



**Petitioner's Confidential Information Has Been Redacted From the Following  
GRAS Report**

**Generally Recognized As Safe  
(GRAS) Determination for the  
Use of Chromax® Chromium  
Picolinate as a Nutrient  
Supplement in Food**

Prepared for:

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## I. GRAS EXEMPTION CLAIM

*A. NAME AND ADDRESS OF NOTIFIER*

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Table 1. Food-Grade Specifications for Chromax® Chromium Picolinate

Parameter	Specification	Test Method
<b><u>General Specifications</u></b>		
Appearance	Reddish, free-flowing powder	Visual inspection
Odor	Odorless, or practically so	Organoleptic
Identity A (IR Spectrum)	Matches standard	USP 24 <197> <sup>1</sup>
Identity B (alkaline H <sub>2</sub> O <sub>2</sub> color test)	Characteristic yellow color	NF 19 (page 2438)
Chromium content (anhydrous)	12.18% to 12.68%	Assay: USP 24 (page 407), CrCl <sub>3</sub> modified
Loss on drying	≤ 4.0%	USP 24 <731> <sup>2</sup>
Powder fineness	99% through 80 Mesh	USP 24 <811> <sup>3</sup>
Chloride content	≤ 0.06%	USP 24 <221> <sup>4</sup>
Sulfate content	≤ 0.2 %	USP 24 <221>
Heavy metals (as lead)	<10 ppm	USP 24 <231> <sup>5</sup> Method II
<b><u>Microbial Specifications</u></b>		
<i>Escherichia coli</i>	Negative in 10 g	NF 19 <2021> <sup>6</sup>
<i>Salmonella species</i>	Negative in 10 g	NF 19 <2021>
<i>Staphylococcus aureus</i>	Negative in 10 g	NF 19 <2021>
<i>Pseudomonas aeruginosa</i>	Negative in 10 g	NF 19 <2021>
Total aerobic count	< 3000 cfu/g	NF 19 <2021>
Mold and Yeast	< 300 cfu/g	NF 19 <2021>
<sup>1</sup> USP <197>: Spectrophometric Identification Tests <sup>2</sup> USP <731>: Loss on Drying Assay <sup>3</sup> USP <811>: Powder Fineness Assay <sup>4</sup> USP <221>: Chloride and Sulfate Limit Test <sup>5</sup> USP <231>: Heavy Metals Limit Test <sup>6</sup> NF <2021>: Microbial Limit Tests – Nutritional Supplements Note: < > specifies the particular test as referenced in USP 24 / NF 19 (2000).		

### C. INTENDED USE AND CONSUMER EXPOSURE

Nutrition 21 intends to market Chromax® Chromium Picolinate for addition to several categories of food as a nutrient supplement to increase the dietary intake of trivalent chromium in the U.S. population. Nutrition 21 proposes to use its product in nutritional beverages (i.e., meal replacements, including ready-to-drink products and powder mixes) and bars (i.e., meal replacement bars, energy bars, and diet meal bars). The maximum proposed use level is 2.4 mg of Chromax® Chromium Picolinate per product serving, which is equivalent to 300 mcg trivalent chromium per serving, assuming a trivalent chromium content of Chromax® Chromium Picolinate of 12.4%.



The estimated mean and 90<sup>th</sup> percentile intakes of trivalent chromium resulting from the proposed uses of Chromax® Chromium Picolinate by U.S. consumers age 2 years and older is 304 and 545 mcg per person per day, respectively. Mean and 90<sup>th</sup> percentile intakes are representative of typical and heavy consumers, respectively, of the foods to which Chromax® Chromium Picolinate is proposed to be added. Total cumulative intake of trivalent chromium from all food sources (including dietary supplements) by this same population, consisting of a 90<sup>th</sup> percentile intake from the proposed uses of Chromax® Chromium Picolinate and a 55 mcg per person per day contribution from other dietary sources (including dietary supplements), is estimated to be 600 mcg per person per day.

***D. BASIS FOR GRAS DETERMINATION***

This GRAS determination for the proposed uses of Chromax® Chromium Picolinate at the maximum use level described in Section C of this chapter is based on scientific procedures as described under Title 21 of the Code of Federal Regulations (“21 CFR”) §170.30(b). Using scientific procedures, the estimated intake of trivalent chromium from the intended uses of Chromax® Chromium Picolinate specified in Section C of this chapter, in addition to intakes of trivalent chromium from other dietary sources (including dietary supplements), has been shown to be safe, and GRAS, under the Federal Food, Drug, and Cosmetic Act (“FDCA”). To demonstrate that Chromax® Chromium Picolinate is safe, and is GRAS, under its intended conditions of use, the safety of the intake of trivalent chromium resulting from the consumption of Chromax® Chromium Picolinate in food has been established under its intended conditions of use, taking into account the potential intake of trivalent chromium from other sources in the diet. Then, this cumulative intake of trivalent chromium is determined to be GRAS by demonstrating that the safety of this level of intake is generally recognized by experts qualified by both scientific training and experience to evaluate the safety of substances directly or indirectly added to food, and is based on generally available and accepted information.

Evaluation of the safety of trivalent chromium intake under the intended conditions of use of Chromax® Chromium Picolinate was accomplished through an estimate of the potential exposure to trivalent chromium from both current dietary sources of trivalent chromium and the proposed uses of Chromax® Chromium Picolinate, and then comparing this total cumulative estimated daily intake (“EDI”) with the acceptable daily intake (“ADI”) for trivalent chromium. As long as the EDI is less than (or approximates) the ADI, the proposed uses of Chromax® Chromium Picolinate can be considered safe at the maximum use level.

The total cumulative EDI of trivalent chromium from consumption of Chromax® Chromium Picolinate in food, including 55 mcg per person per day from other dietary sources (including dietary supplements), in the general U.S. population, excluding infants under the age of one year, is estimated to be 600 mcg per person per day for the 90th percentile (or heavy)

consumer of the products to which Chromax® Chromium Picolinate is proposed to be added. The proposed uses of Chromax® Chromium Picolinate are expected to contribute 545 mcg per person per day of trivalent chromium to this cumulative total. This intake estimate reflects 100 percent market penetration of the proposed uses for Chromax® Chromium Picolinate that are listed in Section C of this chapter.

Based on a review of the publicly available toxicity data on trivalent chromium-containing compounds (including chromium tripicolinate), ENVIRON derived an estimated ADI for trivalent chromium (when administered as chromium tripicolinate) of equal to or greater than 900 mcg/day. This ADI estimate was derived from a subchronic animal study by Anderson et al. (1997b) in which a no-observed-adverse-effect level ("NOAEL") for chromium tripicolinate via ingestion was established at a trivalent chromium dose of 15 mg/kg/day, the highest dose administered in the study. Then, applying a safety factor of 1,000 and multiplying the result by an assumed 60-kg body weight, an estimated ADI for trivalent chromium (when administered as chromium tripicolinate) of equal to or greater than 900 mcg/day was derived. In addition, an evaluation of the available clinical efficacy studies employing chromium tripicolinate suggests that this compound has a long history of safe use in humans as a nutritional supplement and, other than isolated case reports, there is no consistent evidence of adverse effects following its use in humans at doses as high as 1,000 mcg per day trivalent chromium. This upper safe limit in humans of 1,000 mcg per day agrees quite favorably with the 900 mcg/day ADI derived from the subchronic animal study, lending further support to the validity of this ADI.

Two other ADI estimates were derived from chronic drinking water studies in animals that employed trivalent chromium-containing compounds other than chromium tripicolinate. In these studies, the trivalent chromium-containing compounds were administered at trivalent chromium doses that were about 20 to 30 times lower than in Anderson et al. (1997b). The resulting ADI estimates were equal to or greater than 276 mcg/day trivalent chromium (when administered as chromium acetate) and equal to or greater than 492 mcg/day trivalent chromium (when administered as chromium chloride), but likely represent underestimates of the true ADI for the reasons outlined in Chapter V of this GRAS document.

Because the cumulative EDI of trivalent chromium of 600 mcg per person per day (resulting from the proposed uses of Chromax® Chromium Picolinate in food combined with intake estimates from other dietary sources, including dietary supplements) is less than the estimated ADI for trivalent chromium of equal to or greater than 900 mcg per person per day, Chromax® Chromium Picolinate is safe under its intended conditions of use.

Determination of the GRAS status of Chromax® Chromium Picolinate for use as a nutrient supplement in foods has been made through the deliberations of Richard A. Anderson, Ph.D., Joseph F. Borzelleca, Ph.D., and Walter H. Glinsmann, M.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information

summarized in this document, including the potential human exposure to trivalent chromium resulting from the intended uses of Chromax® Chromium Picolinate as a nutrient supplement in food, and have concluded:

No evidence exists in the available information on trivalent chromium that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public health when trivalent chromium is used at levels that are now current or that might reasonably be expected from the proposed uses of Chromax® Chromium Picolinate as a nutrient supplement in food.

It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion.

Therefore, Chromax® Chromium Picolinate is safe, and is GRAS, for the proposed uses and at the maximum proposed use level described in Section C of this chapter. Because Chromax® Chromium Picolinate is GRAS for its proposed uses, it is excluded from the definition of a food additive, and thus may be marketed and sold for these uses in the U.S. without the promulgation of a food additive regulation under 21 CFR.

***E. AVAILABILITY OF INFORMATION***

The data and information that serve as the basis for this GRAS determination will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times at the office of James T. Heimbach, Ph.D., Principal, ENVIRON International Corporation, 4350 North Fairfax Drive, Suite 300, Arlington, VA 22203, telephone: (703) 516-2362, facsimile: (703) 516-2390, and e-mail: [jheimbach@environcorp.com](mailto:jheimbach@environcorp.com).

## II. DESCRIPTION OF SUBSTANCE

### A. *CHEMICAL NAME*

The most commonly used chemical name for the substance that is the subject of this GRAS determination is chromium tripicolinate. Chromium tripicolinate is a stable complex of trivalent chromium (Cr (III)) and picolinic acid. Alternate chemical names for this substance are Tris(2-pyridinecarboxylato-N<sub>1</sub>, O<sub>2</sub>)chromium, and chromium(III) trispicolinate.

### B. *TRADE OR COMMON NAME*

The chromium tripicolinate that is the subject of this GRAS determination is marketed by Nutrition 21 under the trade name "Chromax® Chromium Picolinate." This product is often commonly referred to as "CHROMAX®."

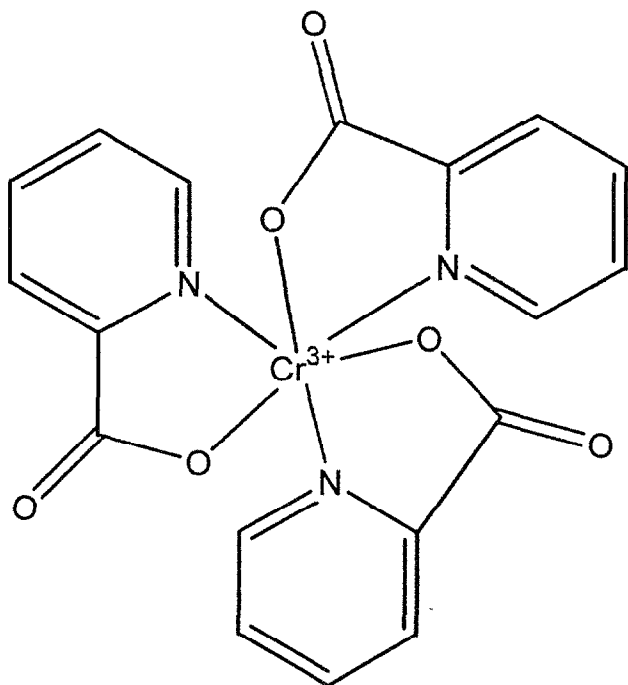
### C. *CAS REGISTRY NUMBER*

The Chemical Abstracts Service ("CAS") Registry Number for chromium tripicolinate is 14639-25-9.

### D. *EMPIRICAL AND STRUCTURAL FORMULAS*

The empirical formula for chromium tripicolinate is C<sub>18</sub>H<sub>12</sub>CrN<sub>3</sub>O<sub>6</sub>. The structural formula for this compound is shown in Figure 1.

Figure 1. Structural Formula for Chromium Tripicolinate



*E. PHYSICAL AND CHEMICAL PROPERTIES*

A brief summary of the physical and chemical properties of chromium tripicolinate are listed below in Table 2.

Table 2. Physical and Chemical Properties of Chromium Tripicolinate

Property	Value
Composition	C - 51.68%, O - 22.95%, Cr - 12.43%, N - 10.05%, H - 2.89%
Molecular Weight	418.31 Daltons
Solubility (in water)	Soluble in water at pH 7 (>100 µg/ml)
Solubility (in chloroform)	Soluble in chloroform (2.0 mM)
UV <sub>max</sub> (in aqueous solution)	264 nm (a <sub>M</sub> 15546 L mol <sup>-1</sup> cm <sup>-1</sup> )

*F. PRODUCTION PROCESS*

*Prepared for Nutrition 21*

**G. PRODUCT CHARACTERISTICS**

**1. Food-Grade Specifications**

Nutrition 21 has developed food-grade specifications for Chromax® Chromium Picolinate that are in accordance with the most current United States Pharmacopoeia (“USP”) and National Formulary (“NF”) monographs. These specifications are intended to establish and maintain the food-grade status of the final product. The latest editions of these monographs (i.e., USP 24/NF 19) published in the year 2000 contain specifications, as well as tests, procedures, and acceptance criteria, that help assure the strength, quality, and purity of listed items. The food-grade specifications established for Chromax® Chromium Picolinate are listed in Table 3.



Table 3. Food-Grade Specifications for Chromax® Chromium Picolinate

Parameter	Specification	Test Method
<b><u>General Specifications</u></b>		
Appearance	Reddish, free-flowing powder	Visual inspection
Odor	Odorless, or practically so	Organoleptic
Identity A (IR Spectrum)	Matches standard	USP 24 <197> <sup>1</sup>
Identity B (alkaline H <sub>2</sub> O <sub>2</sub> color test)	Characteristic yellow color	NF 19 (page 2438)
Chromium content (anhydrous)	12.18% to 12.68%	Assay: USP 24 (page 407), CrCl <sub>3</sub> modified
Loss on drying	≤ 4.0%	USP 24 <731> <sup>2</sup>
Powder fineness	99% through 80 Mesh	USP 24 <811> <sup>3</sup>
Chloride content	≤ 0.06%	USP 24 <221> <sup>4</sup>
Sulfate content	≤ 0.2 %	USP 24 <221>
Heavy metals (as lead)	<10 ppm	USP 24 <231> <sup>5</sup> Method II
<b><u>Microbial Specifications</u></b>		
<i>Escherichia coli</i>	Negative in 10 g	NF 19 <2021> <sup>6</sup>
<i>Salmonella species</i>	Negative in 10 g	NF 19 <2021>
<i>Staphylococcus aureus</i>	Negative in 10 g	NF 19 <2021>
<i>Pseudomonas aeruginosa</i>	Negative in 10 g	NF 19 <2021>
Total aerobic count	< 3000 cfu/g	NF 19 <2021>
Mold and Yeast	< 300 cfu/g	NF 19 <2021>
<sup>1</sup> USP <197>: Spectrophometric Identification Tests <sup>2</sup> USP <731>: Loss on Drying Assay <sup>3</sup> USP <811>: Powder Fineness Assay <sup>4</sup> USP <221>: Chloride and Sulfate Limit Test <sup>5</sup> USP <231>: Heavy Metals Limit Test <sup>6</sup> NF <2021>: Microbial Limit Tests: Nutritional Supplements Note: < > specifies the particular test as referenced in USP 24 / NF 19 (2000).		

These specifications meet those listed for chromium tripicolinate on page 2438 of NF 19.

**2. *Batch Analysis Results***

In order to demonstrate conformance with the food-grade specifications listed in Table 3, Nutrition 21 analyzed five lots or batches of Chromax® Chromium Picolinate. The results of these analyses are displayed in Table 4. These batch analysis results show that all five batches of the final product are in compliance with the food-grade specifications established by Nutrition 21 for this product. These data indicate that the Chromax® Chromium Picolinate production process is under control, and can consistently yield a food-grade product suitable for human consumption.

Table 4. Analysis Results for Five Batches of Chromax® Chromium Picolinate

Parameter	Specification	Batch Number and Results				
		00215053	00215355	00213636	00213638	00213639
Appearance (via visual inspection)	Reddish free flowing powder	PASS	PASS	PASS	PASS	PASS
Odor	Odorless of practically so	PASS	PASS	PASS	PASS	PASS
Identity A: IR Spectrum	Matches standard	PASS	PASS	PASS	PASS	PASS
Identity B: Alkaline H <sub>2</sub> O <sub>2</sub> color test	Characteristic yellow color	PASS	PASS	PASS	PASS	PASS
Total Chromium content (anhydrous)	12.18 – 12.68%	12.29%	12.22%	12.21%	12.32%	12.27%
Moisture	≤ 4.0%	2.30%	2.50%	1.80%	0.70%	0.70%
Powder Fineness	99% through 80 Mesh	99%	99%	99%	100%	100%
Chloride content	≤ 0.06%	PASS	PASS	PASS	PASS	PASS
Sulfate content	≤ 0.2%	PASS	PASS	PASS	PASS	PASS
<b>Metals</b>						
Heavy Metals (as Lead)	< 10 ppm	< 0.15 ppm	< 0.14 ppm	< 0.26 ppm	< 0.25 ppm	< 0.22 ppm
<b>Microbial Analyses</b>						
<i>Escherichia coli</i>	Negative in 10g	PASS	PASS	PASS	PASS	PASS
<i>Salmonella</i> species	Negative in 10g	PASS	PASS	PASS	PASS	PASS
<i>Staphylococcus aureus</i>	Negative in 10g	PASS	PASS	PASS	PASS	PASS
<i>Pseudomonas aeruginosa</i>	Negative in 10g	PASS	PASS	PASS	PASS	PASS
Total Aerobic Count	< 3000 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g
Mold and Yeast	< 300 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g	< 10 cfu/g

### 3. Contaminants

Because Chromax® Chromium Picolinate is produced synthetically employing a fairly simple process, the potential for contamination or for the introduction of impurities is relatively low. However, analyses were conducted on all five batches of Chromax® Chromium Picolinate for the following contaminants in conjunction with the batch analyses described in Table 4:

*Heavy Metals (as Lead):*

Samples from all five batches were analyzed for heavy metals (as lead). The test results yielded a mean value across all samples of 0.20 ppm. The specification for heavy metals is not more than 10 ppm, and thus with no single sample exceeding 0.26 ppm, the test results demonstrate that all batches are within the specification.

*Sulfates:*

Samples from all five batches were analyzed for sulfates. The test results show that all samples yielded values less than 0.20%. The specification for sulfates is not more than 0.20%, and thus with no single sample exceeding 0.20%, the test results establish that all batches are within the specification.

*Chlorides:*

Samples from all five batches were analyzed for chlorides. The test results show that all samples were less than 0.06%. The specification for chlorides is not more than 0.06%, and thus with no single sample exceeding 0.06%, the test results demonstrate that all batches are within the specification.

*Microorganisms:*

Samples from all five batches were cultured for the following microorganisms: *Escherichia coli*, *Salmonella* species, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Results for all samples yielded negative growth in 10 grams of material, and therefore passed the NF specification. Counts for molds and yeasts from all samples were < 10 cfu/g. In addition, the total aerobic count for all samples was also < 10 cfu/g, a value that is well below the NF limit of 3000 cfu/g. Thus, these test results show that all batches meet the established microbial specifications, and demonstrate the absence of microbial contamination.

As previously discussed, specifications have been established by Nutrition 21 for each of these contaminants, and these are listed in Table 3.

#### 4. *Product Stability*

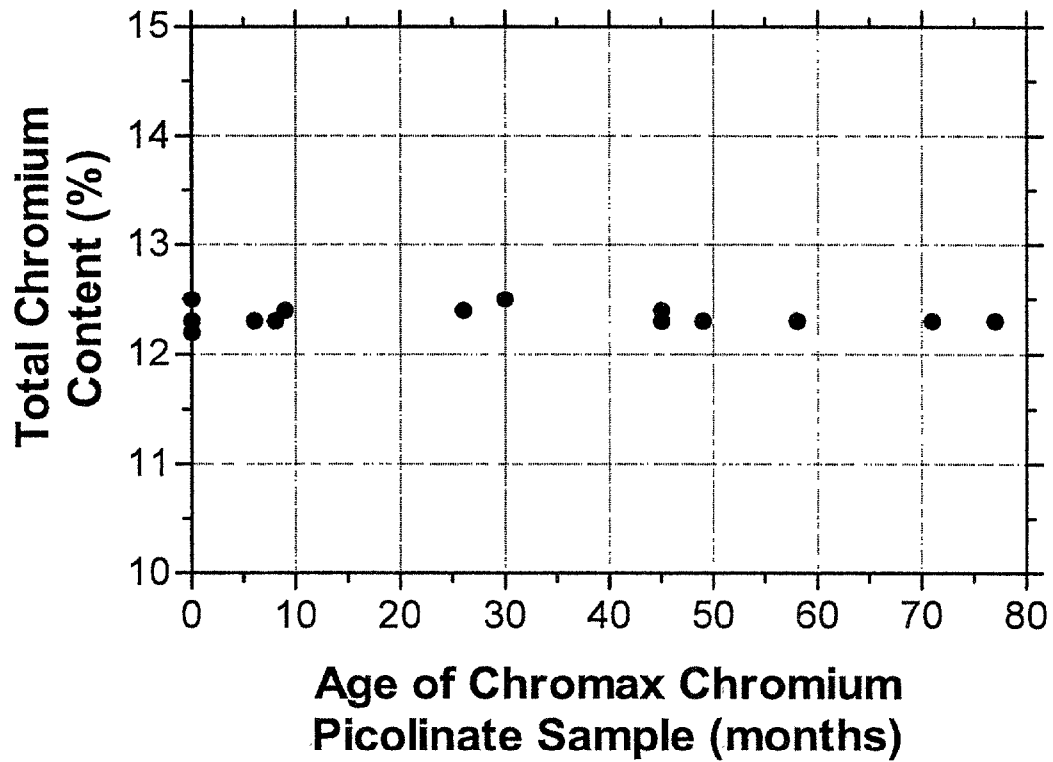
In order to determine the stability of Chromax® Chromium Picolinate over time, Nutrition 21 has had a series of archived samples of its final product analyzed by a certified third-party contract laboratory for total chromium content. Elemental (or total) chromium in these Chromax® Chromium Picolinate samples was measured by titration. These analyzed samples of Chromax® Chromium Picolinate had been archived for varying lengths of time (i.e., 0 to 77 months) prior to analysis in dark bottles at ambient temperature (i.e., 15 to 30° C) and humidity. The results of these analyses are presented in Table 5 and are displayed graphically in Figure 3.

**Table 5. Analysis Results for Total Chromium from Archived Samples of Chromax® Chromium Picolinate**

Age of Sample (months)	Total Chromium Content (% dry weight)
0	12.5
0	12.2
0	12.3
0	12.3
6	12.3
6	12.3
8	12.3
9	12.4
9	12.4
26	12.4
30	12.5
45	12.4
45	12.3
49	12.3
58	12.3
71	12.3
77	12.3

The results of the stability testing shown in Table 5 (and Figure 3) demonstrate that the total chromium content of Chromax® Chromium Picolinate is little affected by storage times of the final product of up to 77 months, which is almost 6.5 years. The product data sheet provided by Nutrition 21 for Chromax® Chromium Picolinate claims a shelf life for this product of 3 years in a dry environment at 25°C, which is supported by the stability results presented above.

**Figure 3. Analysis Results for Total Chromium from Chromax® Chromium Picolinate Samples of Varying Age**



***H. ANALYTICAL METHOD***

The proposed analytical method for the quantification of total chromium in or on food resulting from the proposed uses of Chromax® Chromium Picolinate is the American Association of Analytical Chemists ("AOAC") Official Method 990.08 (*Inductively Coupled Plasma (ICP) Atomic Emission Spectrometric Method*), which was originally designed to determine the presence of metals in solid wastes.

### III. HISTORICAL USE AND CONSUMER EXPOSURE

#### A. *BACKGROUND EXPOSURE*

##### 1. *Food Sources*

Trivalent chromium is present in many commonly consumed foods in the U.S. Dietary sources of chromium are presumably in the trivalent form due to the presence of reducing substances in foods (IOM 2001). A significant portion of chromium present in foods is believed to originate from external sources during growing, processing, preparation, fortification and handling (Anderson et al. 1992). Preparation of food in stainless steel cookware may also contribute to dietary chromium intake (Kuligowski and Halperin 1992).

Fruits, vegetables, and grain products tend to be the best natural sources of dietary chromium (Anderson et al. 1992). Many of these foods provide approximately 1 mcg to more than 20 mcg of chromium per serving. Whole grains and whole grain-based products typically provide higher amounts of chromium than refined grains, and prepared and packaged bread products such as waffles, English muffins, and bagels have been found to have higher levels than less handled bread products (Anderson et al. 1988, Anderson et al. 1992). Meats, poultry, fish, eggs, and legumes are typically lower in chromium, with most of these products providing less than 2 mcg of chromium per serving. Processed meats contain higher levels of chromium (approximately 10 mcg per serving), though a significant amount of this chromium is attributed to transfer from external sources. With a typical chromium concentration of less than 1 mcg per serving, dairy products are poor sources of dietary chromium (Anderson et al. 1992). Spices tend to provide very concentrated sources of chromium; although on a per serving basis, these products contribute negligible amounts to dietary intake (Khan et al. 1990, Anderson et al. 1992). Variable concentrations of chromium have also been detected in beer (Anderson and Bryden 1983).

The USDA nutrient composition database does not include data on the chromium content of foods, and consequently no estimates of chromium intake for the U.S. population based on national food consumption survey data are available. In the absence of nutrient composition data for chromium, results from chromium analyses of self-selected diets may be used to provide estimates of typical chromium intake in the U.S. (Anderson and Kozlovsky 1985, Anderson et al. 1992, Anderson et al. 1993).

Anderson and Kozlovsky (1985) determined the 7-day average chromium content of self-selected diets. The estimated 7-day average chromium intake by 10 adult males was 33 ( $\pm$  3) mcg per day (range 22 to 48 mcg per day), and the estimated intake by 22 adult females was 25 ( $\pm$  1) mcg per day (range 13 to 36 mcg per day). These estimates correspond to approximately



14 mcg chromium per 1000 kcals and 16 mcg chromium per 1000 kcals for males and females, respectively.

In another study of the chromium content of self-selected diets, the average chromium concentration of diets selected by 8 adult males was 18.6 mcg per 1000 kcals, and the average chromium concentration in diets selected by 11 adult females was 12.5 mcg per 1000 kcals (Anderson et al. 1992). Anderson and colleagues later reported the total chromium content of one-day diets self-selected by male and female adults (Anderson et al. 1993). Based on the results of nutrient analyses of duplicate plate samples, the mean chromium content of diets selected by the 8 males was estimated to be 38.8 ( $\pm$  6.5) mcg per day, and the mean chromium content of diets selected by the 11 females was 23.1 ( $\pm$  2.9) mcg per day. In this study, the subjects also consumed a controlled diet for 14 weeks during which their energy requirements were determined. For most subjects, total energy intake while they consumed the freely chosen foods was lower than actual energy requirements. Therefore, the investigators applied correction factors to the nutrient intakes based upon the ratio of energy requirements to energy intake as measured in the duplicate plate analysis; the adjusted estimates of chromium intakes by males and females were 54.1 ( $\pm$  7.2) and 28.7 ( $\pm$  3.1) mcg per day, respectively.

In the study of the chromium content of self-selected diets conducted by Anderson and colleagues, the chromium concentration of 22 balanced diets planned by nutritionists was also analyzed (Anderson et al. 1992). The mean concentration of chromium in the planned diets was 13.4 ( $\pm$  1.1) mcg per 1000 kcals, with values ranging from 8.4 to 23.7 mcg per 1000 kcals. Results from this study indicate that the chromium concentrations in optimal diets (i.e., those designed by a nutritionist) are likely comparable to the concentrations found in self-selected diets.

This estimate of mean chromium concentration in the planned diets (Anderson et al. 1992) was combined with estimates of mean energy intakes by adult populations to establish Adequate Intakes ("AIs") for chromium, as detailed in the recent report of Dietary Reference Intakes ("DRIs") for micronutrients released by the National Academy of Sciences (IOM 2001). The AIs for children were extrapolated from values established for adults. Table 6 provides a summary of the chromium AIs by life stage and gender group. Given that the AIs were developed from estimates of energy intake and dietary chromium concentrations that are comparable to those found in self-selected diets, it follows that the AIs also provide estimates of current chromium intake by the U.S. population.

**Table 6. Chromium Adequate Intakes**

Life Stage/Gender Group	Chromium AI <sup>a</sup> (mcg/day)
0 to 6 mo	0.2
7 to 12 mo	5.5
1 to 3 y	11
4 to 8 y	15
Males, 9 to 13 y	25
Females, 9 to 13 y	21
Males, 14 to 18 y	35
Females, 14 to 18 y	24
Males, 19 to 50 y	35
Females, 19 to 50 y	25
Males, >50 y	30
Females, >50 y	20
Data source: IOM (2001)	
<sup>a</sup> AI = Adequate Intake	

These estimates of dietary chromium intake are lower than the estimated intake of 60 mcg/day from food reported by ATSDR (2000). The ATSDR estimate is based on data published by Kumpulainen et al. (1979) and corroborated by analysis of metals (including chromium) in foods. Increased awareness, improved instrumentation, and optimization for chromium analysis have been attributed to lower estimates of dietary chromium intakes published in the mid to late 1980s as compared to earlier studies (Anderson and Kozlovsky 1985). Therefore, the estimates of up to 35 mcg per day for adults based on the most current data may be more representative of true chromium intakes from dietary sources.

## 2. *Dietary Supplement Sources*

Trivalent chromium is available in single-ingredient dietary supplements and combination formulations including many multivitamin/mineral supplements. Data collected in the Third National Health and Nutrition Examination Survey (“NHANES III”), conducted between 1988 and 1994 (U.S. DHHS 1998), provides the most current information on dietary supplement use in a nationally representative population. Participants were asked to identify what and how many vitamin and/or mineral supplements they used in the previous month. Many

respondents also provided information on use of other dietary supplements. In order to translate data on use of dietary supplements into estimates of vitamin and mineral intake, the staff of the National Center for Health Statistics ("NCHS") created a dietary supplements database (the NHANES III Dietary Supplement Information Data File) containing nutrient/ingredient values and product information for the vitamins, minerals and other dietary supplements reported in the NHANES III adult and youth household questionnaires.

A total of 262 dietary supplement products reported by NHANES III respondents were known to contain trivalent chromium; vitamin-mineral combination supplements accounted for the majority (230) of all chromium-containing products. In the population of respondents ages 2 years and older, approximately 10 percent reported use of a chromium-containing supplement. The mean daily intake of chromium by users of these products is approximately 29 mcg; the mode of supplemental chromium intake is 25 mcg, which corresponds to the most common chromium content of vitamin-mineral combination supplements in NHANES III.

Recent marketing information suggests that use of trivalent chromium supplements has increased in the decade since the NHANES III data were collected. By 1995, trivalent chromium had become one of the fastest-growing minerals in natural food stores and the mass market, and had reached the levels of other minerals such as calcium, magnesium, and zinc. An estimated 10 million individuals regularly ingested chromium picolinate in some supplemental form in 1998, compared to less than one million in 1992 (Nutrition Business International 2001). The majority of trivalent chromium supplements available contain chromium tripicolinate, as Nutrition 21 supplies raw material (chromium tripicolinate) to about 70% of this supplement market. While these data suggest that the percentage of Americans who ingest trivalent chromium supplements has increased since the NHANES III supplement use data were collected, it may be reasonable to assume that mean daily intake by users of these products has not changed substantially from the estimated 29 mcg daily intake based on the NHANES III data, as the amount of chromium tripicolinate delivered in products currently manufactured with Nutrition 21 chromium tripicolinate ranges from 3.1 to 75 mcg per dose (Table 7).

**Table 7. Supplements Containing Chromax® Chromium Picolinate**

Supplement (Manufacturer)	Form	Chromium Tripicolinate (mcg)
Nutri Mega (American Health)	Softgel	3.1
Iron-Free Multiple (Solgar)	Tablets	10
Formula VM-2000 (Solgar)	Tablets	25
Prenatal Nutrients (Solgar)	Tablets	25
Multivitamin-Mineral (Herbalife)	Tablets	33
Multivitamin-Mineral & Herbal (Herbalife)	Tablets	33
More than a Multiple (American Health)	Tablets	50
Meta-Lite (Metabo Lite)	Caplets	75
Appetite Suppressor Rx (Metabo Lite)	Capsules	25
Chromium Herbal 5000 Plus (Metabo Lite)	Capsules	50
Ultimate Performance Max (Rexall)	Bar	5
Body Success (Nature's Bounty)	Drink	7.5
Extreme XXL (Weider)	Drink	22
White Lightning (Weider)	Drink	42
Maximum Fat Burner (Weider)	Drink	48
Data source: Nutrition 21		

**B. INTENDED USE AND CONSUMER EXPOSURE**

**1. Proposed Uses and Maximum Use Level**

Nutrition 21 proposes use of Chromax® Chromium Picolinate in nutritional beverages (i.e., meal replacements, including ready-to-drink products and powder mixes) and nutritional bars (i.e., meal replacement bars, energy bars, and diet meal bars). The target market for these types of products is adults, especially those individuals who are interested in weight loss and/or management, cholesterol control, or enhanced blood sugar and carbohydrate metabolism. The survey data that are available on the consumption of these types of products (in an untargeted marketplace) show that less than two percent of the U.S. population under the age of 13 consume these types of products at all. For the proposed uses listed above, the maximum proposed use level is 2.4 mg Chromax® Chromium Picolinate per product serving, which is equivalent to 300 mcg trivalent chromium per serving, assuming a trivalent chromium content of 12.4 percent in Chromax® Chromium Picolinate.

**2. Estimated Daily Intake**

Using food intake data reported in the United States Department of Agriculture's 1994-96 Continuing Survey of Food Intakes by Individuals ("CSFII") and its 1998 Supplemental Children's Survey (USDA 2000), ENVIRON estimated intake of trivalent chromium that would

result from the proposed uses of Chromax® Chromium Picolinate at the maximum use level. The CSFII provides the most current food consumption data available for the U.S. population.

The CSFII was conducted between January 1994 and January 1997 with non-institutionalized individuals in the United States. In each of the three survey years, data were collected from a nationally representative sample of individuals of all ages. The CSFII 1998 survey was a survey of children ages 0 through 9 years, which was supplemental to the CSFII 1994-96. It used the same sample design as the CSFII 1994-96 and was intended to be merged with CSFII 1994-96 to increase the sample size for children in the survey. The merged surveys are designated as CSFII 1994-96, 1998. In the CSFII 1994-96, 1998, dietary intakes were collected through in-person interviews using 24-hour recalls on two nonconsecutive days approximately one week apart. A total of 21,662 individuals provided data for the first day; of those individuals, 20,607 provided data for a second day. The food record for each individual includes the gram weight and nutrient data for all foods consumed during the day of the recall.

The survey database includes a list of nearly 6,000 food codes for foods that were consumed by survey respondents. ENVIRON identified food codes representative of the proposed uses for Chromax® Chromium Picolinate from the list of food codes and from the CSFII recipe files. The list of food codes representing the proposed uses for Chromax® Chromium Picolinate is provided in Appendix I.

Estimates of 2-day average intakes of trivalent chromium resulting from the proposed uses of Chromax® Chromium Picolinate at the maximum use level were calculated from the food code list and the survey database of diet recalls. All estimates were generated with USDA sampling weights to account for the complex sample design of the CSFII.

Results of the estimates for the U.S. population ages 2 years and older are presented in Table 8; estimates are presented for each proposed use category separately, and for all proposed use categories combined. These estimates were calculated from 2-day average intakes by all individuals who consumed one or more foods from the proposed use categories at least once during the recall period. The estimated mean intake of trivalent chromium from all proposed use categories is 304 mcg per day and the estimated 90th percentile of intake of trivalent chromium from the proposed uses is 545 mcg per day. Results of the estimates of exposure from all proposed use categories combined indicate that approximately 2 percent of the U.S. population ages 2 years and older consume one or more of the foods and beverages included in the list of proposed uses in a 2-day period.

The estimates presented in Table 8 are likely overestimates of exposure to trivalent chromium from foods proposed for supplementation with Chromax® Chromium Picolinate, as these estimates assume that all foods in the proposed use categories are supplemented, and all foods are supplemented at the proposed maximum use level.

<b>Table 8. Estimates of Daily Intake of Trivalent Chromium from Proposed Uses of Chromax® Chromium Picolinate in Nutritional Ready-To-Drink Beverages, Beverage Mixes, and Bars</b>				
<b>Use Category</b>	<b>Users</b>		<b>Estimated Intake per User</b>	
	<b>Number of People</b>	<b>Percent of Survey Population</b>	<b>Mean (mcg/day)</b>	<b>90<sup>th</sup> Percentile (mcg/day)</b>
Ready-To-Drink Nutritional Beverages	110	0.8	340	600
Nutritional Beverage Mixes	146	1.1	290	600
Nutritional Bars	52	0.4	201	344
All Proposed Uses	299	2.2	304	545

Data source: USDA 1994-96, 1998 Continuing Survey of Food Intakes by Individuals. Estimates represent 2-day average intakes reported by users ages 2 years and older. All estimates were generated with USDA sampling weights. All use categories were assumed to contain 300 mcg chromium per serving.

## IV. INTENDED EFFECT

Chromium is an essential element required for normal carbohydrate, lipid, and protein metabolism in humans and animals (Anderson 1998, EPA 1998a). Chromium potentiates the action of insulin *in vivo* and *in vitro* (EPA 1998a, IOM 2001). Chromium deficiency in humans has been associated with diseases such as mature-onset diabetes, cardiovascular disease, and nervous system disorders (EPA 1998a), and may cause such adverse effects in humans as fasting hyperglycemia, impaired glucose tolerance, and elevated plasma insulin, serum total cholesterol and triglycerides (Campbell et al. 1997). In the National Academy of Sciences ("NAS") recent review of micronutrients (IOM 2001), an AI for chromium was established based on estimated mean intakes; sufficient evidence was not available to set an RDA. In this NAS report, the AI was set at 35 mcg/day for young men and 25 mcg/day for young women. In this same report, the NAS noted that few serious adverse effects have been associated with excess intake of chromium from food, but a Tolerable Upper Intake Level ("UL") was not able to be established. Previously, the National Research Council ("NRC") recommended an Estimated Safe and Adequate Daily Dietary Intake ("ESADDI") for chromium of 50 to 200 mcg for adults (NRC 1989). The U.S. Food and Drug Administration ("FDA") Reference Daily Intake ("RDI") for chromium is 120 mcg/day; this value represents the RDI for chromium that is listed on product labels under 21 CFR §101.9.

The estimated normal dietary intake of chromium is 25 mcg/day for women and 33 mcg/day for men, both values less than the minimum ESADDI established by the NRC (Anderson and Kozlovsky 1985). These dietary intake estimates suggest that a significant proportion of the U.S. population may be chromium deficient. Therefore, the intended effect of the addition of Chromax® Chromium Picolinate to food (as described by the proposed uses) is nutrient supplementation as defined under 21 CFR §170.3(o)(20), and more specifically, to increase the dietary intake of trivalent chromium in the U.S. population.

## V. REVIEW OF SAFETY DATA

This chapter presents a critical review of the available toxicity data on chromium tripicolinate, as well as other trivalent chromium compounds, as they relate to the safety of these compounds via ingestion. To accomplish this safety review, ENVIRON relied, in part, on the following three recent toxicological and nutritional reviews of trivalent chromium that are publicly available:

- ***Toxicological Review of Trivalent Chromium*** (EPA 1998a)  
This toxicological review of trivalent chromium was authored by the U.S. Environmental Protection Agency (“EPA”) to provide scientific support and rationale for the hazard and dose-response assessment sections contained in the Integrated Risk Information System (“IRIS”) pertaining to chronic exposure to trivalent chromium.
- ***Toxicological Profile for Chromium*** (ATSDR 2000)  
This Agency for Toxic Substances and Disease Registry (“ATSDR”) toxicological profile for chromium succinctly characterizes the toxicologic and adverse health effects information regarding trivalent and hexavalent chromium. This peer-reviewed profile identifies and reviews the key literature that describes both trivalent and hexavalent chromium’s toxicologic properties.
- ***Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*** (IOM 2001)  
This report by the Panel on Micronutrients of the Food and Nutrition Board of the Institute of Medicine (“IOM”) is one in a series that presents a comprehensive set of reference values for nutrient intakes for healthy U.S. and Canadian populations. One major task of this report was to review the evidence relating intake of micronutrients to reduction of the risk of chronic diseases, and the daily amount needed to maintain normal status based on biochemical indicators and daily body losses.

In addition, ENVIRON also conducted its own independent literature search to ensure that all relevant primary published literature on trivalent chromium compounds was identified and included in this review. The on-line databases searched included MEDLINE, TOXLINE, CANCERLIT, EMBASE, Life Sciences Collection, BIOSIS Previews, SciSearch, NTIS and Chemical Safety Newsbase. ENVIRON also reviewed a number of clinical studies, whose primary objective was to evaluate the efficacy of chromium tripicolinate, to determine if any safety-related information could be obtained from these reports. Finally, ENVIRON evaluated human case reports that have periodically appeared in the published literature that claimed some association between chromium tripicolinate ingestion and the occurrence of adverse events.



Only studies in which the full report was published in English in a peer-reviewed scientific journal were eligible for inclusion in this review. Foreign language articles and published abstracts were not considered for this review.

Historically, the two main issues of potential concern associated with the chronic ingestion of trivalent chromium have been: 1) will trivalent chromium bioaccumulate in the body potentially resulting in tissue concentrations that eventually exceed some (as of yet unknown) toxicity threshold; and 2) what is the potential for ingested trivalent chromium to be carcinogenic in humans. Clearly, to appropriately address the bioaccumulation issue, it is important to understand the bioavailability of trivalent chromium, and more specifically, its absorption, distribution, metabolism, and excretion. Additionally, understanding bioavailability is fundamental for proper interpretation of the toxicity data on trivalent chromium because of the varying bioavailabilities of the different trivalent chromium salts that were employed in the toxicity studies. Therefore, the bioavailability of trivalent chromium will be discussed prior to reviewing the available toxicity data on trivalent chromium.

#### **A. *BIOAVAILABILITY OF TRIVALENT CHROMIUM***

Trivalent chromium bioavailability from trivalent chromium-containing compounds is a function of how the compound is processed by the body following ingestion, i.e., its level of absorption into the body, its distribution to various organ systems in the body (including its possible sites of action), its metabolism within the body, and finally, its elimination from the body. Each of these factors and their impact on bioavailability are further discussed below.

##### **1. *Absorption***

As discussed previously, trivalent chromium is present in many foods, and thus is ingested as a normal part of the daily diet; approximately 0.5 to 2% of dietary trivalent chromium is believed to be absorbed via the gastrointestinal ("GI") tract (Gammelgaard et al. 1999). The ATSDR (2000) states that absorption of ingested trivalent chromium is estimated to be less than 3%. Interestingly, the absorption efficiency of dietary trivalent chromium seems to be inversely related to trivalent chromium dietary intake. At low levels of intake (< 10 mcg/day), absorption is in the range of 0.4 to 0.5%, compared to around 2% at trivalent chromium dietary intakes of > 40 mcg/day (Anderson and Kozlovsky 1985). A number of human and animal studies have confirmed the relatively poor absorption of trivalent chromium from the GI tract, with absorption values generally falling within the range observed following dietary exposures, i.e., 0.5 to 2% (EPA 1998a, ATSDR 2000).

The absorption of trivalent chromium following ingestion is strongly influenced by the chemical complex in which the trivalent chromium is administered. Ingestion of trivalent chromium in the form of inorganic salts, such as chromic oxide or chromium sulfate, result in little or no absorption from the GI tract (i.e., far less than 1%). In contrast, trivalent chromium in

association with a chelating agent or in the form of an organic salt, such as chromium acetate or chromium oxalate, is absorbed from the GI tract to a somewhat greater extent (i.e., in the range of 2 to 3%) (Finley et al. 1996, ATSDR 2000). Ascorbic, picolinic, and nicotinic acids have all been demonstrated to facilitate the absorption of trivalent chromium through the intestinal wall (Anderson et al. 1997b, ATSDR 2000).

To date, very few studies in humans or animals have been conducted that have quantitatively evaluated the absorption of trivalent chromium specifically from ingested chromium tripicolinate. In the only human study to date, chromium tripicolinate dietary supplements were given to 8 human volunteers at doses of 400 mcg trivalent chromium per day on each of 3 successive days (Gargas et al. 1994). Urine samples were collected and analyzed for total chromium. The mean trivalent chromium absorption from the chromium tripicolinate matrix was estimated to be 2.8%, with a range of 1.5 to 5.2%.

In a recent animal study by Juturu et al. (2002), three groups of six rats each were given a single bolus oral dose of 1000 mg/kg of chromium oxide, chromium chloride, or chromium acetate. Urine from each animal was collected over a 24-hour period. All animals were then sacrificed and the liver, kidney, heart, and pancreas were removed, weighed, and analyzed for trivalent chromium. In addition, the 24-hour urine sample from each animal was also analyzed for trivalent chromium. These data provide estimates, based on the total administered dose of trivalent chromium, of trivalent chromium absorption of 0.01, 2.6, and 1.3% when administered as chromic oxide, chromium chloride and chromium acetate, respectively.

In another animal study, Gammelgaard et al. (1999) evaluated the absorption and penetration of chromium tripicolinate into the gut and small intestines of rats relative to the inorganic trivalent chromium salts, chromium chloride and chromium nitrate, employing an *in vitro* system using artificial gastric juice. The study demonstrated that chromium tripicolinate is not disassociated in the stomach, but is available for absorption as a tripicolinate complex when it leaves the stomach and enters the small intestine. In addition, the results of this study showed that chromium tripicolinate permeated the intestinal wall of rats at a rate that was ten times greater than that of chromium chloride or chromium nitrate.

Finally, Sullivan et al. (1984, as cited in ATSDR 2000) reported that trivalent chromium absorption was ten-fold greater in immature rats than adult rats following oral exposures to chromium chloride, suggesting that immature rats may exhibit greater absorption of trivalent chromium compared to adult rats. Other factors that may influence absorption of trivalent chromium include nutritional status (e.g., zinc status and the presence of dietary amino acids) and GI tract status (e.g., fasting vs. fed state) (O'Flaherty 1996).

## 2. *Distribution*

Anderson et al. (1997b) found that chromium concentrations in the kidney and liver of rats fed either chromium tripicolinate or chromium chloride, at concentrations of up to 100 ppm

in the diet for 20 weeks, increased linearly with increasing dose over the course of the study. In this study, the increased bioavailability of trivalent chromium from chromium tripicolinate compared to chromium chloride was clearly apparent; chromium concentrations in the liver and kidney of animals fed chromium tripicolinate were approximately 2- to 6-fold greater than chromium concentrations in these same organs from animals exposed to chromium chloride.

Animal studies on other trivalent chromium compounds have shown that oral exposures result in a wide tissue distribution within the body, including liver, kidneys, spleen, hair, heart, red blood cells, bone, and bone marrow, with the greatest increases in chromium concentrations occurring in the liver and kidneys (EPA 1998a, ATSDR 2000). These findings on other trivalent chromium compounds are consistent with what Anderson et al. (1997b) found for chromium tripicolinate, suggesting that chromium tripicolinate is distributed within the body similarly to these other trivalent chromium compounds.

### 3. *Metabolism*

As previously stated, trivalent chromium potentiates the action of insulin in peripheral tissue (EPA 1998a, IOM 2001), and a number of studies have demonstrated beneficial effects of chromium on circulating glucose, insulin, and lipids in a variety of human subjects and animal species (IOM 2001). However, the exact mechanism by which chromium exerts its beneficial effect is unclear. Once absorbed, at least some of the trivalent chromium is believed to be complexed with other compounds. In its biologically active form, trivalent chromium may occur in either a chromium-nicotinic acid complex referred to as glucose tolerance factor ("GTF") (EPA 1998a, ATSDR 2000) or as a low molecular weight, chromium-binding complex (Yamamoto et al. 1987, 1988). This GTF may function by facilitating interaction between insulin and its receptor site, but additional investigations will be required to elucidate the exact mechanisms involved in the essentiality of trivalent chromium. The low molecular weight, chromium-binding complex identified from such sources as bovine milk and rabbit liver has been shown to have *in vitro* activities comparable to those of GTF with respect to insulin action (Yamamoto et al. 1987, 1988). Recent work by Davis and Vincent (1997, as cited in IOM 2001) and Vincent (1999, as cited in IOM 2001) suggests that this low molecular weight chromium-binding complex may amplify insulin receptor tyrosine kinase activity in response to insulin. The ability of this binding complex to activate insulin receptor tyrosine kinase depends on its chromium content. Progress in this field has been limited by lack of a simple, widely accepted method to identify human subjects who are chromium deficient, and by the difficulty in producing chromium deficiency in animals (IOM 2001).

### 4. *Excretion*

As a consequence of the low absorption of trivalent chromium, the major pathway of excretion following oral exposures is through the feces. Excretion via bile is not a major

contributor to fecal chromium (IOM 2001). The primary route of excretion of absorbed trivalent chromium is via the urine; chromium can also be found in blood, hair, and finger- and toenails. In the human study cited above (Gargas et al. 1994), subjects who ingested 400 mcg of trivalent chromium per day as chromium tripicolinate for 3 consecutive days, showed peak concentrations of trivalent chromium in the urine approximately 7.2 hours post-dose, on average. Of the small amount of trivalent chromium that is absorbed, chromium clearance from the blood is rapid, but clearance from tissues occurs at a slower rate (ATSDR 2000). This observation is consistent with the findings of Anderson et al. (1997b) in which elevated chromium levels were found in certain organs, particularly the liver and kidney, following subchronic exposure to trivalent chromium.

## **5. Conclusions**

The bioavailability of trivalent chromium is a function of its ability to be absorbed into the body, and may be the single most important factor in determining its toxicity (O'Flaherty 1996). Trivalent chromium in foods and inorganic chromium salts, such as chromium chloride, is poorly absorbed following ingestion (i.e., 0.5 to 2%), while trivalent chromium from chromium tripicolinate, an organic salt, is believed to be absorbed at somewhat higher levels (i.e., ~ 3%).

### **B. BIOACCUMULATION OF TRIVALENT CHROMIUM**

Employing both pharmacokinetic modeling and animal data, ingestion of trivalent chromium compounds has been reported to result in the bioaccumulation of trivalent chromium in the body, particularly in the liver and kidneys. Several pharmacokinetic models have been constructed to predict the retention and excretion of trivalent chromium (Lim et al. 1983, Gargas et al. 1994, Stearns et al. 1995b, O'Flaherty 1996). The pharmacokinetic model of O'Flaherty (1996) is based on studies in rats in which chromium compounds were administered orally and intratracheally. Although the usefulness of this model in the safety assessment of trivalent chromium supplementation in humans is limited at this point in time due to a number of factors (e.g., a lack of better understanding of the differences between chromium pharmacokinetics in rats and humans), the author did identify an important uncertainty in modeling the kinetic behavior of chromium. O'Flaherty stated, ". . . it may be that bioaccessibility of chromium to absorption processes will prove to be the most important single characteristic of a chromium source determining its potential absorption and toxicity."

Stearns et al. (1995b) used the Lim et al. (1983) model, which was based on a single intravenous dose of chromium chloride in humans, and the Gargas et al. (1994) model, in which chromium tripicolinate was administered orally to human volunteers for 3 days, to predict that ingested trivalent chromium will accumulate and be retained in human tissues for extended

periods of time. Based on this analysis, Stearns et al. (1995b) further predicted that the cumulative daily intake of trivalent chromium, under exposure conditions such as intake of a nutritional supplement over long periods of time, might result in tissue concentrations that pose a genotoxic risk. This prediction was based upon a comparison of the modeled tissue levels of trivalent chromium following daily dietary supplementation with three pills of chromium tripicolinate (yielding a total trivalent chromium intake of 600 mcg/day) to the concentrations of trivalent chromium that have induced chromosomal aberrations in *in vitro* tests (Stearns et al. 1995a). The relevance of these *in vitro* tests for predicting genotoxic risks *in vivo* will be further discussed later in this document.

Empirically, the results of Anderson et al. (1997b) previously described show that chromium tripicolinate ingestion in animals over a period of 20 weeks resulted in accumulation of trivalent chromium in the liver and kidneys. However, no adverse effects were observed in this study. Regardless, the concern has been raised that bioaccumulation of trivalent chromium in the liver and kidneys from chronic exposure to chromium tripicolinate could result in the exceedance of some (as of yet unknown) toxicity threshold. No long-term (chronic) studies in animals currently exist that specifically evaluate the potential toxicity of chromium tripicolinate from bioaccumulation of trivalent chromium. Therefore, ENVIRON has relied on the available toxicity data from previously conducted long-term animal studies that employed trivalent chromium compounds other than chromium tripicolinate (i.e., MacKenzie et al. 1958, Schroeder et al. 1965). The absence of adverse effects seen in these chronic animal studies suggests that ingested trivalent chromium eventually reaches some kind of equilibrium within the body, and thus never reaches high enough concentrations to exceed some (as of yet unknown) toxicity threshold, even if exposure occurs for a lifetime.

In the first of these chronic animal studies, MacKenzie et al. (1958), as cited in EPA (1998a), provided rats with 25 ppm chromium chloride in drinking water for 12 months and noted no change in body weight, macroscopic or microscopic pathology, or clinical chemistry variables. The EPA believes that this study establishes a no-observed-effect level ("NOEL") or NOAEL of 25 ppm chromium chloride, equivalent to 8.2 ppm trivalent chromium. Then, the EPA, assuming that an average rat weighs 0.35 kg and consumes 0.035 L water/day, adjusted the 8.2 ppm concentration to a dose of 0.82 mg/kg/day trivalent chromium, the highest (and only) dose administered in this study.

In the second chronic study conducted by Schroeder et al. (1965), rats were administered drinking water that contained chromium acetate, an organic salt of trivalent chromium, for their entire lifetime. Again, no adverse effects were observed in these animals at the highest (and only) dose administered of 0.46 mg/kg/day trivalent chromium, establishing this dose as an NOAEL.

In a third chronic animal study of trivalent chromium, three groups of rats fed bread containing concentrations of chromic oxide ranging from 1 to 5% in the diet for 840 days (120

weeks) did not exhibit toxic effects at any dose (Ivankovic and Preussmann 1975). A NOAEL of 1,468 mg/kg/day has been derived from this study by the EPA, and has been employed as the basis for the oral reference dose ("RfD") for trivalent chromium (EPA 1998c). The doses of trivalent chromium ingested by the animals in this study were 2,000- to 10,000-fold higher than those seen in MacKenzie et al. (1958) and Schroeder et al. (1965). This lack of toxicity at such high levels of exposure is most likely due to the absence of absorption of trivalent chromium when administered as chromic oxide, especially in a food matrix. Juturu et al. (2002) confirmed that chromic oxide is essentially unabsorbed when ingested by rats. Therefore, ENVIRON believes this study to be of questionable relevance for determining both the chronic toxicity of trivalent chromium and the potential for trivalent chromium to bioaccumulate.

ENVIRON believes that because Juturu et al. (2002) has shown that trivalent chromium is absorbed from the GI tract to a measurable degree from both chromium chloride and chromium acetate relative to chromic oxide (i.e., 2.6, 1.3, and 0.01 percent, respectively), the two lifetime drinking water studies that employed these compounds (MacKenzie et al. 1958, Schroeder et al. 1965) can be used to address both the chronic toxicity of trivalent chromium and the issue of whether trivalent chromium can attain high enough levels in the body to exceed some (as of yet unknown) toxicity threshold. The lack of adverse effects observed in these two chronic drinking water studies demonstrate that long-term administration of trivalent chromium compounds will not result in the bioaccumulation of trivalent chromium in the body at high enough levels to result in any toxic effects.

In conclusion, trivalent chromium is an essential element, and thus when given as a nutritional supplement, a more bioavailable source should be desirable. However, this increased bioavailability, in conjunction with chronic high-dose exposures, could potentially result in accumulated levels of trivalent chromium in the body that exceed some (as of yet unknown) threshold of toxicity, as suggested by the data of Anderson et al. (1997b) and Stearns et al. (1995b). For trivalent chromium compounds, the chemical complex in which trivalent chromium is ingested greatly influences its bioavailability. As shown by the data summarized in this section, trivalent chromium in chromium tripicolinate is thought to be more bioavailable relative to other trivalent chromium compounds. Whether this increased trivalent chromium bioavailability from chromium tripicolinate ingestion may eventually result in concentrations of trivalent chromium in the body that pose a health risk has been debated in the literature (e.g., McCarty 1996, Stearns and Wetterhahn 1996). However, ENVIRON believes this issue can be addressed through the use of two previously conducted animal toxicity studies that administered trivalent chromium compounds chronically in drinking water without any evidence of adverse effects (i.e., MacKenzie et al. 1958, Schroeder et al. 1965). These results suggest that even with the greater bioavailability of trivalent chromium in chromium tripicolinate, long-term exposures would not be expected to yield any adverse health effects in animals or humans. This conclusion

is further corroborated by the absence of adverse effects observed in the human clinical studies on chromium tripicolinate that will be discussed later in this chapter.

### ***C. NON-CARCINOGENIC TOXICITY***

One true animal toxicity study and numerous human efficacy studies exist in the published literature that have administered chromium tripicolinate at varying doses over varying lengths of time. In animals, the highest dose administered was 15 mg/kg/day trivalent chromium, as chromium tripicolinate, for 20 weeks in Anderson et al. (1997b). In humans, the highest dose administered was 1,000 mcg/day trivalent chromium, as chromium tripicolinate, for eight months in Cefalu et al. (1999). A number of animal toxicity studies are also available in the published literature that have employed other trivalent chromium compounds (e.g., chromic oxide, chromium chloride, and chromium acetate) that are relevant to a safety assessment of chromium tripicolinate. Taken together, these studies provide an adequate database by which to evaluate the non-carcinogenic toxicity of chromium tripicolinate. Summaries of the relevant studies on chromium tripicolinate and other trivalent chromium compounds are presented in Appendices II (*Animal Studies*) and III (*Human Studies and Case Reports*). A brief discussion of the key studies from this database is provided below.

#### ***I. Animal Studies***

##### ***a) Chromium Tripicolinate***

The available animal toxicity data on chromium tripicolinate itself are limited to two studies (Anderson et al. 1997b, Lindemann et al. 1995); however, the results that have been observed in these two studies are consistent with the existing data on other trivalent chromium compounds in demonstrating a very low order of oral toxicity for these compounds. The two animal studies that have employed chromium tripicolinate are summarized in Appendix II. Of these studies, one was a fairly traditional subchronic toxicity study in rats (Anderson et al. 1997b), while the other study examined the reproductive effects of dietary chromium tripicolinate in swine from an animal husbandry perspective (Lindemann et al. 1995), and thus did not evaluate traditional toxicity endpoints. Therefore, this study has limited utility for this safety review.

In the more traditional toxicity study, which has been previously discussed, Anderson et al. (1997b) evaluated the toxicity of chromium tripicolinate and chromium chloride in rats fed diets containing 5, 25, 50, and 100 ppm trivalent chromium, as chromium tripicolinate or chromium chloride, for 20 weeks. Results from this study showed that there were no statistically significant differences in body weight, organ weights, or blood variables among all groups tested at age 11, 17, and 24 weeks. Blood variables measured were glucose, cholesterol, triglycerides, blood urea nitrogen ("BUN"), lactic acid dehydrogenase, transaminases, total protein, and creatinine. Histological evaluation of the liver and kidney of controls and animals fed 100 ppm

trivalent chromium, as chromium chloride or tripicolinate, also did not show any detectable differences. However, both chromium tripicolinate and chromium chloride produced higher chromium concentrations in the liver and kidneys in treated animals compared to controls, and concentrations increased linearly with dose over time. At the highest dose administered, chromium tripicolinate produced about a four-fold increase in chromium levels in the kidney and about a 10-fold increase in chromium levels in the liver. Clearly, these increased chromium concentrations resulted in no apparent adverse effects. The authors concluded that trivalent chromium (as chromium tripicolinate or chromium chloride) is not toxic in rats at up to 100 ppm trivalent chromium in the diet, levels that are several thousand times higher than the upper limit of the ESADDI level for humans of 200 mcg/day established by the NRC. Thus, this study identified a NOAEL for trivalent chromium of 100 ppm in the diet corresponding to a dose of 15 mg/kg/day, assuming a daily dietary intake of 15 g of food and a body weight of 100 g .

In the other published animal study employing chromium tripicolinate, Lindemann et al. (1995) evaluated the reproductive effects of chromium tripicolinate in swine. Animals fed 500 or 1,000 ppb trivalent chromium, as chromium tripicolinate, in the diet (corresponding to a dose of 0.5 or 1 mg/kg/day trivalent chromium, respectively), plus 120 percent of the lysine requirement, during growing, breeding, and reproduction, had greater total and live litter sizes and greater total and live litter weights compared to controls. No effects on serum glucose levels were observed, but pre- and post-feeding insulin levels were decreased. No information on the occurrence of toxic effects was reported, but, again, this study did not specifically evaluate the more traditional toxicity endpoints, which then limits the usefulness of this study for this safety review.

**b) *Other Trivalent Chromium Compounds***

The three comprehensive reviews of trivalent chromium cited at the beginning of this chapter all contain summaries of animal studies that have been conducted employing trivalent chromium compounds other than chromium tripicolinate. Based on these studies, these reviews concur that the oral toxicity of trivalent chromium is very low. In their toxicological review of trivalent chromium, the EPA (1998a) stated that relatively few studies were located in the literature that addressed the oral toxicity of trivalent chromium, but in the studies that were found, no effects, other than reductions of the absolute weights of livers and spleens of rats, have been observed following oral exposure to trivalent chromium. In the review conducted by the IOM (2001), it was observed that ingested trivalent chromium has a low level of toxicity which is due, partially, to its very poor absorption. In addition, the IOM (2001) also noted that several studies have demonstrated the safety of large doses of trivalent chromium, most notably Anderson et al. (1997b).

As alluded to above, several animal studies are available in the published literature that provide toxicity data on ingested trivalent chromium compounds other than chromium



tripicolinate. No significant toxicity was observed in any of these studies. In the study with the highest exposures, Ivankovic and Preussmann (1975) reported the results from two separate studies on chromic oxide, an inorganic salt of trivalent chromium. In the first study, rats were fed baked bread containing up to 5% chromic oxide (corresponding to a dose of approximately 1,400 mg/kg/day trivalent chromium) for 90 days. The only effects observed were reductions in the absolute weights of the livers and spleens of rats in the highest dose group; the EPA determined that these effects did not necessarily represent an adverse effect (EPA 1998a). In the second study by the same investigators, rats were again fed baked bread containing up to 5% chromic oxide (corresponding to a dose of approximately 1,800 mg/kg/day trivalent chromium), 5 days per week, for 120 weeks. Although the primary purpose of this study was to assess the carcinogenic potential of chromic oxide, all major organs were examined histologically and no effects due to chromic oxide treatment were observed at any dose level. However, as explained previously, ENVIRON believes this study to be of questionable relevance for evaluating the chronic toxicity of trivalent chromium due to the absence of absorption of trivalent chromium when administered as chromic oxide, especially in a food matrix.

In another chronic animal study, Schroeder et al. (1965) exposed 54 male and 54 female Swiss mice to drinking water that contained 5 ppm trivalent chromium (as chromium acetate) for life (corresponding to a dose of 0.46 mg/kg/day trivalent chromium). No increase in the incidence of tumors was seen in the treated animals with respect to controls. Similar results were obtained by Schroeder et al. (1965) for Long-Evans rats. In addition, these investigators found little or no evidence of chromium accumulation in the kidney, liver, heart, lung, and spleen, when comparing tissue levels in controls to those in treated animals. This lack of detectable bioaccumulation in these tissues is in contrast to the results of Anderson et al. (1997b), but may be due to the 30-fold lower dose of trivalent chromium employed by Schroeder et al. (1965).

In a third chronic animal study, MacKenzie et al. (1958), as cited in EPA (1998a), provided rats with 25 ppm chromium chloride in drinking water for 12 months and noted no change in body weight, macroscopic or microscopic pathology, or clinical chemistry variables. The EPA believes that this study establishes a NOEL (or NOAEL) of 25 ppm chromium chloride in drinking water, equivalent to a concentration of 8.2 ppm trivalent chromium. Then, the EPA, assuming that an average rat weighs 0.35 kg and consumes 0.035 L water per day, adjusted this 8.2 ppm concentration to a dose of 0.82 mg/kg/day trivalent chromium.

Finally, in a very old study, Akatsuka and Fairhall (1934), as cited in EPA (1998a), fed cats 50 to 100 mg per day of trivalent chromium for 1 to 3 months. No effects on weights or gross or microscopic pathology of major organs were noted. Because little else is known about this study, it is of limited usefulness for this safety assessment.

## 2. *Human Studies*

### a) *Clinical Efficacy Studies*

A number of clinical efficacy studies on chromium tripicolinate supplementation were evaluated for inclusion in this safety review. Sample sizes in these studies ranged from 16 to 155 subjects. The doses administered in these clinical studies ranged from 200 to 1,000 mcg per day trivalent chromium, as chromium tripicolinate, for periods of from 6 weeks to 10 months. The majority of these studies examined the effects of chromium tripicolinate supplementation on glucose tolerance, body composition, or blood lipids. These studies were designed primarily as efficacy trials, and thus their main focus was not safety. However, some of these studies did collect data, such as clinical chemistries, that are nevertheless valuable in documenting whether any potential adverse effects in humans are associated with chromium tripicolinate ingestion. A tabular summary of these clinical studies is presented in Appendix III.

The two most pivotal studies from this clinical database were conducted by Campbell et al. (1997) and Cefalu et al. (1999). These two studies were selected as being the most pivotal because of the high doses of trivalent chromium employed (924 and 1,000 mcg/day, respectively), the relatively long study durations (3 and 8 months, respectively), and the fact that fairly complete clinical (i.e., blood) chemistries were done on all subjects. Evaluation of these clinical chemistry data allow conclusions to be drawn regarding the safety of the chromium tripicolinate doses administered in these efficacy studies.

In Campbell et al. (1997), 18 moderately overweight, middle-aged, healthy male volunteers were recruited to participate in this randomized, double-blind, placebo-controlled study to evaluate the combined effects of resistance training and high-dose chromium tripicolinate supplementation on hematological indices and iron status. These 18 subjects were randomly assigned to receive either 924 mcg per day trivalent chromium (as chromium tripicolinate) or placebo for 12 weeks. The results of this study showed no effect of chromium tripicolinate supplementation on blood chemistry and other hematological parameters, including serum iron levels. More specifically, hematocrit, hemoglobin, red blood cell ("RBC") count, white blood cell ("WBC") count, mean corpuscular volume ("MCV"), mean corpuscular hemoglobin, RBC distribution width, platelet count, and mean platelet volume did not change significantly with chromium tripicolinate supplementation or with resistance training. In addition, serum iron and ferritin concentrations, and total-iron-binding capacity ("TIBC") and transferrin saturation were not affected by chromium tripicolinate supplementation.

Through a personal communication with the principal investigator for this study (i.e., Wayne Campbell), it was also discovered that both liver and kidney function tests were performed on study participants, but the results were not reported in the publication. According to Dr. Campbell, these tests showed no effects of chromium tripicolinate supplementation on

liver or kidney function, supporting the safety of chromium tripicolinate supplementation in humans at doses of up to 924 mcg per day trivalent chromium.

In Cefalu et al. (1999), 29 males and females at high risk for developing type 2 diabetes (because of family history and obesity) were recruited to participate in this randomized, double-blind, placebo-controlled study to assess the effect of high-dose chromium tripicolinate supplementation on insulin sensitivity and body composition. At random, these 29 subjects were assigned to receive either 1,000 mcg per day trivalent chromium (as chromium tripicolinate) or placebo for 8 months. The results of this study showed a significant increase in insulin sensitivity in the chromium tripicolinate group compared to controls. However, no change in glucose effectiveness was observed in comparing treated subjects with controls. In addition, chromium tripicolinate supplementation had no effect on body weight, abdominal fat distribution, and body-mass index ("BMI"). More importantly for this review, no differences in complete blood counts, liver function, renal function, and electrolyte levels were observed in either group at the end of the study when compared against initial values recorded during a five-week baseline period at the beginning of the study.

Furthermore, through a personal communication with the principal investigator (i.e., William Cefalu), it was also revealed that several sets of biochemical tests were done on all subjects both before and after the eight-month treatment period. The specific biochemical tests conducted were:

- A comprehensive metabolic profile, which included albumin, total protein, alkaline phosphatase, ALT, AST, total bilirubin, direct bilirubin, calcium, phosphorus, BUN, creatinine, glucose, sodium, potassium, chloride, and carbon dioxide.
- A hemogram including complete blood counts (i.e., hemoglobin, hematocrit, WBC, platelets, RBC hemoglobin, RBC volume)
- A complete urinalysis, which included a microscopic exam, that assessed ketones, glucose, protein, RBCs, and WBCs in the urine.

In addition, the investigators also did a complete medical history for all subjects including physical, blood pressure, pulse, and electrocardiogram (both pre- and post-treatment). The investigators reported that none of these values changed during therapy, and there were no differences between treatment groups. None of these results were reported in the publication. These results support the safety of chromium tripicolinate supplementation in humans at doses of up to 1,000 mcg per day trivalent chromium.

In addition to the findings summarized above, Anderson et al. (1997a) conducted a randomized, double-blind, placebo-controlled study to determine the role of supplemental chromium tripicolinate in the control of type 2 diabetes, and found "...no evidence of toxicity..." in 52 diabetic subjects administered 1,000 mcg per day of trivalent chromium (as chromium

tripicolinate) for four months. In this study, individuals being treated for type 2 diabetes (180 males and females) were divided randomly into three groups and supplemented with: 1) placebo, 2) 200 mcg per day trivalent chromium (as chromium tripicolinate), or 3) 1,000 mcg per day trivalent chromium (as chromium tripicolinate), for four months. The results showed that supplemental chromium tripicolinate (especially at 1,000 mcg per day trivalent chromium) had significant beneficial effects on HbA<sub>1c</sub> (a glycolated protein which reflects long-term glycemic control), glucose, insulin, and cholesterol in subjects with type 2 diabetes. In addition, Boyd et al. (1998) studied the effects of 13 weeks of supplementation with either chromium tripicolinate (at a dose of 1,000 mcg per day trivalent chromium) or placebo in a double-blind design using 20 college-aged males and females who were participating in a combined aerobic and resistance exercise program. The results showed that exercise alone or coupled with chromium tripicolinate supplementation did not produce significant changes in strength, lean body mass, HDL, triglyceride, ferritin, or glucose levels. However, chromium tripicolinate supplementation with exercise did decrease total cholesterol, LDL, and insulin levels. Although not specifically evaluated, no adverse effects of chromium supplementation were reported in this study. Finally, Campbell et al. (1999) conducted a second study to evaluate the effects of resistance training and chromium tripicolinate supplementation on skeletal muscle size, strength, and power and whole body composition in older men. In this study, 18 men (age 56 to 69 years) were randomly assigned (double-blind) to receive either 924 mcg per day trivalent chromium (as chromium tripicolinate) or placebo for 12 weeks while participating in a twice weekly resistance training program. The results showed that high-dose chromium tripicolinate supplementation did not enhance muscle size, strength, or power development or lean body mass accretion in older men during a resistance training program, which had significant, independent effects on these measurements. Again, although not specifically evaluated, no adverse effects of chromium supplementation were reported in this study.

Although, in general, clinical efficacy studies have observed no adverse effects of chromium tripicolinate supplementation, a couple of studies were found that did observe some potentially adverse effects of chromium tripicolinate supplementation in humans, including potential effects on iron metabolism. However, whether these effects are truly adverse or not is controversial, as they are believed not to be clinically significant, and have been contradicted by findings from other studies. The studies that have identified potentially adverse effects of chromium tripicolinate supplementation in humans are briefly summarized below.

In a study by Lukaski et al. (1996), hematocrit and hemoglobin concentrations did not change, but urinary iron output and transferrin saturation decreased with trivalent chromium supplementation of 200 mcg per day (as either chromium tripicolinate or chromium chloride) for eight weeks in 36 young men participating in resistance training. The investigators indicated that the decrease in urinary iron output in response to chromium tripicolinate supplementation suggests an adverse effect of trivalent chromium on iron absorption, as the resulting iron

deficiency yielded a reduction in iron excretion in an attempt to restore homeostasis. The investigators also indicated that chromium tripicolinate supplementation may affect iron transport and distribution as shown by the decrease in transferrin saturation with chromium tripicolinate supplementation. Furthermore, Lukaski et al. (1996) cited animal and *in vitro* studies that support an effect of trivalent chromium on iron metabolism. However, the results of Campbell et al. (1997) and Boyd et al. (1998) contradict the findings of Lukaski et al. (1996). As discussed above, Campbell et al. (1997) found that high-dose chromium tripicolinate supplementation (at dose five times higher than those employed by Lukaski et al. (1996)) did not affect serum iron and ferritin concentrations. Therefore, these investigators concluded that chromium tripicolinate supplementation did not significantly affect the changes in iron transport observed during the resistance training period, and there was no indication that the subjects in this study were predisposed to compromised iron status or to iron deficiency anemia. Furthermore, Boyd et al. (1998) stated that their data demonstrate that there is little chance of iron deficiency caused by short-term chromium tripicolinate supplementation and exercise. In their study, after 13 weeks of 1,000 mcg per day trivalent chromium supplementation (as chromium tripicolinate), ferritin levels were well above the 10 ng/mL indicative of iron deficiency anemia.

Finally, in women with gestational diabetes, treatment with 4 mcg per kilogram body weight per day trivalent chromium as chromium tripicolinate (240 mcg per day trivalent chromium) resulted in statistically significant changes in several blood chemistry parameters, but the investigators cited these changes as not being clinically significant (Jovanovic et al. 1999). At a trivalent chromium dose of 8 mcg per kilogram body weight per day (480 mcg per day) as chromium tripicolinate in this same study, changes from baseline were observed in triglyceride, HDL, free thyroxine, and alkaline phosphatase levels. The clinical significance of these changes was not discussed by the investigators.

**b) Case Reports**

Large numbers of people have been supplementing their diets with chromium tripicolinate over the last several years. As indicated previously, in the population of NHANES III respondents ages 2 years and older, approximately 10 percent reported use of a chromium-containing supplement. In addition, an estimated 10 million people regularly ingested chromium tripicolinate in some supplemental form in 1998. Given these large numbers of individuals regularly ingesting chromium tripicolinate, few case reports regarding chromium tripicolinate toxicity were found (see Appendix III). In only three of the case reports was chromium tripicolinate supplementation alleged to be associated with the adverse effects observed. In two of these case reports, the subjects were diagnosed with either renal failure secondary to chromium tripicolinate ingestion or chromium-induced nephrotoxicity. One patient consumed 1,200 to 2,400 mcg per day of trivalent chromium as chromium tripicolinate for 4 to 5 months, in

conjunction with no other over the counter drugs (Cerulli et al. 1998). The second patient was on antihypertensive medication and had consumed 600 mcg per day of trivalent chromium as chromium tripicolinate 5 months prior to the report (Wasser et al. 1997). In a third case report, the subject reported muscle weakness, pain, and bilateral cramping after 4 days of supplementation with chromium tripicolinate at 1,200 mcg per day trivalent chromium. In addition to chromium tripicolinate, the patient was also consuming other dietary supplements, although her diet had not been modified except for the addition of chromium tripicolinate, for over 45 days. The authors concluded that chromium tripicolinate supplementation may have been responsible for the development of rhabdomyolysis (Martin and Fuller 1998). In another case report, the subject reported cognitive, perceptual, and motor changes associated with the intake of 200 mcg per day of trivalent chromium as chromium tripicolinate on three separate occasions. The symptoms started 1 to 1½ hour after ingestion of chromium tripicolinate and lasted for about 2 hours (Huszonek 1993). Side effects reported in patients with dysthymic disorder that were treated with chromium tripicolinate (at 200 to 400 mcg per day trivalent chromium) included transient increases in dreaming and insomnia (McLeod et al. 1999). Chest pain, erythema/flushing, dehydration, agitation, dizziness, headache, oral irritation and unspecified bleeding were also reported by individuals consuming chromium tripicolinate at doses ranging from 100 to 6,000 mcg per day trivalent chromium (Gorman and Herrington 1997).

Additionally, a search of the FDA's Special Nutritionals Adverse Event Monitoring System for chromium and chromium tripicolinate revealed that of the 2,621 adverse events reported in the October 20, 1998 SN/AEMS Web Report, a total of 289 and 214 adverse events included chromium or chromium tripicolinate, respectively, as a product ingredient or as part of the product name. A total of 20 of the adverse event reports involved products that identified chromium tripicolinate as the only product ingredient. A variety of adverse events were reported in association with use of these chromium tripicolinate products (CFSAN 2002).

### **3. Conclusions**

In conclusion, these animal and human studies suggest that a low order of toxicity is associated with ingestion of chromium tripicolinate, as well as with other trivalent chromium compounds. Studies in laboratory animals have produced little evidence of toxicity associated with ingested chromium tripicolinate, or other trivalent chromium compounds. In the most pivotal animal study, at levels much higher than the upper limit of the ESADDI for humans, chromium tripicolinate did produce higher chromium concentrations in the liver and kidneys of rats, suggesting that trivalent chromium may bioaccumulate in these tissues, but no adverse effects were associated with these higher tissue levels (Anderson et al. 1997b). Chromium tripicolinate has a long history of safe use in humans as a nutritional supplement and, other than isolated case reports, there is no consistent evidence of adverse effects following its use in

humans at doses as high as 1,000 mcg per day trivalent chromium. There is some indication, however, that individuals with impaired renal function may have a higher risk of adverse effects following high levels of chromium tripicolinate supplementation. Furthermore, there is some concern with respect to the effect of high levels of chromium tripicolinate supplementation on iron absorption, transport, and distribution; although, the available data on this issue are contradictory.

#### **D. CARCINOGENICITY**

The potential carcinogenicity of chromium tripicolinate has been the subject of significant controversy in the scientific literature (e.g., McCarty 1996, Stearns and Wetterhahn 1996). The source of the controversy relates to the chemical nature of chromium, in that it can exist in several oxidation (or valence) states, resulting in a wide spectrum of reactions that can occur in physiological systems. The oxidation state greatly influences the biological fate of chromium in the body, including its bioavailability and carcinogenic potential. The bioavailability of chromium compounds, as it affects the safety of chromium-containing dietary supplements, was previously discussed. In the present section, the potential carcinogenicity of chromium tripicolinate will be examined by first briefly reviewing the mechanisms by which chromium compounds may induce carcinogenicity (and/or genotoxicity), and then examining the available data on the genotoxicity and carcinogenicity of chromium tripicolinate, as well as other trivalent chromium compounds. These genotoxicity studies are summarized and discussed below.

##### **1. Mechanisms of Chromium Genotoxicity/Carcinogenicity**

The most common valence states of chromium compounds in the environment are chromium (III) or trivalent chromium, and chromium (VI), or hexavalent chromium. Hexavalent chromium is classified as carcinogenic to humans following inhalation exposures, based on evidence from human (epidemiological) studies and experiments in laboratory animals (IARC 1990, EPA 1998b). Trivalent chromium compounds are not classifiable as to their carcinogenicity, based on inadequate evidence in humans and animals (IARC 1990, EPA 1998a). The distinctly different carcinogenic (and toxicological) properties of these two chromium species is largely due to differences in their relative abilities to enter cells and interact with DNA (Cohen et al. 1993). Hexavalent chromium exhibits greater genotoxicity than trivalent chromium in *in vivo* test systems and in mammalian *in vitro* cell assays because hexavalent chromium more readily crosses cell membranes, and is therefore taken up more easily and efficiently than is trivalent chromium. The relatively greater membrane permeability of hexavalent chromium is due to the fact that it can enter cells via facilitated diffusion through non-specific anion channels, a more rapid process than the passive diffusion and phagocytosis mechanisms by which trivalent

chromium is absorbed (ATSDR 2000). It should be noted, however, that uptake of trivalent chromium does occur, which may account for the positive genotoxic results observed in some mammalian test systems.

Once inside the cells, the mechanisms by which chromium compounds induce genotoxicity and/or carcinogenicity are not yet fully understood, which is partially due to the complex valence chemistry of chromium compounds. One proposed mechanism is that hexavalent chromium is reduced intracellularly to trivalent chromium, ultimately resulting in the generation of unstable intermediates (e.g., free radicals) capable of inducing DNA damage (Cohen et al. 1993). Other mechanisms have been proposed to account for the positive genotoxicity/carcinogenicity of chromium compounds, such as DNA interactions resulting in the formation of adducts/complexes that interfere with normal DNA replication and transcription or lead to altered gene expression (ATSDR 2000). Both trivalent and hexavalent chromium compounds have shown the ability to interact with DNA to form such adducts/complexes under *in vitro* conditions (Snow 1991, ATSDR 2000). However, as discussed in the following sections, trivalent chromium compounds generally do not induce genotoxicity under *in vivo* conditions, and have not been shown to be carcinogenic in humans or laboratory animals by any route of exposure.

## 2. *In Vivo Genotoxicity Studies*

### a) *Chromium Tripicolinate*

In an *in vivo* chromosomal aberration study in animals (Greenberg et al. 1999), chromium tripicolinate was administered to Sprague-Dawley rats (males and females) by gavage at doses ranging from 33 to 2,000 mg/kg. Animals were sacrificed either 18 or 42 hours post-dose, and the femur excised so that bone marrow cells could be harvested for chromosomal analysis. The results showed that the percentage of cells with damaged chromosomes in the dosed groups were not elevated relative to controls. These results led the investigators to conclude, "...chromium picolinate did not induce chromosomal damage in either males or females after 18 or 42 hours of dosing."

In a human genotoxicity study, Kato et al. (1998) assessed the potential for oxidative damage that may be caused by chromium tripicolinate supplementation by measuring antibodies (using an enzyme-linked immunosorbent assay) recognizing 5-hydroxymethyl-2-deoxyuridine ("HMdU"), which is considered to be a reliable and sensitive biomarker for oxidized DNA damage. To conduct this study, 10 human volunteers ingested chromium tripicolinate supplements at a dose of 400 mcg per day (expressed as trivalent chromium) for 8 weeks. The results showed that urinary chromium levels increased significantly with chromium tripicolinate supplementation relative to baseline levels. However, anti-HMdU titers, expressed either as absolute titers or as percent of baseline, were no different from baseline after supplementation



with chromium tripicolinate. These results led the investigators to conclude that chromium tripicolinate supplementation does not increase oxidative DNA damage in humans under *in vivo* conditions.

**b) Other Trivalent Chromium Compounds**

Trivalent chromium compounds have produced negative results in *in vivo* assays for chromosomal aberrations, DNA or DNA-protein crosslinks and strand breaks, and micronucleated polychromatic erythrocytes (an indicator of chromosomal damage) (ATSDR 2000). These findings are consistent with the fact that the IOM (2001) found that *in vivo* genotoxicity assays for trivalent chromium have been negative.

**3. In Vitro Genotoxicity Studies**

**a) Chromium Tripicolinate**

Chromium tripicolinate did not induce mutations in the Ames bacterial mutation assay with and without the presence of a metabolic activation system (Juturu and Komorowski 2002). It should be noted, however, that the test article, "...was in suspension with particles present..." indicating that the poor water solubility of the test material likely limited the amount of chromium tripicolinate that contacted the bacteria.

There are two reports on the *in vitro* genotoxicity of chromium tripicolinate. Stearns et al. (1995a) examined two different chelated forms of trivalent chromium, chromium tripicolinate and chromium nicotinate, for their ability to induce chromosomal aberrations in Chinese Hamster Ovary ("CHO") cells. The cells were treated with aqueous solutions of chromium tripicolinate, chromium nicotinate, chromium chloride, picolinic acid or nicotinic acid, or with particulate suspensions of chromium tripicolinate or chromium nicotinate in acetone. Treatments were conducted for 24 hours at concentrations ranging from 10 to 200  $\mu$ l. Chromium tripicolinate induced chromosomal aberrations in both treatments (i.e., solution and suspension) in a dose-dependent manner, as did picolinic acid. Neither chromium chloride nor chromium nicotinate induced chromosomal aberrations at doses equivalent to chromium tripicolinate.

Speetjans et al. (1999) studied the ability of chromium tripicolinate to promote DNA cleavage under *in vitro* conditions, but in a subcellular system. Aliquots of plasmid DNA were treated with chromium tripicolinate in aqueous solution employing timed assays of 5 to 180 minutes. Concentrations of chromium tripicolinate used ranged from 0.12  $\mu$ M to 120  $\mu$ M. For some assays, ascorbate or dithiothreitol were added as reducing agents. The conversion of supercoiled plasmid DNA to the circular nicked form was observed, and appeared to be time and concentration dependent. As follow-up, additional assays employing argon bubbles, radical traps, SOD, or hydrogen peroxide were conducted. The results of these assays led the

investigators to conclude, "Cr(pic)<sub>3</sub> in the presence of reductants and air is capable of generating hydroxyl radicals, which in turn can cleave supercoiled DNA."

**b) Other Trivalent Chromium Compounds**

Numerous trivalent chromium compounds have been shown to be genotoxic in *in vitro* assays (EPA 1998a, ATSDR 2000). Mostly negative results have been reported in mammalian cell assays, although positive findings were obtained in CHO cells, mouse fetal cells, and human cell lines. However, in the positive studies, the genotoxic potency of trivalent chromium compounds was several orders of magnitude lower than that of hexavalent chromium compounds tested in the same systems.

In subcellular test systems, where the relative inability of trivalent chromium to cross cell membranes is not a consideration, trivalent chromium compounds have demonstrated the ability to interact with DNA, in some cases with a potency greater than that for hexavalent chromium compounds (Snow 1991, EPA 1998a, ATSDR 2000). However, there is conflicting information with regard to the ability of trivalent chromium to interact with DNA (EPA 1998a).

Additionally, *in vitro* data exist that indicate that trivalent chromium, once absorbed and having crossed cell membranes, can be reduced by naturally occurring reductants in cells (e.g., cysteine and NADH), which, in the presence of peroxides, may lead to the formation of free radicals that could be potentially genotoxic (ATSDR 2000). However, no such events have ever been observed *in vivo*.

**4. Carcinogenicity Studies**

No animal or human studies on the potential carcinogenicity of chromium tripicolinate were identified in the published scientific literature. However, carcinogenicity studies have been conducted on two other trivalent chromium compounds. In one of these studies, as previously discussed, Ivankovic and Preussmann (1975) conducted long-term feeding experiments of chromic oxide in rats to assess its carcinogenic potential. Groups of 60 male and 60 female rats were fed chromic oxide baked in bread at dietary concentrations of 0, 1, 2, or 5 percent, 5 days per week for 840 days. The average total amounts of ingested chromic oxide were reported as 360, 720, and 1,800 g per kg body weight for the 1, 2, and 5 percent treatment groups, respectively. The highest dose of chromic oxide corresponds to an average daily trivalent chromium dose of about 1,500 mg/kg/day. No effects due to chromic oxide ingestion were observed at any dose level in this study.

In another carcinogenicity bioassay, Schroeder et al. (1965) exposed 54 male and 54 female Swiss mice to drinking water that contained 5 ppm trivalent chromium (as chromium acetate) for a lifetime (corresponding to a dose of 0.46 mg/kg/day, expressed as trivalent chromium). No increase in the incidence of tumors was seen in the treated animals with respect to controls. Similar results were obtained by these same investigators for Long-Evans rats. The

dose of trivalent chromium used in this study was 2,000- to 10,000-fold lower than the dose in the Ivankovic and Preussmann (1975) study. However, as previously discussed, chromium acetate is an organic salt of trivalent chromium (like chromium tripicolinate), and is more bioavailable than chromic oxide, which is an inorganic salt of trivalent chromium.

## **5. Conclusions**

In summary, studies show that chromium tripicolinate can be mutagenic *in vitro* under certain test conditions, but it has not been shown to be mutagenic *in vivo*. Clearly, given the chromosomal aberrations observed by Stearns et al. (1995a), chromium tripicolinate, and other trivalent chromium compounds, can be genotoxic under certain *in vitro* conditions. However, the lack of effects seen in the *in vivo* studies by Kato et al. (1998) in humans and Greenberg et al. (1999) in animals provides evidence that chromium tripicolinate is not genotoxic under exposure conditions that are more realistic than the conditions employed in the *in vitro* assays. Consistent with the negative *in vivo* genotoxicity data, there is no evidence of carcinogenicity in humans or animals as a result of ingesting trivalent chromium.

## **E. DERIVATION OF AN ADI FOR TRIVALENT CHROMIUM**

### **1. Safety Considerations**

Trivalent chromium is an essential element required for normal carbohydrate, lipid, and protein metabolism in humans and animals (Anderson 1998). Chromium deficiency in humans is associated with diseases such as mature-onset diabetes, cardiovascular disease, and nervous system disorders (EPA 1998a), and may cause such adverse effects as fasting hyperglycemia, impaired glucose tolerance, and elevated plasma insulin, serum total cholesterol and triglycerides (Campbell et al. 1997). Due to its essentiality, the NRC has recommended an ESADDI for chromium of 50 to 200 mcg for adults (NRC 1989), and the FDA has established an RDI for chromium of 120 mcg/day. By way of comparison, the estimated normal dietary intake of chromium is 25 mcg/day for women and 33 mcg/day for men, both values below the minimum ESADDI (Anderson and Kozlovsky 1985). More recently, the IOM (2001) has set the AI for trivalent chromium at 35 µg/day for young men and 25 µg/day for young women. These AIs provide estimates of current chromium intake by the U.S. population.

As with any essential nutrient, at very low levels of chromium intake, chromium deficiency may occur, which can result in a number of adverse health outcomes that are associated with disruptions in normal carbohydrate, lipid, and protein metabolism. As dietary chromium intake increases, chromium deficiency is overcome and normal metabolism is restored in the body, resulting in a reduction in the adverse effects caused by chromium deficiency. Further intake of chromium may result in optimal functioning of the body's metabolic processes. However, if dietary intake of chromium continues to increase, excess chromium may accumulate

in the body and its tissues; the long-term health consequences of which currently have not been evaluated in humans. Thus, a critical consideration in evaluating the safety of chromium tripicolinate supplementation is determining the exposures at which chromium levels in the body could potentially increase beyond that needed for normal metabolism, which then may be associated with adverse effects.

**a) Bioavailability and Bioaccumulation**

The bioavailability of trivalent chromium is a function of its ability to be absorbed into the body, and may be the single most important factor in determining its toxicity (O'Flaherty 1996). Trivalent chromium compounds in foods and inorganic chromium salts, such as chromium chloride, are poorly absorbed following ingestion (i.e., on the order of 0.5 to 2 percent). Chromium tripicolinate, an organic salt of trivalent chromium, is absorbed at somewhat higher levels (i.e., ~ 3 percent) compared to other trivalent chromium compounds.

Once absorbed, chromium tripicolinate (as well as other trivalent chromium compounds) exhibits a very limited ability to cross cellular membranes and gain access to cellular DNA, particularly with respect to the known inhalation carcinogen, hexavalent chromium. Therefore, this brings into question the relevancy of *in vitro* assays for evaluating the genotoxic and/or carcinogenic potential of trivalent chromium, because in these assays, cells or DNA are exposed directly to relatively high concentrations of trivalent chromium. This is in contrast to *in vivo* assays, where the conditions of exposure more closely mimic what actually occurs when trivalent chromium compounds are ingested. Further evidence that these *in vitro* assays may not be very good predictors of the genotoxic and/or carcinogenic potential of trivalent chromium compounds is that although (as noted above) numerous trivalent chromium compounds have yielded positive findings in *in vitro* mutagenicity assays, trivalent chromium has never been shown to be carcinogenic by any route of exposure in *in vivo* studies in humans or animals.

Although the concentrations of trivalent chromium that induced genotoxicity in the *in vitro* studies were extremely large relative to dietary intake levels, Stearns et al. (1995a,b) have hypothesized that the bioaccumulation of chromium following long-term ingestion of nutritional supplements could result in tissue levels that approach these genotoxic levels. In support of this contention, there is evidence that chromium bioaccumulates in tissues from both animal studies and human pharmacokinetic models. However, there are not sufficient data to determine whether these tissue levels will continue to rise with long-term use of chromium-containing supplements, at what rate these concentrations will increase, and what the threshold concentration for genotoxicity or other adverse effects is *in vivo*, or even if such a threshold exists.

**b) Non-Carcinogenic Toxicity**

The available data on the toxicity of trivalent chromium reveals a very low order of oral toxicity. In fact, there is little evidence, other than isolated case reports, of any toxic effects following oral exposures in humans or laboratory animals. However, trivalent chromium has been found to accumulate in the liver and kidneys of rats exposed to very high levels of chromium tripicolinate and chromium chloride with concentrations increasing linearly with dose over time (Anderson et al. 1997b). While this bioaccumulation resulted in no observed toxicological effects, it is a finding worthy of consideration when determining what would be considered to be an appropriate acceptable intake level for trivalent chromium when ingested as a direct food ingredient.

**c) Carcinogenicity**

The concern regarding the potential carcinogenicity of chromium tripicolinate is primarily based on positive findings in *in vitro* mutagenicity assays, which is entirely consistent with existing studies that have evaluated the mutagenicity of other trivalent chromium compounds *in vitro*. However, in contrast to the *in vitro* results, *in vivo* genotoxicity studies employing both chromium tripicolinate and other trivalent chromium compounds have generally yielded negative results. Consistent with the *in vivo* genotoxicity data, there is no evidence of carcinogenicity in humans or animals following ingestion of trivalent chromium. In fact, trivalent chromium has never been shown to be carcinogenic by any route of exposure in *in vivo* studies in humans or animals.

**d) Conclusions**

The growing sales and consumption of trivalent chromium-containing compounds as nutritional supplements has resulted in heightened awareness of several issues that relate to the safety of these supplements. For example, the chemical form of chromium that occurs in the diet (and the most abundant form in the environment) is trivalent chromium, which is also referred to as chromium in the 3+ oxidation state, or chromium (III). This is an important distinction, as hexavalent chromium, which is chromium in the 6+ oxidation state and designated as chromium (VI), is classified as carcinogenic to humans via inhalation (IARC 1990, EPA 1998b). As a consequence of the carcinogenicity of hexavalent chromium (at least via inhalation), concern has been expressed regarding the potential carcinogenic effects of ingested trivalent chromium. Based on our review of the available genotoxicity and carcinogenicity data on chromium tripicolinate and other trivalent chromium compounds, there is no evidence to suggest that ingestion of chromium tripicolinate as a direct food additive at the levels proposed herein would result in an increased cancer risk in the human population.

Another issue that has drawn attention with respect to the safety of trivalent chromium-containing supplements is the potential for trivalent chromium to accumulate in the body as a

result of chronic ingestion, such as might result from the long-term use of chromium tripicolinate as a dietary supplement. Although trivalent chromium is a normal constituent of the diet, some nutritional supplements contain trivalent chromium as part of a complex that results in a greater percentage of trivalent chromium absorption into the body following ingestion than that which occurs from the diet. Chromium tripicolinate, in which trivalent chromium is complexed with picolinic acid, is a popular trivalent chromium supplement primarily *because* the picolinic acid complex results in the more efficient uptake of trivalent chromium from the GI tract into the body. Therefore, the concern relates to whether the addition of trivalent chromium via nutritional supplements to the normal dietary intake of trivalent chromium, combined with the increased efficiency of absorption of trivalent chromium from these supplements, will result in the bioaccumulation of trivalent chromium in the body to levels that may exceed some threshold of toxicity. Given the very low order of toxicity associated with ingested trivalent chromium compounds, as demonstrated from both laboratory animal and human exposures, we believe the risk of exceeding the toxicity threshold for chromium tripicolinate when ingested as a direct food additive to be very low, if indeed, such a threshold even exists.

## **2. *Derivation of an Acceptable Daily Intake (ADI)***

An ADI represents the maximum amount of a substance that can be safely consumed on a daily basis for a lifetime (FDA 1993). The FDA has specified that an ADI is usually established by application of a safety factor of at least 100 to the highest dose at which no effects were observed in a chronic study in the most sensitive animal species. When multiple animal studies have been performed that have identified either a NOEL or a NOAEL, the lowest NOEL or NOAEL is selected from these studies. The FDA states that except where evidence is submitted that justifies use of a different safety factor, a safety factor of 100 to 1 is used when applying chronic animal experimentation data to man; that is, tolerance for the use of a human food ingredient will not exceed 1/100th of the maximum amount demonstrated to be without harm to experimental animals (FDA 1993). However, the FDA recommends that a safety factor of 1,000 be used when calculating an ADI based on a NOAEL from a subchronic animal study.

Therefore, in order to assess the overall safety of ingested trivalent chromium resulting from the proposed uses of Chromax® Chromium Picolinate, one must derive an estimated ADI for trivalent chromium (i.e., an estimate of a safe exposure over a lifetime likely to be without adverse effects). The derivation of an estimated ADI for trivalent chromium is outlined below.

Four candidate studies are available for consideration for use in deriving an ADI for trivalent chromium. That is, each of these studies identified either a NOEL or a NOAEL for trivalent chromium. The first study is the subchronic animal study by Anderson et al. (1997b), in which chromium tripicolinate was fed to rats for 20 weeks. Although this is a subchronic study, the investigators employed chromium tripicolinate, the specific compound of interest for this GRAS determination. In this study, rats exhibited no signs of toxicity even at the highest dose

tested, which was estimated to be 15 mg/kg/day of trivalent chromium. Thus, this dose represents a NOAEL, but because this NOAEL occurred at the highest dose tested, the true NOAEL could be equal to or greater than 15 mg/kg/day.

The three remaining animal studies that could be used for ADI derivation are chronic studies that employed trivalent chromium compounds other than chromium tripicollinate. In the first study, MacKenzie et al. (1958), who administered 25 ppm chromium chloride to rats in drinking water for 12 months and noted no change in body weight, macroscopic or microscopic pathology, or clinical chemistry variables, identified a NOEL (or NOAEL) at a dose of 0.82 mg/kg/day trivalent chromium, according to the EPA (1998a). Again, because this NOEL (or NOAEL) occurred at the highest (and only) dose tested, the true NOEL (or NOAEL) could be equal to or greater than 0.82 mg/kg/day.

In another chronic exposure study, Schroeder et al. (1965) exposed 54 male and 54 female Swiss mice to drinking water that contained 5 ppm trivalent chromium (as chromium acetate) for a lifetime (corresponding to a dose of 0.46 mg/kg/day, expressed as trivalent chromium). No increase in the incidence of tumors was seen in the treated animals with respect to controls. Similar results were obtained by these same investigators for Long-Evans rats. This study then identified a NOAEL for trivalent chromium of 0.46 mg/kg/day. As in the other two studies, this NOAEL occurred at the highest dose tested; thus, the actual NOAEL could be equal to or greater than 0.46 mg/kg/day.

Finally, as discussed previously, Ivankovic and Preussmann (1975) conducted lifetime feeding experiments of chromic oxide in rats to assess its carcinogenic potential. This study serves as the basis for EPA's RfD for the insoluble salts of trivalent chromium. The RfD is an estimate of a daily exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime. Thus, an RfD is comparable to an ADI. The RfD was derived by adjusting the 5-day per week daily dose for the highest dose tested in the chromic oxide study to a 7-day per week daily dose (or about 1,500 mg/kg/day), and then applying a 1,000-fold safety factor to this dose. This safety factor accounts for the variability in the toxic response between rats and humans, variability in the toxic response among humans (i.e., sensitive subpopulations), and for lack of data on the reproductive effects of this compound. Application of the 1,000-fold safety factor to the daily dose results in an RfD for the insoluble salts of trivalent chromium of 1.5 mg/kg/day (EPA 1998a). Application of an additional safety factor of 10 to account for the lack of data on reproductive effects is partially in response to reproductive effects (decreased fertility) observed in mice following high-dose exposures to chromium chloride in drinking water of up to 5,000 mg/L (Elbetieha and Al-Hamood 1997, as cited in EPA 1998a). However, the EPA believes there were significant deficiencies in this study, precluding its use in a risk assessment for trivalent chromium compounds (EPA 1998a).

In regards to the use of the RfD as an ADI, it is important to note that the RfD adopted by EPA "is limited to metallic chromium (III) of *insoluble* salts." Examples of insoluble salts cited

by the EPA include chromic (III) oxide ( $\text{Cr}_2\text{O}_3$ ) and chromium (III) sulfate ( $\text{Cr}_2[\text{SO}_4]_3$ )” (EPA 1998c). The available bioavailability data clearly show that differences in the chromium complex ingested can have marked effects on the absorption, and therefore, the toxicity of trivalent chromium. The amount of trivalent chromium available in the body following chromic oxide exposure is expected to be significantly less than after equivalent exposure to chromium tripicolinate or other organic salts of trivalent chromium, such as chromium acetate. Juturu et al. (2002) demonstrated this fact by showing that chromic oxide exhibited negligible absorption from the GI tract in rats, while chromium acetate yielded an absorption of around 1 to 2 percent. Therefore, this suggests that the oral RfD for trivalent chromium established by the EPA based on Ivankovic and Preussman (1975) should not be used as the basis for calculating the ADI for trivalent chromium. Thus, one of the other three studies summarized above would be best for deriving an ADI estimate for trivalent chromium.

As indicated above, the FDA (1993) states that when multiple animal studies have been performed that have identified either a NOEL or a NOAEL, the lowest NOEL or NOAEL is selected from these studies for purposes of ADI derivation. This suggests that Schroeder et al. (1965), with a NOAEL of 0.46 mg/kg/day trivalent chromium, should be selected for ADI derivation. However, in all of the studies, the dose at which the NOAEL or NOEL was identified was the highest dose administered, and thus an upper bound on the NOAEL (or NOEL) was not established in any of these studies. Therefore, it is useful to evaluate all of the candidate studies, except for Ivankovic and Preussman (1975) for the reasons cited above, to help in “bracketing” the ADI for trivalent chromium.

In the study by Schroeder et al. (1965), the NOAEL of 0.46 mg/kg/day identified from this chronic drinking water study in rats employing chromium acetate was divided by a safety factor of 100 to yield an acceptable intake of 4.6 mcg/kg/day. This acceptable intake was then multiplied by 60 kg, the assumed body weight of a human, to yield an estimated ADI of equal to or greater than 276 mcg/day trivalent chromium, when administered as chromium acetate.

In the study by MacKenzie et al. (1958), the NOEL (or NOAEL) of 0.82 mg/kg/day trivalent chromium identified from this chronic drinking water study in rats employing chromium chloride was divided by a safety factor of 100 to yield an acceptable intake of 8.2 mcg/kg/day. This acceptable intake was then multiplied by 60 kg, the assumed body weight of a human, to yield an estimated ADI of equal to or greater than 492 mcg/day trivalent chromium, when administered as chromium chloride.

In the study by Anderson et al. (1997b), the NOAEL of 15 mg/kg/day trivalent chromium identified from this subchronic feeding study in rats employing chromium tripicolinate was divided by a safety factor of 1,000 to yield an acceptable intake of 15 mcg/kg/day. This safety factor is 10-fold larger than the 100-fold factor typically used by FDA to account for the subchronic nature of the Anderson et al. (1997b) study, and thus is consistent with the FDA’s guidance in employing subchronic studies to derive ADIs. This acceptable intake was then



multiplied by 60 kg, the assumed body weight of a human, to yield an estimated ADI of equal to or greater than 900 mcg/day trivalent chromium, when administered as chromium tripicolinate.

The three estimated ADIs for trivalent chromium derived above are equal to or greater than 276 mcg/day (when administered as chromium acetate), 492 mcg/day (when administered as chromium chloride), and 900 mcg/day (when administered as chromium tripicolinate), and are based on the following three NOAELs or NOELs, 0.46 mg/kg/day, 0.82 mg/kg/day, and 15 mg/kg/day, respectively. The NOAEL from the subchronic study (15 mg/kg/day) is approximately 20 to 30 times higher than the other two NOAELs or NOELs. The absorption of trivalent chromium from the three chromium compounds on which these NOAELs or NOELs are based (i.e., chromium acetate, chromium chloride, and chromium tripicolinate, respectively) is similar enough (i.e., in the range of 1 to 3 percent) that this factor alone cannot explain the differences in these values. Most likely, the two NOAELs or NOELs derived from the chronic studies (0.46 mg/kg/day and 0.82 mg/kg/day) are too low because of the very low doses employed in these studies, and the fact that a subchronic study exists that identified a higher NOAEL. Furthermore, the NOAEL from the subchronic study is also probably too low an estimate because it occurred at the highest dose administered, and thus no upper bound on the NOAEL was established. Therefore, ENVIRON believes that the true chronic NOAEL lies somewhere between 0.82 mg/kg/day and 15 mg/kg/day, and most likely will yield an ADI greater than 900 mcg/day trivalent chromium. By way of example, if a future chronic study identifies a NOAEL of 2 mg/kg/day, which certainly is plausible given the current data, the estimated ADI from this study would be 1,200 mcg/day trivalent chromium, quite a bit higher than the estimated ADI of 900 mcg/day derived from the current subchronic study employing chromium tripicolinate.

In addition, an evaluation of the available clinical efficacy studies employing chromium tripicolinate suggests that this compound has a long history of safe use in humans as a nutritional supplement and, other than isolated case reports, there is no consistent evidence of adverse effects following its use in humans at doses as high as 1,000 mcg per day trivalent chromium. This upper safe limit in humans of 1,000 mcg per day agrees quite favorably with the 900 mcg/day ADI derived from a subchronic animal study, lending further support to the validity of this ADI.

## **VI. SAFETY ASSESSMENT AND GRAS DETERMINATION**

### **A. INTRODUCTION**

This chapter presents an assessment that demonstrates that Chromax® Chromium Picolinate is safe, and is GRAS, under the FDCA for use as a nutrient supplement at the specified maximum use level (i.e., 2.4 mg/serving) in nutritional beverages and bars. This safety assessment/GRAS determination entails a two-step process. In step one, the safety of Chromax® Chromium Picolinate under its intended conditions of use is demonstrated. Safety is established by comparing the EDI of trivalent chromium resulting from Chromax® Chromium Picolinate consumption under its intended conditions of use with the ADI for trivalent chromium derived from human and/or animal studies. A substance directly added to food is considered safe for its intended use if the EDI of the substance under its intended conditions of use is less than, or approximates, its ADI (FDA 1993). In the second step, Chromax® Chromium Picolinate is determined to be GRAS by demonstrating that the safety of this substance under its intended conditions of use is generally recognized among qualified scientific experts.

The regulatory framework for establishing whether a substance is GRAS, in accordance with Section 201(s) of the FDCA, is set forth under 21 CFR §170.30. This regulation states that general recognition of safety may be based on the view of experts qualified by scientific training and experience to evaluate the safety of substances directly or indirectly added to food. A GRAS determination may be made either: 1) through scientific procedures under §170.30(b); or 2) through experience based on common use in food, in the case of a substance used in food prior to January 1, 1958, under §170.30(c). This GRAS determination employs scientific procedures established under §170.30(b).

A scientific procedures GRAS determination requires the same quantity and quality of scientific evidence as is needed to obtain approval of the substance as a food additive. In addition to requiring scientific evidence of safety, a GRAS determination also requires that this scientific evidence of safety be generally known and accepted among qualified scientific experts. This "common knowledge" element of a GRAS determination consists of two components: 1) the data and information relied upon to establish the scientific element of safety must be generally available; and 2) there must be a basis to conclude that there is a consensus among qualified experts about the safety of the substance for its intended use.

The criteria outlined above for a scientific procedures GRAS determination are applied below in an analysis of whether Chromax® Chromium Picolinate, employed as a nutrient supplement, is safe, and is GRAS, at a maximum use level of 2.4 mg/serving in nutritional beverages and bars. If Chromax® Chromium Picolinate is determined to be GRAS for its

intended use, it is permitted to be used for that purpose because it is not (by definition) a food additive, and therefore does not require promulgation of a food additive regulation under 21 CFR prior to being marketed and sold in the U.S.

***B. SAFETY OF CHROMAX® CHROMIUM PICOLINATE***

A scientific procedures GRAS determination requires that information about the substance establish that the intended use of the substance is safe. The FDA has defined “safe” or “safety” for food additives under 21 CFR §170.3(i) as “a reasonable certainty in the minds of competent scientists that the substance is not harmful under its intended conditions of use.” This same regulation specifies that three factors must be considered in determining safety. These three factors are:

- The probable consumption of the substance and of any substance formed in or on food because of its use (i.e., the EDI);
- The cumulative effect of the substance in the diet, taking into account any chemically- or pharmacologically-related substance or substances in such diet; and
- Safety factors which, in the opinion of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients, are generally recognized as appropriate.

After consideration of these factors, an EDI and an ADI are typically derived for the substance. An EDI for the substance is derived based on the probable human consumption of the substance and of any substance formed in or on food because of its use. The ADI represents the maximum amount of the substance that has been shown to be safe for consumption by humans on a daily basis for a lifetime. Finally, the EDI for a substance is compared against its ADI. As long as the EDI is less than (or approximates) the ADI, the substance can be considered safe for its intended use (FDA 1993).

***1. EDI of Trivalent Chromium***

As indicated above, 21 CFR §170.3(i) requires that, in evaluating the safety of the proposed use of a new food additive, the probable consumption (i.e., the EDI) of the substance and of any substance formed in or on food because of its use be considered, as well as the cumulative effect of the substance in the diet, taking into account any chemically- or pharmacologically-related substance or substances in such diet. Thus, because a scientific procedures GRAS determination requires the same quantity and quality of evidence as is required to obtain approval of the substance as a new food additive, a scientific procedures GRAS determination must also consider the probable consumption and cumulative effect of the substance in the diet. The EDI derivation described below provides a conservative estimate of

the intake of trivalent chromium under the intended conditions of use of Chromax® Chromium Picolinate.

As described in Chapter III, using food intake data reported in the USDA's 1994-96, 1998 CSFII (USDA 2000), ENVIRON estimated exposure to trivalent chromium that would result from the proposed uses of Chromax® Chromium Picolinate. The estimated mean and 90<sup>th</sup> percentile intake of trivalent chromium resulting from consumption of Chromax® Chromium Picolinate from all proposed use categories by users aged 2 years and older is 304 mcg/day and 545 mcg/day, respectively, assuming 2.4 mg of Chromax® Chromium Picolinate per serving or 300 mcg of trivalent chromium per serving.

## **2. *ADI for Trivalent Chromium***

Based on the review presented in Chapter V, ENVIRON derived an estimated ADI for trivalent chromium of equal to or greater than 900 mcg/day, when administered as chromium tripicolinate. This ADI was derived from a subchronic animal study by Anderson et al. (1997b), and supported by safety data from clinical efficacy studies employing chromium tripicolinate at doses as high as 1,000 mcg/day trivalent chromium. In Anderson et al (1997b), a NOAEL for chromium tripicolinate via ingestion was established at a trivalent chromium dose of 15 mg/kg/day. Then, applying a safety factor of 1,000 and multiplying by a 60-kg body weight, an estimated ADI for trivalent chromium of equal to or greater than 900 mcg/day was derived. Two other ADI estimates were derived from chronic animal studies of trivalent chromium-containing compounds other than chromium tripicolinate that were administered at very low doses. These ADIs for trivalent chromium were equal to or greater than 276 mcg/day (when administered as chromium acetate) and equal to or greater than 492 mcg/day (when administered as chromium chloride), but likely represent underestimates of the true ADI for the reasons outlined in Chapter V of this document.

## **3. *Establishing the Safety of Chromax® Chromium Picolinate***

As a result of the proposed uses and maximum use level of Chromax® Chromium Picolinate described in Chapter III, the EDI of trivalent chromium from Chromax® Chromium Picolinate is estimated to be no more than 545 mcg per person per day. To this EDI of trivalent chromium from Chromax® Chromium Picolinate added to food, the potential contribution of trivalent chromium from dietary sources must be added. ENVIRON has estimated that the trivalent chromium intake from these dietary sources could contribute as much as 55 mcg per person per day to the EDI of 545 mcg per person per day resulting from the consumption of food containing Chromax® Chromium Picolinate, yielding a cumulative EDI for trivalent chromium of 600 mcg per person per day.

This cumulative EDI of trivalent chromium of 600 mcg per person per day, due to the addition of Chromax® Chromium Picolinate to the specified foods at the proposed maximum

use level and intake from dietary sources, does not exceed the estimated ADI for trivalent chromium of equal to or greater than 900 mcg per person per day established by ENVIRON's review of published toxicity studies of trivalent chromium compounds, including chromium tripicolinate. Thus, Chromax® Chromium Picolinate for the proposed uses at the maximum proposed use level can be considered safe.

**C. GENERAL RECOGNITION OF THE SAFETY OF CHROMAX® CHROMIUM PICOLINATE**

The proposed uses and maximum use level of Chromax® Chromium Picolinate in food described in this document have been determined to be safe through scientific procedures set forth under 21 CFR §170.30(b). This safety was established by first estimating potential human exposure to trivalent chromium from the intended uses of Chromax® Chromium Picolinate in food to be no more than 545 mcg per person per day. Next, ENVIRON's conclusion that an intake of trivalent chromium of more than 900 mcg per person per day is safe was employed to establish an ADI for trivalent chromium from Chromax® Chromium Picolinate of equal to or greater than 900 mcg/day. Then, the probable human exposure, or EDI, for trivalent chromium, resulting from the proposed uses and maximum use level of Chromax® Chromium Picolinate in food, was compared to the ADI for trivalent chromium. Because the EDI is less than (or approximates) the ADI, the substance (i.e., Chromax® Chromium Picolinate) can be considered safe for its intended uses at the maximum use level. Finally, because this safety assessment satisfies the common knowledge requirement of a GRAS determination, this intended use can also be considered GRAS.

Determination of the safety and GRAS status of Chromax® Chromium Picolinate for direct addition to foods under its intended conditions of use at the maximum use level has been made through the deliberations of Richard A. Anderson, Ph.D., Joseph F. Borzelleca, Ph.D., and Walter H. Glinsmann, M.D. These individuals are qualified by scientific training and experience to evaluate the safety of food and food ingredients. These experts have carefully reviewed and evaluated the publicly available information summarized in this document, including the potential human exposure to trivalent chromium resulting from the intended use of Chromax® Chromium Picolinate as a nutrient supplement in food, and have concluded:

No evidence exists in the available information on trivalent chromium that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public health when trivalent chromium is used at levels that are now current or that might reasonably be expected from the proposed uses of Chromax® Chromium Picolinate as a nutrient supplement in food.

It is their opinion that other qualified and competent scientists reviewing the same publicly available data would reach the same scientific conclusion. Therefore, Chromax® Chromium Picolinate is safe, and is also GRAS, for the proposed uses at the maximum proposed

use level described in this document. Because Chromax® Chromium Picolinate is GRAS under its intended conditions of use, it is excluded from the definition of a food additive, and thus may be marketed and sold for the proposed uses described herein in the U.S. without the promulgation of a food additive regulation under 21 CFR.

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**APPENDIX I**

**FOOD CODES FOR NUTRITIONAL READY-TO-DRINK  
BEVERAGES, BEVERAGE MIXES, AND BARS IN THE  
1994-1996, 1998 CONTINUING SURVEY OF  
FOOD INTAKES BY INDIVIDUALS (USDA 2000)**

**APPENDIX I:**

**Food Codes for Nutritional Ready-To-Drink Beverages, Beverage Mixes, and Bars from the 1994-96, 1998 Continuing Survey of Food Intakes by Individuals (USDA 2000)**

Food Category <sup>a</sup>	Food Code	Food Name	Serving Size (g) <sup>b</sup>
RTD Bev	11611000	INSTANT BREAKFAST, FLUID, CANNED	313
RTD Bev	11621000	DIET BEVERAGE, LIQUID, CANNED	313
RTD Bev	11623000	MEAL SUPPLEMENT / REPLACEMENT, PREPARED, RTD	341
RTD Bev	11631000	HIGH CALORIE BEV, CANNED OR POWDERED, RECONSTITUTED	250
RTD Bev	11641000	MEAL REPLACEMENT, MILK BASED, HIGH PROTEIN, LIQUID	256
RTD Bev	41440010	MEAL REPLACEMENT/SUPPLEMENT, LIQUID, HI PROTEIN	252
RTD Bev	41440020	ENSURE W/ FIBER, LIQUID	248
RTD Bev	41440050	ENSURE PLUS LIQUID NUTRITION	252
RTD Bev	41440100	MEAL REPLACEMENT, LIQUID, SOY-BASE (ISOCAL, OSMOLITE)	247
Bev mix	11612000	INSTANT BREAKFAST, POWDER, MILK ADDED	279
Bev mix	11613000	INSTANT BFAST, PWDR, SWT W/ LO CAL SWT, MILK ADDED	247
Bev mix	11830800	INSTANT BREAKFAST POWDER, NOT RECONSTITUTED	37
Bev mix	11830810	INSTANT BFAST, PWDR, SWT W/ LO CAL SWT, NOT RECONSTITUTED	20
Bev mix	11830850	HIGH CALORIE MILK BEVERAGE, POWDER, NOT RECONSTITUTED	30
Bev mix	11830900	PROTEIN SUPPLEMENT, MILK BASED, DRY POWDER	43
Bev mix	11830940	MEAL REPLACEMENT, PROTEIN, MILK BASED, FRUIT JUICE MIX	31
Bev mix	11830970	MEAL REPLACEMENT, PROTEIN TYPE, MILK-BASE, POWDER	58
Bev mix	11830990	NUTRIENT SUPP, MILK-BASE, POWDER (INCL SUSTAGEN)	31
Bev mix	11831500	NUTRIENT SUPPLEMENT, MILK-BASE, HIGH PROT, NOT RECONSTITUTED	27
Bev mix	11832000	MEAL REPLACEMENT, MILK-&SOY-BASE, POWDER, NOT RECONSTITUTED	44
Bev mix	11835100	MEAL REPLACEMENT, POSITRIM DRINK MIX, DRY POWDER	43
Bev mix	11835200	LOSE-IT (NANCI), MEAL REPLACEMENT, POWDER	21
Bev mix	41430000	PROTEIN POWDER, NFS	28
Bev mix	41430010	PROTEIN SUPPLEMENT, POWDERED	28
Bev mix	41430100	FORMULATED DIET MEAL, POWDER, SOY PROTEIN ISOLATE	28.35
Bev mix	41430200	MEAL REPLACE / SUPP, SOY-MILK-BASE, POWD, WATER ADDED	218
Bev mix	41430310	PROTEIN DIET POWDER W/ SOY & CASEIN	20
Bar	41435110	HIGH PROTEIN BAR, CANDY-LIKE, SOY & MILK BASE	50
Bar	53541100	BREAKFAST BAR, DIET MEAL TYPE	25
Bar	53541200	MEAL REPLACEMENT BAR (INCL SLIM FAST BAR)	34
Bar	53544450	POWERBAR (FORTIFIED HIGH ENERGY BAR)	65

<sup>a</sup> RTD Bev = Ready-To-Drink Beverage; Bev mix = Beverage mix  
<sup>b</sup> Default serving sizes (in grams) for each of the food codes were identified using the "Quantity not specified" gram weights in the USDA Survey Food Coding Database (USDA 2000).

**APPENDIX II**

**TABULAR SUMMARY OF ANIMAL TOXICITY  
STUDIES ON CHROMIUM TRIPICOLINATE**

**Tabular Summary of Animal Toxicity Studies on Chromium Tripicolinate**

Reference	Objective	Study design	Duration	Intake/Dose	Results	Conclusions/Comments
<p>Anderson et al. 1997</p> <p><i>J Am Coll Nutr</i> 16(3):273-279</p>	<p>To evaluate the safety of chromium chloride and chromium tripicolinate in rats</p>	<p>Rats 4 wks of age fed stock diet, stock diet + CrPic, or stock diet + CrCl for 20 weeks.</p> <p>Fasting blood samples collected after 11 and 17 wks of age (control and high-dose grps only) and at 24 wks of age (all animals).</p> <p>Animals were sacrificed at 24 wks of age. Body weight, organ weight, and blood chemistry (serum glucose, cholesterol, triglycerides, BUN, total protein, creatinine, lactate dehydrogenase, alanine amino transferase, and aspartate amino transferase) measurements were taken. Histologic evaluation of the liver and kidney of control and high-dose animals conducted.</p> <p>Liver and kidney tissues measured for Cr, Cu, Fe, and Zn concentrations.</p>	<p>20 weeks</p>	<p>0, 5, 25, 75, and 100 mg Cr per kg diet</p> <p>(0.75, 3.75, 7.5, and 15 mg/kg/day, assuming intake of 15 g diet per day and a bw of 100 g)</p>	<p>All animals appeared normal throughout the study; no visible differences were observed among the groups. There were no significant differences among the control or test groups in body weight, organ weights, glucose, cholesterol, triglycerides, BUN, protein, lactate dehydrogenase, or ALT/SGPT at 11, 17, or 24 wks of age. Random variations occurred in AST/SGOT at 17 and 24 wks of age and in creatinine at 24 wks of age.</p> <p>Histologic examination of the liver and kidneys of the control and high-dose animals revealed no differences between groups.</p> <p>Higher Cr concentrations were found in the liver and kidneys of test group animals compared to the controls, with higher Cr levels occurring in the liver than the kidney. Liver and kidney Cr concentrations increased linearly. CrPic produced higher Cr concentrations compared to CrCl; CrPic produced about a 4-fold increase in Cr levels in the kidneys and about a 10-fold increase in Cr levels in the liver at the highest dose level compared to CrCl. There were no apparent adverse effects of the increased Cr concentrations.</p>	<p>The investigators concluded that trivalent Cr is not toxic in rats at levels that are several thousand times higher than the upper limit of the estimated safe and adequate daily dietary intake (ESADDI) level for humans of 200 µg/day.</p>



Reference	Objective	Study design	Duration	Cr/Dose	Results	Conclusions/Comments
Lindemann et al. 1995  <i>J Anim Sci</i> 73:457-465	To examine the effect of different doses of Cr (as CrPic) at various protein levels on growth performance, carcass composition, clinical chemistry, sow fecundity, and body weight changes in growing-finishing pigs	Pigs randomly assigned to the following feeding groups and allowed water and feed <i>ad libitum</i> :  1. Male and female pigs fed test or control diets; carcass and back-fat depth measurements taken about every 2 wks.  2. Male and female pigs fed test diets; blood samples taken 2 wks before end of study and analyzed for clinical chemistry parameters; carcass, back-fat, and loin muscle area measurements taken 1 day after sacrifice.  3. Females from Group 2 fed test or control diet through growth trial and then through breeding and reproduction (to 2 generations). Gestation weight gain, lactation weight changes, lactation feed intake, total number and weight of offspring, number and weight of pigs alive at birth, day 21, weaning, and the weaning-to-estrus period determined; blood glucose and insulin measurements collected at about 9 months on some animals.	Group 1: 60 days  Group 2: 106 days  Group 3: 106 days + breeding and reproduction (to 2 generations)	1. 0, 250, or 500 ppb Cr from CrPic (0, 0.25, 0.5 mg/kg/day Cr)  2. 0, 100, 200, 500, and 1,000 ppb Cr from CrPic $\pm$ 100 or 120% of lysine requirement (0, 0.1, 0.2, 0.5, or 1 mg/kg/day Cr)  3. (a) 0 ppb Cr $\pm$ 100-120% lysine req. during growth trial, 0 ppb Cr during breeding and reproduction  (b) 200 ppb Cr (0.2 mg/kg/day) $\pm$ 100-120% lysine req. during growth trial, 200 ppb Cr (0.2 mg/kg/day) during breeding and reproduction  (c) 500 or 1,000 ppb Cr (0.5 or 1 mg/kg/day) + 120% lysine req. during growth trial, 0 ppb Cr during breeding and reproduction	1. Cr did not affect average daily gain and feed intake; Cr added to diet improved gain:feed ratio; increase in longissimus muscle area. No signs of illness or disease observed in any test animal.  2. Only effect of Cr noted in serum chemistry parameters was an increase in potassium at the 200 ppb supplementation level. Average daily gain not affected; 200 ppb + normal lysine level (i.e., 100%) improved gain:feed ratio, decreased daily feed intake, reduced back-fat, and increased longissimus muscle area; 200 ppb + 120% lysine resulted in no improvement in gain:feed ratio or change in feed intake.  3. There was a tendency for greater weight gain during gestation in animals with prior Cr supplementation vs. those w/o prior supplementation ( $p < 0.09$ ). Cr supplementation during growth, breeding, and reproduction (group b) resulted in a significant increase in total litter size ( $p < 0.03$ ), live litter size ( $p < 0.02$ ), total litter weight ( $p < 0.02$ ), and live litter weight ( $p < 0.01$ ). The increase in litter size was observed up to a mean weaning age of 29 days. Increased litter weight was maintained only through day 21. There were no differences observed in individual animal weights at birth. Animals with no prior Cr supplementation weighed more at weaning than supplemented pigs. Cr supplementation during the growth period only (group c) resulted in intermediate litter sizes and intermediate weights at day 21 and weaning. >90% of animals in all groups survived to day 21 and weaning. The number of animals completing the 1 <sup>st</sup> and 2 <sup>nd</sup> litters were: 11 and 6, respectively in group a, and 11 and 10, respectively in groups b and c (initially 13 animals/group).  No effects on serum glucose levels were observed. CrPic resulted in decreased pre- and post-feeding insulin levels and decreased insulin:glucose ratio ( $p < 0.003$ ).	Investigators concluded that CrPic produces favorable biological responses in growing and reproducing swine.

**APPENDIX III**

**TABULAR SUMMARY OF HUMAN CLINICAL STUDIES  
ON CHROMIUM TRIPICOLINATE**

### Human Clinical Studies on Chromium Tripicolinate

Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/ Conclusions	Adverse Effects
Anderson et al. 1997  <i>Diabetes</i> 46:1786-1791	To determine the role of Cr in the control of type 2 diabetes.	Randomized, double-blind, placebo-controlled.	4 months	Low Cr group: 3.85 $\mu\text{mol/d}$ of Cr as Cr(pic) (200 $\mu\text{g/d}$ of Cr(III))  High Cr group: 19.2 $\mu\text{mol/d}$ of Cr as Cr(pic) (1000 $\mu\text{g/d}$ of Cr(III))  Placebo group	155 subjects who were being treated for type 2 diabetes at 2 hospitals in Beijing, China.  Low Cr group: n=53  High Cr group: n=52  Placebo group: n=50	Fasting and 2-hour glucose was lower in the high Cr(pic) group after 2 and 4 months of supplementation. Fasting and 2-hour insulin decreased in both low and high Cr(pic) groups after 2 and 4 months of supplementation. Total cholesterol decreased in high Cr(pic) group after 4 months of supplementation. HbA <sub>1c</sub> decreased in high and low Cr(pic) groups after 4 months of supplementation. No significant effect of Cr(pic) on HDL, TG, BUN, weight, or BMI.	None reported.  Authors stated, "There was no evidence of toxicity in this study."

### Human Clinical Studies on Chromium Tripicolinate

Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/Conclusions	Adverse Effects
Boyd et al. 1998 <i>J Nutr Biochem</i> 9:471-475	To determine the effects of Cr supplementation on healthy, young exercising men and women.	Double-blind, placebo-controlled.	13 weeks	1000 µg/d of Cr(III) as Cr(pic)  Placebo group	25 healthy college-aged students participating in an exercise class.	No significant differences in serum ferritin, fasting glucose, HDL or TG levels between the two groups. No significant differences in weight loss, lean body mass or strength in either group. Significant differences in serum TC and insulin between the Cr(pic) group and placebo group post exercise.	Subjects in either group reported no complaints or side effects.  No significant effect on serum ferritin levels.

### Human Clinical Studies on Chromium Tripicolinate

Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/Conclusions	Adverse Effects
Campbell et al. 1997  <i>Am J Clin Nutr</i> 66:944-949	To evaluate the combined effects of resistive training and high-dose Cr(pic) supplementation on hematological indices and iron status in older men.	Randomized, double-blind, placebo-controlled.	12 weeks	924 µg/d Cr(III) (17.4 µ mol Cr) as Cr(pic)  Placebo group	18 moderately overweight men (56-69 years) without diabetes, uncontrolled hypertension, abnormal cardiac, liver or renal function.  Cr(pic) group: n=9  Placebo group: n=9	No effect of Cr(pic) supplementation on muscle strength was observed.  <u>Conclusion:</u> Cr(pic) supplementation did not affect hematological indices and iron status. It also did not influence the resistive training-related changes in TIBC and transferrin saturation.	Hematocrit, hemoglobin, RBC count, WBC count, MCV, mean corpuscular hemoglobin, RBC distribution width, platelet count, and mean platelet volume did not change significantly with Cr(pic) supplementation or with resistive training.  Serum iron and serum ferritin concentrations, TIBC and transferrin saturation were not affected by Cr(pic) supplementation. Serum iron and ferritin concentrations were not affected by resistive training, but TIBC decreased ( $p < 0.0001$ ) and transferrin saturation increased ( $p = 0.050$ ) over time with resistive training.

### Human Clinical Studies on Chromium Tripicolinate

Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/Conclusions	Adverse Effects
Campbell et al. 1999  <i>J Appl Physiol</i> 86(1):29-39	To determine the effect of high-dose Cr(pic) supplementation on body composition and strength in older men participating in resistance training.	Randomized, double-blind, placebo-controlled.	12 weeks	924 µg/d of Cr(III) as Cr(pic)  Placebo group	18 men aged 50-75 years. All subjects participated in a resistance training program.  Cr(pic) group: n=9  Placebo group: n=9	Cr(pic) supplementation had no effect on body composition and strength in older men.  Urinary Cr excretion increased by approximately 50-fold with Cr(pic) supplementation compared to baseline.  At baseline, the mean absorption of Cr ranged from 0.25 to 0.37%. Cr absorption increased with Cr(pic) supplementation and ranged from 0.93 to 1.15%.	None reported.

### Human Clinical Studies on Chromium Tripicolinate

Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/Conclusions	Adverse Effects
<p>Cefalu et al. 1999</p> <p><i>The Journal of Trace Elements in Experimental Medicine</i> 12:71-83</p>	<p>To determine the effect of Cr supplementation on body composition and insulin sensitivity in subjects at high risk of developing type 2 diabetes.</p>	<p>Randomized, double-blind, placebo-controlled.</p>	<p>8 months  (5-week baseline period)</p>	<p>1000 µg/d Cr(III) as Cr(pic)  Placebo group</p>	<p>29 subjects (14 men, 15 women) at high risk of developing type 2 diabetes.</p> <p>Cr(pic) group: n=15  Placebo group: n=14</p>	<p>Significant increase in insulin sensitivity at the midpoint (p&lt;0.05) and at the end of the study (p&lt;0.05) was observed in the Cr(pic) group compared to the control group. No change in glucose effectiveness was observed in the Cr(pic) or control group. No effect on body weight, abdominal fat distribution, and BMI was observed with Cr(pic) supplementation.</p>	<p>No difference in complete blood count, liver function, renal function, and electrolyte levels was observed between the baseline period and the end of the study in either group.</p>
<p>Cheng et al. 1999</p> <p><i>The Journal of Trace Elements in Experimental Medicine</i> 12:55-60</p>	<p>To determine the effects of Cr supplementation on fasting and postprandial blood glucose and on diabetic symptoms (fatigue, thirst and frequent urination) in a follow-up survey.</p>	<p>Observational</p>	<p>10 months</p>	<p>500 µg/d Cr(III) as Cr(pic)</p>	<p>833 subjects with type 2 diabetes.</p>	<p>Fasting and postprandial blood glucose decreased significantly after 1 month of supplementation and continued to remain lower after 10 months of supplementation. &gt;85% of the subjects experienced reduction in thirst, fatigue and frequency of urination during the supplementation period.</p>	<p>No confirmed negative side effects of Cr(pic) supplementation were reported.</p>

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Clancy et al. 1994  <i>International Journal of Sports Nutrition</i> 4 :142-153	To determine the effects of Cr(pic) on lean body mass and strength in football players.	Randomized, double-blind, placebo-controlled.	9 weeks	200 µg/d of Cr(III) as Cr(pic)  Placebo group (flour and beet powder)	36 University of Massachusetts football players.	No effect of Cr(pic) supplementation on anthropometric measures, body composition, and strength.	None reported.



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Grant et al. 1997  <i>Med Sci Sports Exerc</i> 29(8):992-998	To determine if Cr(pic) would alter body weight and composition, glucose tolerance, and plasma lipids favorably, and if these effects could be augmented with exercise. To determine the effectiveness of Cr nicotinate supplementation combined with exercise.	Randomized	9 weeks	400 µg/d of Cr(III) as Cr(pic)  400 µg/d of Cr(III) as Cr(pic) + exercise (E/CP group)  Placebo + exercise (E/P group)  400 µg/d of Cr(III) as Cr(nic) + exercise (E/CN group)	43 obese, sedentary females (age 18-35 years).  Subjects participated in a cross-training program.	Body weight increased in the CP group. No change in body weight was observed in the E/CP and E/P groups. Body weight decreased in the E/CN group.  No effect on plasma glucose, plasma insulin, glucose tolerance curve was seen in the DP, E/CP, E/CN and E/P groups. Insulin response decreased significantly after an oral glucose tolerance test in the E/CN group, but not in the other groups. No significant changes in TG, TC, LDL and HDL levels were observed after any treatment.	None reported.
Hallmark et al. 1996  <i>Med Sci Sports Exerc</i> 28(1):139-144	To examine the effects of Cr(pic) supplementation in combination with a progressive resistive exercise training program on body composition, muscle strength, and urinary Cr excretion in untrained young male subjects.	Randomized, double-blind, placebo-controlled.	12 weeks	200 µg/d of Cr(III) as Cr(pic)	16 untrained healthy young men (age 18-35 years).  Cr(pic) group: n=8  Placebo group: n=8	Urinary Cr excretion (24-hour) increased 9-fold compared to baseline levels after 6 weeks of supplementation, and was unchanged at 12 weeks of supplementation with Cr. No significant change in Cr excretion in the placebo group was observed.  Strength gain in the Cr(pic) group was not significantly different from the placebo group. Body composition in the Cr(pic) and placebo group did not change significantly in response to resistive training.	None reported.

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Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/Conclusions	Adverse Effects
Hansen et al. 1992  <i>International Journal of Sport Nutrition</i> 2:343-350	To assess the effects of Cr(pic) supplementation on body composition and strength in beginning weight training students.	Randomized, double-blind, placebo-controlled.	12 weeks	200 µg/d of Cr(III) as Cr(pic)  Placebo group  4 groups: 1) Male Cr(pic) group (M-Cr(pic)) 2) Female Cr(pic) group (F-Cr(pic)) 3) Male placebo group (M-P) 4) Female placebo group (F-P)	59 healthy students (37 males and 22 females) ages 18-36 years who were enrolled in a beginning weight training program.  M-Cr(pic): n=18 F-Cr(pic): n=12  M-P: n=19 F-P: n=10	Body weight increased significantly in females receiving Cr(pic) compared to the other 3 groups. A significant increase in sum-of-circumference and significant decrease in sum-of-skin folds was observed in all groups. No effect of Cr supplementation was observed on strength measurements.	None reported.
Jovanovic et al. 1999  <i>J Trace Elem Exp Med</i> 12:91-97	To examine the efficacy of Cr supplementation for control of gestational diabetes.	Randomized, placebo-controlled	8 weeks	4 µg/kg bw/d of Cr(III) as Cr(pic)  8 µg/kg bw/d of Cr(III) as Cr(pic)  Placebo group	30 gestational diabetic women aged 25-43 years.  Low Cr(pic) group: n=10  High Cr(pic) group: n=10  Placebo group n=10	In the low Cr(pic) group, the HbA <sub>1c</sub> levels decreased after supplementation (p<0.05), but no change in the high Cr(pic) and placebo group. Hyperglycemia and hyperinsulinemia improved in both Cr(pic)-supplemented groups. Insulin or C-peptide levels were not affected with the higher dose of Cr(pic).	In the low Cr(pic) group, free thyroxine decreased significantly (p=0.035) and BUN increased significantly (p=0.031) compared to the baseline levels. In the high Cr(pic) group, TG (p=0.045) and alkaline phosphatase (p=0.023) increased significantly and HDL (p=0.003) and free thyroxine (p=0.043) decreased significantly compared to the baseline levels. HDL levels in the placebo group decreased significantly (p=0.049) compared to the baseline levels.

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Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/Conclusions	Adverse Effects
Kaats et al. 1996  <i>Current Therapeutic Research</i> 57(10):747-756	To investigate the effects of Cr(pic) supplementation on body composition.	Randomized, double-blind, placebo-controlled.	72 days	200 µg/d of Cr(III) as Cr(pic)  400 µg/d of Cr(III) as Cr(pic)  Placebo group	154 subjects (mean age 45.7 years for men and 46.5 for women) who responded to a news story on local TV.  Low Cr(pic) group: n=33  High Cr(pic) group: n=66  Placebo group: n=55	Significant positive changes (decrease in fat mass and/or increase in lean mass) in body composition were observed in both Cr(pic)-supplemented groups compared to the placebo group, but no differences were observed between the groups receiving 200 and 400 µg/d of Cr(III) as Cr(pic).	None reported.

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Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/Conclusions	Adverse Effects
Kaats et al. 1998  <i>Current                      Therapeutic                      Research</i> 59(60):379-388	To determine if previous results on changes in body fat composition with Cr(pic) supplementation can be replicated by controlling for caloric intake and energy expenditure; using other measures of body composition; and reducing the dropout rate.	Randomized, double-blind, placebo-controlled.	90 days	400 µg/d of Cr(III) as Cr(pic)  Placebo group	122 subjects (mean age 42.3 years, 17 men and 105 women) were recruited from fitness centers and athletic clubs.	Significant reduction in percent body fat, fat mass, and body weight was observed after controlling for caloric intake and energy expenditure in the Cr(pic) group compared to the placebo group.	The subjects were asked to report any side effects due to the treatment on a weekly basis.  None reported.
Kaats et al. 1999  <i>JANA</i> 2(1):42-49	To determine the effects of a behavior modification plan (BMP), which included nutritional supplements, on total and LDL cholesterol.	Randomized, double-blind, placebo-controlled.	60 days	400 µg/d of Cr(III) as Cr(pic)  Placebo group	80 subjects  Cr(pic) group: 39  Placebo group: 41	No effect of Cr(pic) supplementation on TC was observed (entire treatment group) compared to the placebo group. After dividing the subjects into low, high and desirable groups based on their baseline TC levels, a significant decrease in TC was observed in the group with baseline TC > 200mg/dl supplemented with Cr(pic)	None reported.

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Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/ Conclusions	Adverse Effects
Lee, NA 1994  <i>Diabetes Care</i> 17(12):1449-1452	To determine the effects of Cr(pic) supplementation on lipids in Hispanic NIDDM patients.	Prospective, double-blind, placebo-controlled, cross-over.	2 months on either Cr(pic) or placebo  (2-month wash-out period)	200 µg/d of Cr(III) as Cr(pic)  Placebo	28 (men and women) NIDDM patients (age 32-65 years) from diabetes clinics.	All patients had detectable serum Cr levels after 2 months of supplementation with a mean concentration of 0.85 µg/l.  Mean body weight increased during the placebo (0.6%) and Cr(pic) period (0.2%).  No difference in glucose control between the groups was observed. The LDL and HDL levels did not change, but TG was reduced significantly after Cr(pic) supplementation.	No adverse reactions from Cr(pic) supplementation were reported.

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Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/ Conclusions	Adverse Effects
Lukaski et al. 1996  <i>Am J Clin Nutr</i> 63:954-965	To determine the effects of Cr(pic) and CrCl <sub>3</sub> on body composition and strength gain in young men participating in a resistance training program.	Double-blind, placebo-controlled.	8 weeks	3.3 μmol Cr(III) as Cr(pic)  3.5 μmol Cr(III) as Cr Cl <sub>3</sub>  Placebo group	36 young men (age 19-29 years). All subjects participated in a resistance training program for 8 weeks.  Cr(pic) group: n=12  CrCl <sub>3</sub> group: n=12  Placebo group: n=12	Cr supplementation in any form did not have beneficial effects on body composition or strength gain in young men.  Serum Cr levels and urinary Cr excretion increased with Cr supplementation with no differences due to the form of Cr administered.	Hematocrit and hemoglobin concentrations did not change with Cr supplementation. Transferrin saturation decreased with resistance training and was further reduced in men supplemented with Cr(pic) (24%) compared to men receiving CrCl <sub>3</sub> (13%) or placebo (10%).  Chromium supplementation decreased the urinary iron output significantly compared to the placebo group. The effect of Cr(pic) was greater than CrCl <sub>3</sub> . The output in the placebo group was higher compared to Cr supplemented groups.  Ceruloplasmin activity was greater after resistance training in men supplemented with CrCl <sub>3</sub> compared to men supplemented with Cr(pic). Immunoreactive ceruloplasmin concentrations remained unchanged with Cr supplementation. The ratio of enzymatic to immunoreactive ceruloplasmin was less with CrCl <sub>3</sub> than with placebo or Cr(pic). Chromium supplementation did not affect SOD activity.  Plasma Mg levels decreased with Cr supplementation regardless of the form of Cr compared to the placebo. Urinary Mg was not affected by Cr supplementation.  Plasma and urinary Zn levels were not affected by Cr supplementation.

### Human Clinical Studies on Chromium Tripicolinate

Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/Conclusions	Adverse Effects
Press et al. 1990  <i>West J Med</i> 152:41-45	To determine the effectiveness of Cr(pic) in humans.	Randomized, double-blind, placebo-controlled, cross-over.	6 weeks on either Cr(pic) or placebo  (2-week wash-out period)	200 µg/d of Cr(III) as Cr(pic)  Placebo group (Calcium phosphate)	28 volunteers aged 25-80 years with high TC levels (220-320 mg/dl).	Cr(pic) supplementation significantly decreased serum TC, LDL, and apolipoprotein B levels and significantly elevated apolipoprotein A-I levels. A nonsignificant increase in HDL levels was observed with Cr(pic) supplementation. Slight but nonsignificant elevations occurred in LDL, TC, and apolipoprotein A-I levels with the placebo. Apolipoprotein B increased significantly during the placebo period.  Serum TG, weight, blood pressure, temperature, or heart rate did not change significantly with either the placebo or Cr(pic) treatment	None reported.
Ravina et al. 1995  <i>The Journal of Trace Elements in Experimental Medicine</i> 8:183-190	To evaluate the effects of Cr(pic) supplementation on blood glucose, insulin, and glycated hemoglobin in type I and II diabetic subjects.	Double-blind, placebo-controlled for 10 subjects.	10 days (162 subjects)  3 months (10 subjects)	200 µg/d of Cr(III) as Cr(pic)	162 volunteers ages 7-80 years with type I (n=48) or type II (n=114) diabetes.  10 subjects used for double-blind placebo-controlled trial.	Both type I and type II diabetic patients showed improvement with Cr(pic) supplementation. The insulin dose was reduced in IDDM patients. The insulin dose or oral hypoglycemic drug dose was reduced or withheld in the NIDDM patients.	None reported.

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Reference	Objective	Study Design	Duration	Intake/Dose	Subjects	Results/Conclusion	Adverse Effects
Trent and Thiedling-Cancel 1995  <i>J Sports Med Phys Fitness</i> 35(4):273-280	To evaluate the potential role of Cr(pic) as a weight reduction adjuvant in the Navy's remedial conditioning programs.	Double-blind, placebo-controlled.	16 weeks	400 µg/d of Cr(III) as Cr(pic)  Placebo group (Calcium chloride)	95 Navy personnel (79 men and 16 women, mean age 30.3 years) from eight command programs enrolled in remedial conditioning program.  Cr(pic) group: n=15  Placebo group: n=44	No effect of Cr(pic) supplementation was observed on body weight, percent body fat, and lean body mass compared to the placebo. The group as a whole lost some body weight and body fat.	No evidence of somatopsychological effects was observed due to Cr(pic) supplementation.



**ADDENDUM TO:  
Generally Recognized as Safe (GRAS)  
Determination for the Use of  
Chromax® Chromium Picolinate as a  
Nutrient Supplement in Food**

Prepared for:  
Nutrition 21, Inc.

Prepared by:  
  
ENVIRON  
Health Sciences Institute

April 2003

**ENVIRON**

Chromax® chromium tripicolinate is sold by Nutrition 21, Inc., with offices in Purchase, NY, and was determined to be Generally Recognized as Safe (GRAS) in July 2002 by a panel of experts qualified by scientific training and experience to evaluate the safety of food and food ingredients (ENVIRON 2002). The safety of chromium tripicolinate under its intended conditions of use was based upon a review of available safety data, as well as a comparison of the Estimated Daily Intake (EDI) of chromium per day from all food and supplement sources with its Acceptable Daily Intake (ADI) based upon rodent and human safety studies. The chromium EDI of 600 mcg per day was lower than its ADI of 900 mcg per day, and thus consumption of chromium tripicolinate was concluded to be safe under its intended conditions of use.

Since preparation of the GRAS determination document in 2002, additional studies have become available to assess the safety of chromium tripicolinate. This addendum to the 2002 GRAS determination reviews the impact of the findings from those studies on the conclusion that chromium tripicolinate is GRAS.

The 2002 GRAS document noted that several *in vitro* assays demonstrated that gene mutations, DNA damage, and chromosome damage were seen in cultured cells and DNA preparations treated with relatively high concentrations of various chromium salts, including chromium tripicolinate. However, no increases in mutagenic damage were seen in an Ames *Salmonella* mutation assay conducted with chromium tripicolinate in the presence and absence of a rat liver homogenate metabolic activation system (Juturu and Komorowski 2002). Chromium tripicolinate was reported to produce increases in chromosome damage in Chinese hamster ovary cells within a concentration range of 0-3.0 mM and damage in isolated plasmid DNA after relatively rigorous treatments for periods from 5 to 180 minutes at concentrations from 0.12 uM

to 120 uM (Speetjens et al. 1999). In contrast to some reports of mutagenic effects with *in vitro* test systems, no evidence of *in vivo* DNA damage was observed in urine samples of 10 human subjects consuming 400 mcg per day of chromium as chromium tripicolinate for approximately 56 days, as determined by measurements of 5-hydroxymethyl-2-deoxyuridine that was used as an indicator molecule for detecting oxidative DNA strand breakage (Kato et al. 1998). In addition, there were no adverse clinical signs of toxicity in rodents dosed with 5-100 ppm of chromium tripicolinate in the diet for 20 weeks (Anderson et al. 1997). The absence of adverse effects in the rodent study was in agreement with lack of clinical effects in human subjects that consumed 400 mcg per day of chromium as chromium tripicolinate. The lack of evidence of DNA damage in the repeat-dose study with human subjects led to the conclusion that *in vitro* genotoxic activity observed in cellular test systems was not relevant for evaluating potential risk to humans or for determining the GRAS status of chromium tripicolinate.

A review of the literature on chromium tripicolinate published since the compilation of the GRAS determination indicates that additional studies have generally confirmed the original observations of *in vitro* effects from chromium tripicolinate. Manygoats et al. (2002) noted that ultrastructural damage was produced in Chinese hamster ovary cells after treatments with chromic chloride or chromium tripicolinate. They noted that chromium tripicolinate, administered for an extensive 48-hour culture period at substantial doses of 1 mM (440 mcg/ml) to 3 mM (1340 mcg/ml), was the more active compound at producing mitochondrial damage.

Stearns et al. (2003) reported that chromium tripicolinate at concentrations of 0.75 to 3.0 mM was mutagenic to Chinese hamster ovary cells *in vitro* and produced increases in the numbers of mutant cells resistant to 6-thioguanine caused by a specific gene mutation. Chromic chloride was also mutagenic in this same test and produced a 10-fold increase in drug-resistant

mutants relative to numbers of mutant cells found in control cultures. Although the authors considered chromium tripicolinate to be more highly active than chromic chloride, mutation effects in this *in vitro* test are apparently related to treatment of the CHO cells at concentrations of chromium (in any form) that far exceed concentrations that could ever be achieved *in vivo*.

The National Toxicology Program (NTP) has recently completed independent *in vitro* and *in vivo* genotoxicity assays of both chromium tripicolinate (anhydrous) as well as chromium tripicolinate monohydrate (NTP 2003). The NTP reported summary results that showed that chromium tripicolinate (anhydrous) did not produce chromosome damage in the *in vivo* mouse micronucleus assay and was also negative in two Salmonella (Ames) bacterial mutation assays. Chromium tripicolinate monohydrate was also observed to lack potential to produce adverse effects on chromosomes in the mouse micronucleus assay with males, with equivocal findings in females (NTP 2003). The absence of genetic toxicity findings *in vivo* are consistent with results reported by Greenberg et al. (1999) in studies with rats given chromium tripicolinate orally with doses of up to 2,000 mg/kg body weight. No increases in chromosome aberrations were seen following evaluation of chromosomes in bone marrow cells harvested at two time intervals following dosing to determine potential damage. The absence of genetic toxicity effects *in vivo* in mice in the NTP studies of two different forms of chromium tripicolinate confirm the absence of chromosome damage seen in rats (Greenberg et al. 1999) as reviewed in the GRAS document in 2002. The absence of genetic toxicity findings from *in vivo* results is also consistent with the lack of genotoxicity in DNA damage studies with human subjects as evaluated in the 2002 GRAS document (Kato et al. 1998). Thus, these additional studies confirm the conclusions in the GRAS review (ENVIRON 2002) that chromium tripicolinate is not genotoxic *in vivo* and the lack of significant genotoxicity findings contrasts directly to the reported effects detected in the

artificial environment of cell culture screening tests. *In vitro* tests are conducted at relatively high concentrations of chromium tripicolinate, and may magnify the effect of potential chemical reactions with chromium tripicolinate reported by some authors (Bagchi et al. 1997; 2002). The bioavailability of dietary chromium from trivalent chromium salts is very low following ingestion (approximately 0.5 to 2%, with somewhat higher levels of 3% for chromium tripicolinate, as evaluated in animal and human studies (O'Flaherty 1996; Campbell 1999)). Therefore, the amount of chromium tripicolinate distributed in the bloodstream is several orders of magnitude lower than the concentrations tested *in vitro*. Therefore, *in vitro* effects appear to have limited (if any) relevance to living animals or to human risk assessment because of the high doses used, and the consistent lack of significant genetic toxicity seen with *in vivo* animal and human tests.

Additional genotoxicity and *in vitro* cytotoxicity studies have also been published since the preparation of the 2002 GRAS determination, but these appear to have limited application to safety assessment of chromium tripicolinate. Hepburn et al. (2003) evaluated chromium tripicolinate prepared in their laboratory for mutagenic potential in a wild-type strain of fruit flies (*Drosophila melanogaster*). Concentrations of chromium tripicolinate from 10.4 to 260 mcg/kg, given as a component of the standard diet, did not produce any adverse effects on viability, fertility or behavior in adult flies. Larvae exposed to a similar concentration range were reported to undergo developmental delays and decreased pupation success, but there is no substantive way to use these findings with an insect larva for assessing potential mammalian (much less human) toxicity. An unspecified concentration of chromium tripicolinate (described only as “dietary concentrations equivalent to those in human Chromium supplementation”) was reported to produce increases in X-linked lethal mutations and dominant female sterility. The significance

of mutagenic effects in fruit flies has questionable relevance to human risk assessment because of the significant differences in physiology and metabolism between insects and mammals, as well as the impossibility of extrapolating dosage effects from insects to humans. As noted by experts in genetic toxicology (Hoffman 1996), “the means of exposure, measurement of doses, metabolism and gametogenesis in *Drosophila* differ from those in mammalian toxicology. Mammalian assays therefore provide the best basis for assessing risk to human germ cells and hold a central place in genetic toxicology...” The absence of genotoxic effects seen with *in vivo* assays in rats and mice described previously confirms that increases in mutations in insects have little if any significance in assessing mammalian genotoxicity of chromium tripicolinate.

Hepburn and Vincent (2003) attempted to determine the tissue distribution of chromium picolinate in rats following dosing by intravenous injection into the tail vein with radiolabelled <sup>51</sup>Chromium-tripicolinate with sampling at six time intervals from 30 minutes to 24 hours after dosing. The authors noted that “for [chromium(picolinate)<sub>3</sub>] to have a deleterious effect on DNA via production of reactive oxygen species, the compound needs to enter cells intact and remain intact long enough to produce a quantity of reactive oxygen species.” However, attempts by the authors to detect chromium tripicolinate itself in tissues and body fluids failed with the analytical detection methods employed. Because only <sup>51</sup>Chromium marker was followed in the study, it is not possible to distinguish the form in which chromium was found in the cells and tissues. In addition, this intravenous administration is not relevant to extrapolations of the fate of chromium picolinate ingested orally by humans. Thus, although the results of this study confirm reports by others on distribution of chromium from chromium tripicolinate in specific body tissues, the report fails to provide information on chromium tripicolinate itself or its fate following ingestion.

## Conclusion

Additional studies on *in vitro* cytotoxicity and genotoxicity of chromium tripicolinate have been published since preparation of a safety assessment document for this food supplement that concluded chromium tripicolinate was GRAS under its intended conditions of use. A review of these new studies show increases in genetic changes *in vitro* as noted previously. However, new studies conducted by the NTP confirm the absence of *in vivo* effects in mammalian test systems reported in the GRAS review document, and also do not show increases in mutations in the standardized Ames bacterial mutation test system. The relatively high doses used in the *in vitro* tests relative to the amounts ingested by humans, and the low degree of absorption from the diet, show that these *in vitro* test systems have no relevance to determination of human safety. The consistent lack of adverse toxicological or genetic effects *in vivo* supports the GRAS determination reviewed and agreed to by the GRAS panel convened in July 2002 (ENVIRON 2002).

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