived from Versar, Versar was charged with reviewing only non-cancer data for all the metals. This is because the reports that formed the basis for ASTM F 963, the Netherlands National Institute for Public Health and the Environment (RIVM, 2006, and the previous European Toy Safety Standard (Council Directive 88/378/EEC, 1988), derived their values from non-cancer endpoints.

Staff systematically analyzed the ADIs developed by Versar, compared the derived values to the permissible intake levels, ingestion levels that have been determined to pose little to no risk of adverse effects provided in the EU's toy safety directive, and drafted a staff document that addressed the comparison for each chemical. Because the values from Versar and the EU directive were derived under different assumptions and procedures, staff adjusted the derived ADIs in the same way that the EU's limits were handled. Staff converted the Versar ADIs to daily intake levels in milligrams per day (mg/day), by applying the same percentage of allowed

[25] The reduction of the dietary intake value by half, and reduction to between 0.1 percent and 10 percent of the value to derive the contribution of toys to the dietary intake level, appear to be based not on any specific data, but rather, on assumptions deemed reasonable by the report's authors. CPSC staff found insufficient information in the RIVM report for evaluating the validity of these assumptions.

[26] The assumed 12 kg body weight for children was reported in the RIVM (2006), but was not further documented. Twelve kg is equivalent to about 26.5 pounds, and using reasonably current data, corresponds to children who are approximately 1 year old (Ogden *et al.,* 2004).

daily intake for exposure from toys as was done in the European analysis. Thus, staff multiplied the ADI by the percent allowed for toys (0.1% – 10%), and multiplied the result by the average weight of a 1-year-old child (12 kg).

In an effort to determine the migration of metals from toys, CPSC staff also requested that the contractor review current data showing migration of metals from children's toys. However, little or no data for metals used in toys or migration of metals from children's toys was found. This lack of data was also noted in the RIVM report (2006). Staff believes that additional testing will be required to identify the use of metals in children's toys and to quantify the potential migration of the metals from children's toys.

In February 2011, CPSC staff initiated an external scientific peer review of the staff document and the Versar contract report related to the review and analysis of the metals in the ASTM F 963-07 toy safety standard. Two reviewers were selected by Versar, and three reviewers from other U.S. federal agencies were selected by CPSC staff. The remainder of this memo summarizes the peer-review comments and provides CPSC staff's responses to those comments. Staff revised the CPSC staff metals document (Tab B), as appropriate, based on the peer-review comments. The Versar contract report did not require any revisions.

IV. **Discussion**

A. **Overall Comments**

Nineteen comments were received that questioned the purpose of the staff document, general issues related to migration of chemicals from products, and possible reliance on other safety standards.

Several reviewers questioned why staff was developing new safety levels when other agencies have, or currently are working on updating current safety levels for the metals. They also requested that staff review and consider other agency work, including creating a table of such work.

Two comments were related to the migration information presented in the staff document. One reviewer was unclear about the purpose of this section of the document. A reviewer requested that a more complete description of migration be included to help determine whether there is a problem, and another reviewer suggested that the information relate back to the migration limits in ASTM F 963-07.

One reviewer was unclear of the role of the RIVM 2006 document in staff's analysis and noted that there is a more recent RIVM document, released in 2008. In addition, this reviewer believed that it was inappropriate to use the modifying factors from ASTM F 963-07 on the proposed ADIs. This reviewer also noted that many of the proposed ADI levels were below the recommended daily nutritional intake levels and should be reviewed carefully.

One reviewer requested that staff clarify which form of the element is being considered. The reviewer also asked for clarification on terminology, such as "free standing NOAEL[27]" and "all sources," asking whether "all sources" meant all oral exposures or all exposures from any route.

Staff Response

Staff has added several tables to the revised document containing standards and other information developed by other agencies. Staff notes that assessment of the effectiveness of ASTM F 963-07 is required by section 106(d)(i) of the CPSIA, and while work related to the same chemicals may be underway by others, staff must conduct the present analyses. To the extent that other work was relevant to the CPSC's activities, staff incorporated such work in its evaluations.

At the request of CPSC staff, Versar reviewed literature on the migration of the discussed metals from children's toys. Little or no data was found concerning the presence and concentrations of metals in toys or that related to the bioavailability of the metals that might be found in toys. This lack of data was also identified in the RIVM document (2006).

The reviewer indicates correctly the existence of a more recent RIVM document (2008) that discusses metals in toys. Staff has reviewed both documents and finds that the information provided in each is identical.

Staff recognizes that there are nutritional requirements for some of the elements under discussion, and staff has noted this, where appropriate, in the revised document.

Staff agrees that the form of each element under discussion should be identified clearly , and staff has clarified this information, where necessary, in the revised document The term "free-standing NOAEL" refers to the fact that only one dose was looked at in comparison to control animals. No effects were seen at this dose, allowing for this single dose to be considered the NOAEL for the data set. Without any other data points to support the finding that the given dose is the actual NOAEL for this data set, the dose is considered "free-standing." In addition, "all sources" refers to the acceptable daily intake (ADI) not being limited to one source, such as toys, personal care products, and food, unlike the values found in EN71-3 and F 963, which are identified as values allotted for toy materials.

B. Antimony (Sb) Comments

Five comments specifically addressed antimony. Four requested clarification of the key studies used in developing the proposed ADI for antimony; one requested that staff review the proposed ADI for accuracy. A reviewer asked why Poon et al. was credited for the data used to derive the proposed ADI, when an NTP study is the actual source of the data. In addition, this reviewer did not believe that the document fully discussed all the key studies considered and that staff should include a discussion of the Valli and Lynch reviews (1998 and 1999).

Staff Response

CPSC staff reviewed the derivation of the proposed ADI of 6 µg antimony/kg/day. Staff agrees that NTP (1992) is the key study and that the identified NOAEL of 6 mg antimony/kg/day and an

[27] No Observed Adverse Effect Level.

uncertainty factor of 1000 (10 for intra- species variation, 10 for interspecies variation, and 10 for use of a subchronic study) is appropriate (CPSC, 1992). Staff has corrected the citation of the key study to the NTP (1992), rather than Poon et al. (1998). Staff finds that the discussion of the Valli and Lynch reviews are sufficient for the purpose of the staff document because the discussion provided the main points of discussion from the Valli and Lynch reviews. Those needing more detailed information are also able to identify the primary source for their inquiry.

C. Arsenic (As) Comments

Nine comments were received on arsenic. One reviewer believed that staff should consider cancer as an endpoint because the reviewer believes that the ASTM F 963-07 value for arsenic considers cancer.

One reviewer noted that the average daily intake used to derive the ASTM F 963-07 value was much higher than the actual exposure levels in the key study that was used to derive the proposed ADI. Therefore, this reviewer indicated that staff should re-evaluate the appropriateness of using this study. In addition, the reviewer believed that the comparison of the ASTM F 963-07 value and the proposed ADI was flawed because the existing ASTM F 963-07 standard level is based on total arsenic in the diet, and the proposed ADI is based on inorganic arsenic. This same reviewer also recommended that staff highlight the differences between risk- and diet-based approaches and explain why a risk-based approach is more appropriate.

One reviewer believed that the NOAEL identified in the key study, Wasserman et al., was inappropriate because the reviewer found that the data in the article actually suggests that the NOAEL is not definable.

One reviewer asked for an explanation of why the proposed ADI is such a significant departure from other existing dose response assessments.

Two reviewers believed that a more detailed justification of the uncertainty factors used in developing the proposed ADI is needed, especially the use of the uncertainty factor of 3 instead of the default factor of 10. In addition, one reviewer believed that an uncertainty factor for human sensitivity is not needed because sensitive individuals were the ones responding at the LOAEL[28] in the study used to develop the proposed ADI.

Staff Response

The European Union's Toy Safety Directive is based on an analysis of dietary intake that resulted in estimates of allowable limits for the heavy metals. Staff did not find that cancer data was used in the analysis. For some of the metals, the percent contribution to exposure allowed from toys was reduced compared to other metals, in part based on information about carcinogenicity and other significant and/or severe toxicity. The end result of this approach is the derivation of a lower intake level (*i.e.*, a lesser amount of the metal might be allowed). In preparing the proposed ADI for comparison with the ASTM F 963-07 existing values, CPSC staff used the same modifying factors in order to match the process used in the existing toy safety standard. This process does not explicitly incorporate carcinogenicity data, but it is accounted for as described above.

[28] Lowest Observed Adverse Effect Level.

A risk-based approach to deriving ADIs is one in which well-conducted toxicity and epidemiology studies are used to identify the lowest exposure doses that are associated with adverse health effects. This is the approach that CPSC staff and the contractor used. The dietary intake approach that forms the basis of the EU toy safety standard, and the current ASTM F 963-07 standard, considers data about typical exposures to the chemicals of interest within the population, and then through assumptions and adjustments, derives permissible daily limits. These approaches have clear differences. For example, the dietary approach looks at typical exposures, not the possible adverse effects that may occur from those exposures, and it does not adjust the values using uncertainty factors. The ADI is defined as the amount of a chemical that a person may be exposed to on a daily basis without the chemical posing a significant risk of adverse health effects. The dietary approach does not necessarily result in an exposure level that is not associated with adverse effects (*i.e.*, typical dietary exposures may actually be associated with a risk for adverse effects). Further, the dietary approach may not account for specific forms of a chemical that are likely used in toys or other products and that may have differing levels of toxicity. The goal in this case was to make the same adjustments to a derived exposure limit, regardless of the method used to derive it, in order to facilitate the comparison of new values with the existing values.

In the review of articles chosen for arsenic, a LOAEL was identified from the Wasserman et al. paper. As portrayed by the author and in agreement with the reviewer, no NOAEL could be determined. While the Wasserman et al. paper is listed in the documents, along with several others that could be used for deriving an ADI, this paper was not chosen as the key study for development of the proposed ADI.

Staff's method of developing ADIs is consistent with current risk-assessment practice. The study group used in the key study (Ahsan et al., 2006) had lifelong chronic exposure, including exposures during childhood, to arsenic through well water. It is standard practice to use an uncertainty factor of 10 to convert a LOAEL to a NOAEL. Staff agrees with the reviewer's logic that the subset of test subjects responding to the LOAEL dose is presumably the sensitive population for the examined population. However, application of an uncertainty factor is warranted to account for any additional sensitivity that is not present in the population under study. Staff believes that an uncertainty factor of 3 is appropriate to account for human variability in this case.

D. Barium (Ba) Comments

Three comments addressed barium. One reviewer requested a correction of the statement that the uncertainty factor of 300 that was applied to the ATSDR's MRL[29] be changed to reflect that the uncertainty factor was applied to the NOAEL. In addition, this reviewer believed that an uncertainty factor of 3 for an incomplete database for developmental toxicity was inappropriate. Another reviewer questioned why animal data was used to develop the proposed ADI, when RIVM has a lower TDI[30] based on human data.

[29] Minimal Risk Level.
[30] Tolerable Daily Intake.

Staff Response

It was not staff's intention to suggest that the uncertainty factor of 300 was applied to ATSDR's MRL. Staff has revised the document to state clearly that the uncertainty factor was applied to the NOAEL. The development of the ADI in the draft document was developed, in part, using ATSDR's analysis, which included the use of an additional uncertainty factor of 3 for an incomplete database for developmental toxicity. This is not consistent with CPSC staff's guidelines for use of uncertainty factors, which do not allow for an uncertainty factor for incomplete databases. This section of the draft document has been revised and no longer contains the uncertainty factor for an incomplete database for developmental toxicity.

CPSC staff reviewed the human data found in the RIVM document and found that it is not suitable for developing the proposed ADI. The human study cited is a 1985 study that concluded no effect on blood pressure. RIVM did not use this data to develop the TDI. RIVM found that it was more appropriate to use the same approach used by ATSDR in 2005, to develop their chronic MRL, which is based on increased kidney weights in female rats and nephropathy in male rats (NTP, 1994).

E. Cadmium (Cd) Comments

Seven comments addressed cadmium. One reviewer asked for a better explanation of why the Suwazono et al. paper was chosen as the key study over the other studies. In addition, this reviewer asked why Suwazono et al. used a $BMDL_{05}$[31] as the background risk. Another reviewer requested information on the conversion of the biomarker to intake dose for cadmium in the key study.

Two comments addressed the use of uncertainty factors. One comment was directed at the rationale for the use of an uncertainty factor of 3 instead of the standard 10 for intraspecies (human) variability. The other reviewer stated that there was no context concerning whether an uncertainty factor of 3 for intraspecies variability was sufficient to cover child vulnerabilities. This reviewer also asked whether the CPSC results agreed or disagreed with the IRIS RfD.

Finally, one reviewer requested clarification on the reference, RIVM, cited for the limits in the existing toy safety standard.

Staff Response

Staff's review of the available literature included epidemiological studies of populations in which individuals were exposed chronically to cadmium throughout their lifetimes, including childhood (CPSC, 2010). Staff focused on studies of populations that did not have exposures to specific industrial or environmental sources of cadmium (*i.e.*, populations without a particular identified source).

Staff chose Suwazono *et al.* (2006)[32] as the key study for a number of reasons, including that the study was based on a large, well-characterized population without any identified source of environmental or occupational exposure; the modeling of the data included multiple covariates to

[31] Benchmark Dose Lower confidence limit - 5 percent above background

[32] Suwazono Y, Sand S, Vahter M, Filipsson AF, Skerfving S, Lidfeldt J, Akesson A (2006) Benchmark dose for cadmium-induced renal effects in humans. Environ Health Perspect 114:1072–1076.

account for potential confounders; the application of a benchmark dose method modified for continuous, rather than categorized data; and the estimated cadmium exposures associated with adverse health effects were among the lowest of several studies.

This paper reported 0.5 micrograms cadmium per gram creatinine in the urine (0.5 µg/g creatinine) as the lower confidence limit of the cadmium concentration benchmark dose level (*i.e.*, BMDL associated with a 5 percent additional risk of protein in the urine, or $BMDL_{05}$).

As to the reviewer's question about the use of the $BMDL_{05}$ as the background risk at 0 exposure, staff believes that this relates to the benchmark dose modeling approach used by Suwazono *et al.* Because the adverse response under study (protein in urine) is not a unique result of cadmium exposure, the study's modeling incorporates a 5 percent background probability of an adverse response based on a hypothetical control distribution at urinary cadmium concentration equal to zero in the population under study. This approach replaces the use of a reference population exposed at a low dose to define the adverse response, and it has the advantage of avoiding the effects of cadmium exposure in a reference population. The $BMDL_{05}$ is the estimated additional risk in the population with a 5 percent background risk.

The 0.5 µg/g creatinine value is the same critical value chosen by the Agency for Toxic Substances and Disease Registry (ATSDR) in the most recent draft Toxicological Profile (ATSDR 2008),[33] which is based on a meta-analysis of the same data evaluated by CPSC staff.

Because the BMDL is a measure of cadmium excreted in urine, additional analysis is required to estimate the corresponding level of cadmium intake into the body. This can be done using modeling techniques. In this case, staff chose to accept the analysis conducted by ATSDR as presented in the draft Toxicological Profile for Cadmium. For a 0.5 µg/g creatinine urinary cadmium concentration, the ATSDR analysis resulted in an estimated level of cadmium intake of 0.33 µg/kg/day.

The epidemiological data for chronic cadmium exposure is extensive, consisting of many large studies that included thousands of individuals in different parts of the world. Studied populations included individuals exposed in occupational settings to particular sources in their environment, such as industrial sources, or to general sources of cadmium, such as food and drinking water. The studies, in most cases, also include individuals exposed to cadmium throughout their lifetimes, including childhood, such as in the Suwazono *et al.* (2006) analysis chosen as the key study. Because of the large amount of information in this case, staff believes that an uncertainty factor of 3, rather than the default uncertainty factor is appropriate. This reduced uncertainty factor is consistent with the ATSDR evaluation.

Thus, using somewhat different approaches to analyze the available data, CPSC and ATSDR staff each estimated an acceptable daily intake level (ADI) (called the minimal risk level or MRL by ATSDR) of 0.1 µg/kg/day.

The EPA's IRIS review[34] for cadmium includes the derivation of a reference dose (similar to an ADI or MRL). The analysis, last revised in 1994, includes an oral reference dose (RfD) of 1

[33] ATSDR (2008) Toxicological Profile for Cadmium (Draft). Agency for Toxic Substances and Disease Registry. U.S. Department of Health and Human Services. Available at http://www.atsdr.cdc.gov/toxprofiles/tp5.html.
[34] EPA (1994) Integrated Risk Information System, Cadmium (CASRN 7440-43-9). U.S. Environmental Protection Agency. http://www.epa.gov/iris/subst/0141.htm.

µg/kg/day for food intake and 0.5 µg/kg/day for cadmium in drinking water (different levels of absorption of cadmium from food or from water account for the different RfD values). Both of these RfD values are higher (as much as an order of magnitude) than staff's estimated ADI. The difference between CPSC staff's ADI and the EPA's RfD stems from using different epidemiological studies and a different approach to estimating cadmium intake. Given the data available to the EPA at the time the EPA developed the RfD, staff consider EPA's estimate to be reasonable.

The RIVM document was cited as the source of information related to the limits for migration of elements specified in the ASTM F 963-07 toy safety standard, rather than the European standard, EN 71-3, because EN 71-3 does not provide the basis for the standard's limits. Background information, analysis of available data, and development of exposure limits are presented in the RIVM report.

F. Chromium (Cr) Comments

Seven comments addressed chromium. One reviewer stated that it was inappropriate to compare Cr in the diet (Cr III) and a risk-based ADI (predominately Cr VI). In addition, this reviewer believed that it was inappropriate to include a review of Cr III. The reviewer also wanted an explanation of why a source allocation of 1 percent for Cr was chosen versus a lower percentage, such as 0.1 percent.

In the area of uncertainty factors used, one reviewer believed that there was no need for a NOAEL to LOAEL uncertainty factor of 10, or they believed that the uncertainty factor for use of LOAEL could be reduced to 3 because the LOAEL appears to be close to background levels.

One reviewer questioned the need for an assessment of chromium that is different from existing dose response assessments for chromium. In addition, this reviewer thought that staff should consider the risk of dermal sensitization from Cr VI.

Finally, one reviewer was unclear about whether the purpose of the document was to develop an ADI for total chromium (aggregate of all forms) or a standard for Cr VI.

Staff Response

The basis of the current ASTM F 963-07 toy safety standard restrictions for the migration of certain elements, including chromium, is the European Union's toy safety standard. The requirements of the latter standard are based on the analysis of dietary intakes of the elements. In the current work, for the purpose of comparison to the ASTM F 963-07 existing values, draft ADIs were developed using the same approach and modifying factors used by the EU.

As discussed above, the dietary intake approach that forms the basis of the EU toy safety standard and the current ASTM F 963-07 standard considers data about typical exposures to the chemicals of interest within the population and then, through assumptions and adjustments, derives permissible daily limits. In contrast, a more typical risk-based approach, in which well-conducted toxicity and epidemiology studies are used to identify the lowest exposure doses that are associated with adverse health effects, is the approach that CPSC staff and the contractor used. These approaches have clear differences. Notably, the dietary approach may not account for specific forms of a chemical that are likely used in toys or other products and that may have

differing levels of toxicity. However, the goal in this case was to make the same adjustments to a derived exposure limit, regardless of the method used to derive it, in order to facilitate the comparison of new values with the existing values.

As noted by the reviewer, one important issue in the original assessment for the European standard is that dietary chromium is predominantly Cr III. Cr VI is the more toxic form of chromium, and because environmental or biological conditions may cause Cr III and Cr VI to interconvert, the more health protective approach is to consider that chromium is present as Cr VI. Because either form of chromium may be found in products, both were included in the Versar review. However, the ADI is based on Cr VI.

With respect to uncertainty factors, staff prefers the use of the default factor of 10^{35} to account for the use of a LOAEL rather than a NOAEL (CPSC, 1992). Staff does not believe that the available information justifies use of a reduced uncertainty factor in this case.

CPSC staff agrees that dermal sensitization and Cr VI is an important topic. However, dermal sensitization is not considered in this exercise because the toy safety standard is dealing with oral exposures.

G. Mercury (Hg) Comments

Five comments addressed mercury. One reviewer expressed the belief that it was inappropriate to compare dietary mercury exposure, which is predominantly methyl mercury, with inorganic mercury, the form of mercury used to develop the proposed ADI. This reviewer also stated that the dietary intake of methyl mercury is not a benign baseline.

One reviewer questioned the need for an assessment of mercury that is different from existing dose-response assessments for mercury. In addition, this reviewer noted that the ADI, based on inorganic mercury, is lower than the U.S. EPA's RfD[36] for methyl mercury, which is considered to be the most toxic form of mercury.

One reviewer commented that because there are similar rat and human dietary intakes of mercury, staff should use a reduced uncertainty factor for interspecies extrapolation because effects in the key study in rats are not observed in humans.

Staff Response

As discussed above, the dietary intake approach that forms the basis of the EU toy safety standard and the current ASTM F 963-07 standard considers data about typical exposures to the chemicals of interest within the population and then, through assumptions and adjustments, derives permissible daily limits. In contrast, a more typical risk-based approach, in which well-conducted toxicity and epidemiology studies are used to identify the lowest exposure doses that are associated with adverse health effects, is the approach that CPSC staff and the contractor used in this case. These approaches have clear differences. Notably, the dietary approach may

[35] The uncertainty factor is intended to account for deficiencies in the available data. Without data to guide the choice of the numerical value for the uncertainty factor, the default value of 10 is used by CPSC staff and others. See CPSC's Chronic Hazard Guidelines, 1992 for additional discussion on the use of uncertainty factors. Available at http://www.cpsc.gov/businfo/chronic.pdf.

[36] Oral Reference Dose.

not account for specific forms of a chemical that are likely used in toys or other products, and that may have differing levels of toxicity. However, the goal in this case was to compare the exposure limits of the existing standard to limits derived using up-to-date data and information and standard assessment approaches.

CPSC staff does not believe that the available data adequately defines the differences in the toxicity of mercury between humans and animals. Thus, staff does not agree that use of an uncertainty factor other than the default of 10 is warranted at this time.

Staff has approached the available data, and the assessment of the data, with standard risk analysis procedures. Staff recognizes that this analysis results in a draft ADI that is lower than previous assessments.

H. Selenium (Se) Comments

Six comments specifically addressed selenium. One reviewer noted that the proposed ADI for selenium is below the required nutritional intake level, and below the U.S. EPA's RfD. Another reviewer stressed that there is a fine line between required amounts of selenium and toxic amounts. However, another reviewer recommended that a different approach should be used to assess selenium because the risk-assessment paradigm is problematic for essential elements.[37]

In the areas of uncertainty factors used and study discussions, one reviewer recommended that staff should incorporate supportive animal studies for selenium in the discussions, describe the full uncertainty factors that could be used, and discuss the compromises in the assessment that may be required because of dietary intake and the recommended daily allowance for selenium.

Staff Response

For this analysis, staff chose to use traditional risk analysis methods. Staff recognizes that the nutritional essentiality of elements and substances may complicate the use of such approaches. Because of the issue of nutritional requirements for selenium and the potential for toxicity with excess selenium exposure, any ongoing staff work will need to include an evaluation of a number of factors that could influence the ADI or related toy safety requirements.

With respect to the comment concerning animal studies, staff notes that the data summary in the draft report already references animal data that supports the analysis.

Regarding uncertainty factors, staff prefers the use of the default factor of 10 to account for the use of a LOAEL rather than a NOAEL. At this time, staff has not developed recommendations for the application of specific ADI values.

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[37] Required for normal body functioning.

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