

## Effects of Pre- and Postnatal Exposure to Chromium Picolinate or Picolinic Acid on Neurological Development in CD-1 Mice

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**Abstract** Chromium picolinate, Cr(pic)<sub>3</sub>, a popular dietary supplement marketed as an aid in fat loss and lean muscle gain, has also been suggested as a therapy for women with gestational diabetes. The current study investigated the effects of maternal exposure to Cr(pic)<sub>3</sub> and picolinic acid during gestation and lactation on neurological development of the offspring. Mated female CD-1 mice were fed diets from implantation through weaning that were either untreated or that contained Cr(pic)<sub>3</sub> (200 mg kg<sup>-1</sup> day<sup>-1</sup>) or picolinic acid (174 mg kg<sup>-1</sup> day<sup>-1</sup>). A comprehensive battery of postnatal tests was administered, including a modified Fox battery, straight-channel swim, open-field activity, and odor-discrimination tests. Pups exposed to picolinic acid tended to weigh less than either control or Cr(pic)<sub>3</sub>-exposed pups, although the differences were not significant. Offspring of picolinic acid-treated dams also appeared to display impaired learning ability, diminished

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olfactory orientation ability, and decreased forelimb grip strength, although the differences among the treatment groups were not significant. The results indicate that there were no significant effects on the offspring with regard to neurological development from supplementation of the dams with either Cr(pic)<sub>3</sub> or picolinic acid.

**Keywords** Chromium · Chromium picolinate · Picolinic acid · Neurological development · Mice

## Introduction

Chromium(III) is generally thought to be an essential trace element, enhancing glucose tolerance, protein and lipid metabolism, and serum cholesterol [1–8]. As dietary chromium is not readily absorbed (about 0.5–2%), the coupling of chromium to a suitable ligand is necessary to increase its bioavailability. Chromium picolinate [Cr(pic)<sub>3</sub>] has traditionally been one of the most popular forms of supplemental chromium. It has been added to dietary supplements, “weight loss” vitamins, and “health” shakes for years and is touted as an aid in promoting fat loss and lean muscle gain. Cr(pic)<sub>3</sub> has also been suggested as a treatment for metabolic disorders, such as type 2 diabetes, as some studies indicate that it is able to increase insulin sensitivity and lower serum glucose. However, the heterogeneity across these studies and their overall limitations restrict their ability to provide firm conclusions [8–10].

Recently, Cr(pic)<sub>3</sub> has been proposed to be an effective antidepressant, particularly for those who suffer from type 2 diabetes. Approximately 8% of US adults with diabetes have major depression, and recent studies suggest that depression is not only a major risk factor for the development of type 2 diabetes but that it may also accelerate the onset of diabetic complications [11, 12]. Insulin acts peripherally on skeletal muscle to increase amino acid uptake, resulting in increased blood–brain transport of tryptophan, which ultimately results in an increase in serotonin (5-HT) [13]. Insulin resistance has long been correlated with depression, although the exact nature of the relationship has not been elucidated. Chromium has been proposed to function as an antidepressant by enhancing insulin function (and therefore increasing tryptophan transport), increasing serotonin levels by modulating serotonin (5-HT<sub>2A</sub>) receptors, or some combination of the above [14, 15]. Significant increases in tryptophan, 5-HT, melatonin, and noradrenaline were noted after sub-chronic dosing with chromium picolinate in a study by Franklin and Odontiadis [15]. Other studies have indicated that Cr(pic)<sub>3</sub> may be effective at alleviating symptoms of depression in individuals with metabolic disorders, presumably by enhancing insulin function, without the side effects typically associated with more traditional treatments, such as monoamine oxidase inhibitors [13, 16–19]. However, the clinical studies with Cr(pic)<sub>3</sub> [16, 17, 19] utilized very limited subject pools and require larger trials to firmly establish whether the supplement can beneficially affect atypical depression.

The safety of Cr(pic)<sub>3</sub> supplementation remains controversial. Stearns and coworkers were the first to report deleterious effects of Cr(pic)<sub>3</sub> when they demonstrated that it was clastogenic [20] and later that it was mutagenic in Chinese hamster ovary cells [21]. Chromium from Cr(pic)<sub>3</sub> accumulates in cells and has nuclear affinity, implying that supplemental levels over long periods of time might be harmful [22]. While Cr(pic)<sub>3</sub> was not found to be mutagenic in *Salmonella typhimurium*, it was found to induce mutagenic responses in the L5178Y mouse lymphoma mutation assay [23]. Chromium picolinate has

also been found to be capable of cleaving DNA under physiologically relevant conditions [24]. Recent studies have failed to demonstrate that commercially prepared  $\text{Cr}(\text{pic})_3$  was clastogenic or mutagenic [25, 26]. However, these apparently conflicting results have recently been reconciled, as the studies in which damage was not observed used dimethyl sulfoxide, a free radical trap which could quench reactive oxygen species, as a solvent for  $\text{Cr}(\text{pic})_3$  [27]. Recently, our laboratory published findings that prenatal exposure to high levels of chromium picolinate resulted in an increased incidence of cervical arch defects [28]. However, although our more recent findings were similar in direction, the differences from control values for this parameter were not statistically significant [29].

The controversy surrounding the safety and efficacy of  $\text{Cr}(\text{pic})_3$  and picolinic acid extends to the neurological realm. Picolinic acid, a naturally occurring catabolite of tryptophan metabolism, has been shown to be neuroprotective against the toxic effects of quinolinic acid, an excitotoxic tryptophan metabolite [30]. Its ability to function as a protective immunomodulator has been demonstrated in several studies involving intracerebral infection and tumor growth [31, 32]. However, at high doses, picolinic acid has been suggested as being neurotoxic, inducing injury to neuronal cell bodies and spongiosis of the hippocampus [33].

Although chromium picolinate has been suggested as a therapy for women with gestational diabetes, the effects of chromium picolinate or picolinic acid on the neurological development of offspring following exposure during pregnancy have not been reported. Aggressive treatment of gestational diabetes has been associated with better pregnancy outcomes and a lower incidence of postpartum depression, and  $\text{Cr}(\text{pic})_3$  may be helpful in lowering glycated hemoglobin levels [34, 35]. As over 200,000 cases of gestational diabetes are diagnosed annually in the USA [36] and the incidence of type 2 diabetes in women of child-bearing age is skyrocketing, a significant number of fetuses could potentially be exposed to  $\text{Cr}(\text{pic})_3$  during pregnancy if it is used as a supplement to traditional therapy. Thus, the current study examined the effects of prenatal and lactational exposure of chromium picolinate and picolinic acid on the neurological development of CD-1 mice.

## Materials and Methods

### Animals and Husbandry

Male and female CD-1 mice, obtained from Charles River Breeding Laboratories, International (Wilmington, MA, USA) were housed in an Association for Assessment and Accreditation of Laboratory Animal Care, International-approved animal facility in rooms maintained at  $22 \pm 2^\circ\text{C}$ , with 40–60% humidity and a 12-h photoperiod. Animals were bred naturally, two females with one male, after a 2-week acclimation period. Observation of a copulation plug designated gestation day (GD) 0. Mated females were individually housed in shoe-box-type cages with hardwood bedding and were allowed to feed on Harlan-Teklad LM-485 rodent diet and tap water ad libitum.

### Test Chemicals

Chromium(III) picolinate,  $\text{Cr}(\text{pic})_3$ , was synthesized according to the methods of Press et al. [7], and authenticity was established by high-resolution electron impact mass spectrometry

[37]. Picolinic acid was purchased from Fisher Scientific (Pittsburgh, PA, USA). LM-485 milled rodent diet was purchased from Harlan Teklad (Madison, WI, USA). Either  $\text{Cr}(\text{pic})_3$  or picolinic acid was added to milled rodent chow in sufficient quantities to achieve the appropriate concentration of the test compound. All calculations were based on data from previous studies, which indicated that pregnant CD-1 mice consume an average of 7 g diet per day. Extensive stability studies indicate that  $\text{Cr}(\text{pic})_3$  is extremely stable and that no degradation in the diet would be expected [37].

Because the purpose of this study was to determine the effects of pharmaceutical levels of chromium, no special measures were taken to prevent exposure of the mice to small amounts of chromium that may be introduced into the diet through methods of feed preparation or from the cage hardware. The diet purchased, Teklad LM-485 (7012), contained added chromium in the form of chromium potassium sulfate (0.48 mg/kg of diet).

Mated females were randomly assigned to one of three treatment groups: (1) control, untreated rodent diet; (2)  $\text{Cr}(\text{pic})_3$ , 200 mg/kg body mass per day (25 mg  $\text{Cr kg}^{-1} \text{ day}^{-1}$ ); or (3) picolinic acid, 174 mg/kg body mass per day. The study was performed in four replicates for a total of 15 litters (166 pups) in the control group, 17 litters (210 pups) in the  $\text{Cr}(\text{pic})_3$  group, and 17 litters (213 pups) in the picolinic acid (PA) group (Table 1). All test diets were administered from GD 6 until weaning at postnatal day (PND) 21. Food consumption was measured for the intervals GD 6–9, 9–13, and 13–17, and clinical signs were recorded daily. After birth, the pups were not dosed directly and were not culled. The dosage of chromium picolinate was based on results from a previous study, in which 25 mg  $\text{Cr kg}^{-1} \text{ day}^{-1}$  as  $\text{Cr}(\text{pic})_3$  was associated with a significant increase in the incidence of cervical arch defects compared to control animals [28]. After weaning on PND 21, the offspring were separated and gang-housed with same-sex littermates in shoebox-type cages and were allowed access to untreated Harlan Teklan LM-485 rodent diet and tap water ad libitum. All behavioral testing was conducted at The University of Alabama's Animal Care Facility in a standard animal room with fluorescent lighting. No special lighting or sound-dampening equipment was used.

## Neurological Testing

### *Modified Fox Battery*

From PND 5–9, offspring were assessed for somatic and neurobehavioral development by use of a modified Fox battery [38]. Assessments of behavioral ontogeny are highly recommended as part of neurotoxicity test panels, and the Fox battery is a robust, sensitive method of evaluation of behavioral development that has been in use for over 30 years [39–

**Table 1** Prewaning Behavior

	Treatment and Dose (mg $\text{kg}^{-1} \text{ day}^{-1}$ )		
	Control (0)	$\text{Cr}(\text{Pic})_3$ (200)	Picolinic Acid (174)
Litters/pups examined	15/166	17/210	17/213
Day 11 homing ( $\pm$ SEM) (s)	38.50 $\pm$ 6.33	41.31 $\pm$ 5.41	48.84 $\pm$ 5.10
Day 13 hanging bar time ( $\pm$ SEM) (s)	6.54 $\pm$ 0.97	8.21 $\pm$ 0.79	5.57 $\pm$ 0.48

41]. The pups were weighed prior to the beginning of assessment each day. The battery was administered during the light period between 1300 and 1700 hours, and the tests were given in the same order for each pup during each testing period. The following reflexes and responses were scored, and unless otherwise noted, they were scored as yes/no responses:

*Righting Reflex* Pup turns over with all four paws on the ground after being placed on its back. This test was measured as the amount of time that the pup took to perform the task to criterion. A maximum time of 30 s was given to complete the task. Pups not able to complete the task were given the maximum score of 30 s, and the failure to complete the task was noted.

*Postural Flexion and Extension* Pup spreads its forelimbs out to the side when picked up by the scruff of the neck.

*Vibrissae Placing* Pup places its forepaw onto a cotton swab stroked across its vibrissae.

*Forelimb/Hindlimb Grasp* Pup grips a wooden stick when stimulated with it on the plantar surface of its fore- or hindpaws.

*Visual Placing* Pup raises its head and extends its forelimbs in a placing response when suspended by the tail and slowly lowered toward a tabletop.

*Cliff Aversion* Pup will turn and crawl away from the edge after being placed with nose and forepaws at the edge of a tabletop or raised level surface.

*Negative Geotaxis* Pup will turn so that its nose is 180° from the starting position when placed facing down a 25° incline. The incline used was covered with a slip-resistant surface material so that the pups would not slide. The time taken to complete the test to criterion was recorded, and no time limits were imposed.

#### *Olfactory Orientation and Forelimb Grip Strength*

On PND 11, an olfactory orientation (“homing”) test was conducted on all pups. Prior to testing, each pup was weighed. Large rat cages were used, and one cup of clean, unused bedding material was placed at one end of the cage. One cup of soiled bedding from the pup’s home cage was placed at the opposite end of the cage. Each bedding patch was arranged so that each side was uniform in depth and in width. The pup was placed in the center of the cage, equidistant from the two piles of bedding, with its nose facing parallel to the two piles so that it was not facing either pile. The pup was given 120 s to explore the cage, and the amount of time it took to reach and bed in the home (soiled) litter was noted. If the pup never reached the soiled litter, or bedded in the clean litter, it was given a score of 120 s, and failure to complete the task to criterion was noted.

On PND 13, a forelimb grip strength test was administered to all pups. Prior to testing, all pups were weighed. An apparatus was constructed by taping a wooden dowel between two standard-size mouse cages so that when the pup was suspended it would be approximately 13 cm from the table surface. The pup was placed so that it was gripping the dowel with its forepaws and, after ensuring that the pup was gripping the bar, was gently

released by the experimenter. The time it took for the pup to lose its grip and fall was recorded, and a maximum time of 60 s was allotted.

### *Postweaning Tests*

Beginning on PND 60, a series of postweaning tests was administered. These tests were designed to measure the potential for lasting effects of chromium picolinate or picolinic acid exposure on motor coordination, anxiety, and memory.

A straight-channel swim test (modified from Broening et al. [42]) was administered to measure motor skills. The straight-channel swim test is a method of evaluation commonly used to assess motor skills as well as a comparison of the motivation of the subjects prior to other tasks requiring these skills [42–46]. Briefly, a 76×30 cm tank was filled with tap water to a depth of 23 cm the day before testing. The water was allowed to equilibrate to room temperature. A visible escape platform was mounted at one end of the tank. Each mouse was picked up by the scruff of the neck and placed in the water at the end opposite from the platform. The time that the mouse took to escape the water by crawling completely onto the platform was measured, and the swimming pattern was noted (straight, circular, wall hugging), as well as any unusual behaviors (jumping out of the tank, diving, etc.). A maximum of 90 s was given to complete the task, and if the task was not completed in the time allotted, the mouse was removed from the water by an experimenter. Four trials were administered over the course of 2 days, with two trials per day. Between the two daily swim trials, mice were dried and placed in a cage containing clean bedding with a heating pad under it until they were completely dry. The mice rested approximately 20 min between daily trials. The average time of the four trials was compared.

An open-field test (modified from Matzel et al. [47]) was conducted. This test is widely used to evaluate the behavior of the mice in novel situations and to assess locomotor function [47–49]. Arenas (60×60 cm) were constructed with white expanded polyvinyl chloride. The bottom of each arena was marked to make a 4×4 grid (16 total squares). Each mouse was placed in the center of the arena, and each trial lasted 5 min. Four trials were administered 24 h apart. The numbers of lines crossed (as measured by the mouse's front paws crossing a line), the numbers of rearing incidences, the total time spent in the center four squares, and the numbers of incidences of urination and defecation were measured.

An olfactory recognition memory test, which assesses an animal's ability to discriminate and remember odor-associated food rewards (modified from Matzel et al. [47]), was conducted in four same-day trials, with no maximum time limit. At the end of the light cycle, 2 days prior to testing, all food was removed from each cage. The mice were food-deprived for 24 h. They were then given milled rodent chow in standard feed jars in their cages and were allowed to feed for 15 min. At the end of 15 min, the feed jars were removed, and pelleted rodent chow was placed on top of their cages. The mice were then allowed to feed for 90 min, after which the feed was again removed until after testing the following day. This resulted in a food-deprivation period of approximately 16 h prior to testing. A cotton swab containing 25  $\mu$ l of one of three extracts (mint, almond, or orange) was placed in a food jar that contained milled rodent chow. The jars containing swabs with either the almond or orange extracts had mesh placed over their openings so that the feed was not accessible. The remaining jar that contained the cotton swab marked with the mint extract was designated the "target" jar and had accessible feed (no mesh). These odor-marked feed jars were placed in three corners of the arenas used for the open-field test. In the first trial only (the training trial), pelleted food was placed on top of the target jar. The

mouse was placed in the empty corner and allowed to find the target jar and eat the available food. The latency to retrieve the food and the number of errors were recorded. An error was noted if the animal went to the incorrect jar or went to the target jar but did not retrieve food. In each subsequent trial, the corner location of the target feed jar as well as its position relative to the other jars was changed, but its associated odor (mint) remained consistent through the four trials. The number of mistakes in each trial was averaged for the litter and analyzed. Cotton swabs were replaced every 5 min or at the end of a trial if the trial lasted longer than 5 min.

### *Histological Analysis*

After completion of all tests, offspring from the third and fourth replicates were euthanized by a CO<sub>2</sub> overdose. The brain of each mouse was removed whole, sectioned midsagittally, and placed in a 50% glutaraldehyde solution (Fisher Scientific, Fair Lawn, NJ, USA). One half of each brain was sent to Mass Histology (Worcester, MA, USA) for processing. Briefly, the brains were processed using a Tissue-Tek VIP processor, cut into 6- $\mu$ m sections with an Olympus 4060, and stained with Bielschowsky's silver stain. The CA3 region of the hippocampus of each brain half was examined for gross morphological defects, and the numbers of pyramidal neurons were counted in one section from each brain. Any specimen in which the hippocampus was not readily identifiable, appeared to have sustained damage during processing, or in which the CA3 region was not distinguishable from surrounding regions was not analyzed.

### *Statistical Analysis*

The litter served as the statistical unit for analyses. Body mass measurements and other non-categorical data, such as righting time from individual pups, were averaged for littermates for each observation day. Categorical data, such as results of the forelimb grasp or hindlimb grasp tests, were examined as the percentage of the litter that performed the various behaviors on each observation day.

The data from the swim test trials were averaged across the four trials. Open-field exploration data (number of lines crossed and number of rearing incidences) were also averaged across the four trials. In the odor-discrimination test, the data for each trial were averaged and analyzed separately. All tabular data are presented as the mean $\pm$ SEM. Data were analyzed by one-way analysis of variance (ANOVA), followed by a least significant difference post hoc test to determine specific significant differences ( $P\leq 0.05$ ) by use of the SPSS program (SPSS, Chicago, IL, USA).

Data from the histological examinations were averaged by litter and analyzed by ANOVA with the SPSS program (SPSS).

## **Results**

### Maternal Data

Maternal weight gain was not affected by the administration of Cr(pic)<sub>3</sub>. No signs of maternal toxicity were observed for dams in any of the groups. No difference in food consumption was noted among the groups, and average food consumption was

approximately 7 g diet per day. Cannibalism of some of the offspring did occur, but no differences in event frequency were observed among the treatment groups.

### Fetal Development

Offspring of picolinic acid-treated dams tended to weigh less than those of control- or Cr(pic)<sub>3</sub>-exposed dams, although the differences were not statistically significant (Fig. 1). No gross malformations were observed in offspring of the dams in any of the treatment groups, and no consistent differences in reflex development, as measured by the modified Fox battery, were observed among the treatment groups (data not shown).

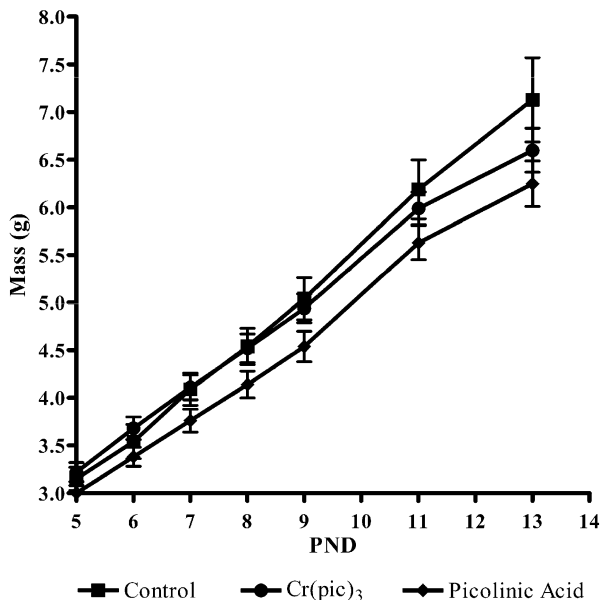
### Olfactory Orientation and Forelimb Grip Strength

Offspring of picolinic acid-exposed dams appeared to have diminished olfactory orientation ability compared to control or Cr(pic)<sub>3</sub>-exposed dams; however, these differences were not statistically significant. Forelimb grip strength also appeared to be diminished in the picolinic acid-exposed offspring, but this difference also was not statistically significant (Table 1).

### Postweaning Tests

Compared to control and Cr(pic)<sub>3</sub>-exposed offspring, picolinic acid-exposed offspring exhibited increased swim time in the straight-channel swim test and reduced overall activity (fewer lines crossed and incidences of rearing) in the open-field activity test. However, none of these differences was statistically significant. The offspring of picolinic acid-treated dams consistently made more mistakes in the odor-discrimination test,

**Fig. 1** Growth rates of mouse pups from dams treated with 200 mg kg<sup>-1</sup> day<sup>-1</sup> Cr(pic)<sub>3</sub>, 174 mg kg<sup>-1</sup> day<sup>-1</sup> picolinic acid, or untreated during pregnancy and lactation





**Table 2** Postweaning Behavior

	Treatment and Dose (mg kg <sup>-1</sup> day <sup>-1</sup> )		
	Control (0)	Cr(Pic) <sub>3</sub> (200)	Picolinic Acid (174)
Litters/pups examined	15/166	17/210	17/213
Open arena/lines crossed (number±SEM)	161.43±4.92	146.96±7.80	141.37±6.86
Open arena/rearing (number±SEM)	43.14±1.79	37.97±2.20	36.41±2.60
Swim time (±SEM) (s)	14.21±1.67	13.39±1.24	15.49±1.21
Odor-discrimination errors			
Trial 1 (number±SEM)	9.0±1.1	10.4±1.3	12.4±1.6
Trial 2 (number±SEM)	7.3±1.0	8.1±1.1	9.7±1.2
Trial 3 (number±SEM)	6.1±0.7	7.7±0.7	8.1±0.7
Trial 4 (number±SEM)	5.8±0.7	6.1±0.6	7.4±0.6

implying an impaired ability to learn, but the differences among groups were not significant (Table 2).

### Histological Examination

The treatments did not appear to affect the number of pyramidal neurons in the CA3 region of the hippocampus (Table 3). Picolinic acid-exposed mice had, on average, more neurons in the region than controls, but the difference was not statistically significant ( $P>0.05$ ). No evidence of gross morphological anomalies was found (e.g., spongiosis).

### Discussion

With the incidence of type 2 and gestational diabetes on the rise, supplements such as chromium picolinate, that promise to decrease insulin resistance and improve glucose metabolism, are very attractive. Although chromium picolinate has been suggested as therapeutic for women diagnosed with gestational diabetes [35], no published literature to date has investigated its effects on the neurological development in offspring exposed to it. This study examined chromium picolinate and its constituent ligand, picolinic acid, on such development. No significant differences were found among the treatment groups.

Most of the research involving kynurenine metabolites to date has focused on the excitotoxic properties of quinolinic acid [50–52]. Picolinic acid is a quinolinic acid partial antagonist, alleviating many of the seizures and behavioral deficits caused by intracerebral quinolinic acid administration, while not inducing any neurotoxic effects itself [50]. However, a 1995 study by Beskid et al. [33] indicated that administration of high levels of exogenous picolinic acid resulted in neurological insult to pyramidal neurons in the CA3

**Table 3** CA3 Pyramidal Neurons in the Hippocampus

	Treatment and Dose (mg kg <sup>-1</sup> day <sup>-1</sup> )		
	Control (0)	Cr(Pic) <sub>3</sub> (200)	Picolinic Acid (174)
Litters/samples examined	6/42	10/73	10/88
Number of neurons (number±SEM)	131.8±3.42	134.4±7.71	141.2±7.11

region of the hippocampus and accompanying spongiosis. Several possible explanations were offered, including interference with zinc metabolism, interference with L-kynurenine metabolism, and non-specific toxicity.

The connection between zinc and picolinic acid is an interesting one. Zinc is absolutely essential for cognitive development and continuing cognitive function, as studies with rodents and Rhesus monkeys have indicated that zinc deficiency results in severe cognitive deficits, particularly in spatial working memory [53, 54]. Similar results have been observed in humans, with zinc-deficient subjects displaying ataxia, depression, hallucinations, and deficiencies in memory [55–57]. While not all of the cellular and molecular roles of zinc are understood, zinc deficiency appears to affect memory by reducing the number of *N*-methyl-D-aspartate receptors, which have recently been shown to play a role in learning and memory, particularly in hippocampal long-term potentiation [58, 59]. Zinc deficiency during development results in cognitive deficits that appear to be permanent. Studies have indicated that such deficits persist long after zinc-adequate diets have been fed, if mild zinc deficiency is induced during gestation and lactation [60, 61].

Picolinic acid is a known zinc chelator. In fact, the chelation of endogenous zinc by picolinic acid has been proposed as the mechanism by which picolinic acid alleviates quinolinic acid toxicity [62]. In a study by Seal and Heaton [63], administration of exogenous picolinic acid in the diet resulted in a tenfold increase in urinary zinc loss and a 25-fold increase in excretion of injected  $^{65}\text{Zn}$ . Those authors concluded that picolinic acid increased zinc turnover without increasing retention, eventually leading to zinc depletion in the absence of adequate zinc supplementation. Also, subcutaneous administration of picolinic acid has been reported to lower plasma zinc concentrations in rats [64].

The offspring of picolinic acid- and, to a lesser extent, chromium picolinate-treated dams in this study tended to display behavior patterns similar to offspring of zinc deficient dams in other studies, e.g., hypoactivity, deficiencies in motor coordination, and impaired learning ability, although the differences observed were not significantly different from control offspring. Many of the neurobehavioral tests were conducted a significant time after weaning and therefore well past the time of potential exposure to picolinic acid. Thus if any of the apparent deficits that were observed occurred as a result of transplacental exposure or consumption of test diet by pups prior to weaning, they may have been long-term or permanent effects. Chelation of endogenous zinc (as has been shown by Seal and Heaton [63]) and exogenous zinc from the diet by the high amounts of picolinic acid administered in this study may have resulted in a zinc deficiency sufficient to impair neurological development in the offspring. Chromium picolinate appears to break down in the acidic environment of the stomach. It is cleaved into chromium and picolinate, which can be absorbed into the bloodstream [65]. The amount of picolinic acid released from the  $\text{Cr}(\text{pic})_3$  complex may have induced very mild, but nonetheless effective, maternal zinc deficiency, resulting in the data presented here.

In direct contrast to the results of Beskid et al. [33], we saw no evidence of spongiosis or overt insult to the pyramidal neurons of the CA3 region of the hippocampus. The differences in observations can most likely be attributed to differences in administration and dosage. In the Beskid et al. [33] study, the picolinic acid was administered via daily intraperitoneal injection, ensuring maximal absorption. In contrast, we administered the picolinic acid in the diet, better mimicking the normal route of human intake. Thus, only ~2% of the daily dose of picolinic acid in our study was likely absorbed. In addition, hippocampal insult was observed at dosages of 60 or 100 mmol/day in the Beskid et al. [33] study or approximately 7.38 or 12.31 g/day. Our study utilized a dosage of 0.007 g/day (based on an average mouse mass of 0.40 kg and a dosage of  $0.174 \text{ g kg}^{-1} \text{ day}^{-1}$ ), an

approximately 1,000-fold lower dosage. The dosages employed in our study are considerably higher than typical human exposures. Thus, it is highly unlikely that the dosages employed in the Beskid et al. [33] study would ever be encountered in human usage.

## Conclusions

Non-physiologically relevant concentrations were used in this study, and therefore, caution must be used in the interpretation of these results with regard to potential risk to humans. Our results indicate that there were no significant effects on the offspring with regard to neurological development from supplementation of the dams with either  $\text{Cr}(\text{pic})_3$  or PA.

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**Conflict of Interest Statement** J.B.V. is the inventor or co-inventor of four patents on the use of  $\text{Cr}_3$ ,  $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^+$ , as a nutritional supplement or therapeutic agent.

## References

1. Abraham A, Brooks B, Eylath U (1992) The effects of chromium supplementation on serum glucose and lipids in patients with and without non-insulin dependent diabetes. *Metabolism* 41:768–771
2. Anderson R, Polansky M, Bryden N, Canary J (1991) Supplemental-chromium effects on glucose, insulin, glucagon, and urinary chromium losses in subjects consuming controlled low-chromium diets. *Am J Clin Nutr* 54:909–916
3. Anderson R, Polansky M, Bryden N, Roginski E, Mertz W, Glinsmann W (1983) Chromium supplementation of human subjects: effects on glucose, insulin, and lipid parameters. *Metabolism* 32:894–899
4. Glinsmann W, Mertz W (1966) Effects of trivalent chromium on glucose tolerance. *Metabolism* 15:510–519
5. Gurson C, Saner G (1971) Effect of chromium on glucose utilization in marasmic protein-calorie nutrition. *Am J Clin Nutr* 24:1313–1319
6. Hopkins LJ, Ransome-Kuti O, Majaj A (1968) Improvement of impaired carbohydrate metabolism by chromium(III) in malnourished infants. *Am J Clin Nutr* 21:203–211
7. Press R, Gellar J, Evans G (1990) The effects of chromium picolinate on serum cholesterol and apolipoprotein fractions in human subjects. *West J Med* 152:41–45
8. Shinde U, Sharma G, Xu Y, Dhalla N, Goyal R (2004) Insulin sensitizing action of chromium picolinate in various experimental models of diabetes mellitus. *J Trace Elem Med Biol* 18:23–32
9. Anderson R (2000) Chromium in the prevention and control of diabetes. *Diabetes Metab* 26:22–27
10. Balk E, Tatsioni A, Lichtenstein A, Lau J, Pittas A (2007) Effect of chromium supplementation on glucose metabolism and lipids: a systematic review of randomized controlled trials. *Diabetes Care* 30:2154–2163
11. Li C, Ford E, Strine T, Mokdad A (2008) Prevalence of depression among U.S. adults with diabetes: findings from the 2006 behavioral risk factor surveillance system. *Diabetes Care* 31(3):420–426
12. Musselman D, Betan E, Larsen H, Phillips L (2003) Relationship of depression to diabetes types 1 and 2: epidemiology, biology, and treatment. *Biol Psychiatry* 54:317–329
13. McCarty M (1994) Enhancing central and peripheral insulin activity as a strategy for the treatment of endogenous depression—an adjuvant role for chromium picolinate? *Med Hypotheses* 43:247–252
14. Attenburrow M, Odontiadis J, Murray B, Cowen P, Franklin M (2002) Chromium treatment decreases the sensitivity of 5-HT<sub>2A</sub> receptors. *Psychopharmacology* 159:432–436
15. Franklin M, Odontiadis J (2003) Effects of treatment with chromium picolinate on peripheral amino acid availability and brain monoamine function in the rat. *Pharmacopsychiatry* 36:176–180

16. Davidson J, Abraham K, Connor K, McLeod M (2003) Effectiveness of chromium in atypical depression: a placebo-controlled trial. *Biol Psychiatry* 53:261–264
17. Docherty J, Sack D, Roffman M, Finch M, Komorowski J (2005) A double-blind, placebo-controlled, exploratory trial of chromium picolinate in atypical depression: effect on carbohydrate craving. *J Psychiatr Pract* 11:301–314
18. Khanam R, Pillai K (2006) Effect of chromium picolinate on modified forced swimming test in diabetic rats: involvement of serotonergic pathways and potassium channels. *Basic Clin Pharmacol Toxicol* 98:155–159
19. McLeod M, Golden R (2000) Chromium treatment of depression. *Int J Neuropsychopharmacol* 3:311–314
20. Stearns D, Wise SJ, Patierno S, Wetterhahn K (1995a) Chromium(III) picolinate produces chromosome damage in Chinese hamster ovary cells. *FASEB J* 9:1643–1648
21. Stearns D, Silveira S, Wolf K, Luke A (2002) Chromium(III) tris(picolinate) is mutagenic at the hypoxanthine (guanine) phosphoribotransferase locus in Chinese hamster ovary cells. *Mutat Res* 513:135–142
22. Stearns D, Belbruno J, Wetterhahn K (1995b) A prediction of chromium(III) accumulation in humans from chromium dietary supplements. *FASEB J* 9:1650–1657
23. Whittaker P, San R, Clarke J, Seifried H, Dunkel V (2005) Mutagenicity of chromium picolinate and its components in *Salmonella typhimurium* and L5178Y mouse lymphoma cells. *Food Chem Toxicol* 43:1619–1625
24. Speetjens J, Collins R, Vincent J, Woski S (1999a) The nutritional supplement chromium(III) tris(picolinate) cleaves DNA. *Chem Res Toxicol* 12:483–487
25. Gudi R, Slesinski R, Clarke J, San R (2005) Chromium picolinate does not produce chromium damage in CHO cells. *Mutat Res* 587:140–146
26. Slesinski R, Clarke J, San R, Gudi R (2005) Lack of mutagenicity of chromium picolinate in the hypoxanthine phosphoribosyltransferase gene mutation assay in Chinese hamster ovary cells. *Mutat Res* 585:86–95
27. Coryell V, Stearns D (2006) Molecular analysis of *hprt* mutations induced by chromium picolinate in CHO AA8 cells. *Mutat Res* 610:114–123
28. Bailey M, Boohaker J, Sawyer R, Behling J, Rasco J, Jernigan J, Hood R, Vincent J (2006) Exposure of pregnant mice to chromium picolinate results in skeletal defects in their offspring. *Birth Defects Res B* 77:244–249
29. Bailey M, Sturdivant J, Jernigan P, Townsend M, Bushman J, Ankareddi I, Rasco J, Hood R, Vincent J (2007) Comparison of the potential for developmental toxicity of prenatal exposure to two dietary chromium supplements, chromium picolinate and  $[\text{Cr}_3\text{O}(\text{O}_2\text{CCH}_2\text{CH}_3)_6(\text{H}_2\text{O})_3]^{+}$ , in mice. *Birth Defects Res B* 83(1):27–31
30. Beninger R, Colton A, Ingles J, Jhamandas K, Boegman R (1994) Picolinic acid blocks the neurotoxic but not the neuroexcitant properties of quinolinic acid in the rat brain: evidence from turning behaviour and tyrosine hydroxylase immunohistochemistry. *Neuroscience* 61:603–612
31. Blasi E, Mazzolla R, Pitzurra L, Barluzzi R, Bistoni F (1993) Protective effect of picolinic acid on mice intracerebrally infected with lethal doses of *Candida albicans*. *Antimicrob Agents Chemother* 37(11):2422–2426
32. Leuthauser S, Oberley L, Oberley T (1982) Antitumor activity of picolinic acid in CBA/J mice. *JNCI* 68:123–126
33. Beskid M, Jachimowicz J, Taraszewska A, Kukulska D (1995) Histological and ultrastructural changes in the rat brain following systemic administration of picolinic acid. *Exp Toxic Pathol* 47:25–30
34. Crowther C, Hiller J, Moss J, McPhee A, Jeffries W, Robinson J (2005) Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *New Engl J Med* 352:2477–2486
35. Jovanovic L, Gutierrez M, Peterson C (1999) Chromium supplementation for women with gestational diabetes mellitus. *J Trace Elem Exp Nutrition* 12:91–97
36. A.D. Association (2004) Gestational diabetes mellitus. *Diabetes Care* 27:S88–S90
37. Chakov N, Collins R, Vincent J (1999) A re-investigation of the electronic spectra of chromium(III) picolinate complexes and high yield synthesis and characterization of  $\text{Cr}_2(\mu\text{-OH})_2(\text{pic})_4\cdot 5\text{H}_2\text{O}$  (Hpic = picolinic acid). *Polyhedron* 18:2891–2897
38. Fox W (1965) Reflex-onotogeny and behavioural development of the mouse. *Anim Behav* 13:234–241
39. Bignami G, Musi B, Dellomo G, Laviola G, Alleva E (1994) Limited effects of ozone exposure during pregnancy on physical and neurobehavioral development of CD-1 mice. *Toxicol Appl Pharmacol* 129:264–271
40. Calamandrei G, Venerosi A, Branchi I, Valanzano A, Puopolo M, Alleva E (1999) Neurobehavioral effects of prenatal lamivudine (3TC) exposure in preweaning mice. *Neurotoxicol Teratol* 21:365–373

41. Petruzzi S, Fiore M, Dell'omo G, Bignami G, Alleva E (1995) Medium and long-term behavioral effects in mice of extended gestational exposure to ozone. *Neurotoxicol Teratol* 17:463–470
42. Broening H, Morford L, Inman-Wood S, Fukumura M, Vorhees C (2001) 3,4-Methylenedioxymethamphetamine (ecstasy)-induced learning and memory impairments depend on the age of exposure during early development. *J Neurosci* 21:3228–3235
43. Vorhees C, Schaefer T, Williams M (2007) Developmental effects of  $\pm$ 3,4-methylenedioxymethamphetamine on spatial versus path integration learning: effects of dose distribution. *Synapse* 61:488–499
44. Vorhees C, Skelton M, Williams M (2007) Age-dependent effects of neonatal methamphetamine exposure on spatial learning. *Behav Pharmacol* 18:549–562
45. Williams M, Moran M, Vorhees C (2003) Refining the critical period for methamphetamine-induced spatial deficits in the Morris water maze. *Psychopharmacology* 168:329–338
46. Williams M, Morford L, Wood S, Wallace T, Fukumura M, Broening H, Vorhees C (2003) Developmental D-methamphetamine treatment selectively induces spatial navigation impairments in reference memory in the Morris water maze while sparing working memory. *Synapse* 48:138–148
47. Matzel L, Han Y, Grossman H, Karnik M, Patel D, Scott N, Specht S, Gandhi C (2003) Individual differences in the expression of a “general” learning ability in mice. *J Neurosci* 23:6423–6433
48. Palenicek T, Hlinak Z, Bubenikova-Valesova V, Votava M, Horacek J (2007) An analysis of spontaneous behavior following acute MDMA treatment in male and female rats. *Neuro Endocrinol Lett* 28(6):781–788
49. Palenicek T, Votava M, Bubenikova V, Horacek J (2005) Increased sensitivity to the acute effects of MDMA (“ecstasy”) in female rats. *Physiol Behav* 86:546–553
50. Boegman R, Jhamandas K, Beninger R (1990) Neurotoxicity of tryptophan metabolites. *Ann N Y Acad Sci* 585:261–273
51. Giordano M, Calderon S, Norman A, Sandberg P (1987) The quinolinic acid model of Huntington’s disease: dose-dependent effects on behavior. *Soc Neurosci Abstr* 13:1360
52. Lapin I, Prakhie I, Kiseleva I (1986) Antagonism of seizures induced by the administration of the endogenous convulsant quinolinic acid into rat brain ventricles. *Neural Transm* 65:177–185
53. Caldwell D, Oberleas D, Clancy JJ, Prasad A (1970) Behavioral impairment in adult rats following acute zinc deficiency. *Proc Sc Exp Biol Med* 133:1417–1421
54. Golub M, Keen C, Gershwin M, Hendrickx A (1995) Developmental zinc deficiency and behavior. *J Nutr* 125:2263S–2271S
55. Henkin R, Patten B, Re P, Bronzert D (1975) A syndrome of acute zinc loss. Cerebellar dysfunction, mental changes, anorexia, and taste and smell dysfunction. *Arch Neurol* 32:745–751
56. Kay R, Tasman-Jones C, Pybus J, Whiting R, Black H (1976) A syndrome of acute zinc deficiency during total parenteral alimentation in man. *Ann Surg* 183:331–340
57. Moynahan E (1976) Letter: Zinc deficiency and disturbances of mood and visual behavior. *Lancet* 1:91
58. Browning J, O’Dell B (1995) Zinc deficiency decreases the concentration of *N*-methyl-D-aspartate receptors in guinea pig cortical synaptic membranes. *J Nutr* 125:2083–2089
59. Zhao M, Toyoda H, Lee Y, Wu L, Ko S, Zhang X, Jia Y, Shum F, Xu H, Li B et al (2005) Roles of NMDA NR2B sybtype receptor in prefrontal long-term potentiation and contextual fear memory. *Neuron* 47:859–872
60. Halas E, Eberhardt M, Diers M, Sandstead H (1983) Learning and memory impairment in adult rats from mildly zinc deficient dams. *Physiol Behav* 30:371–381
61. Halas E, Hunt C, Eberhardt M (1986) Learning and memory disabilities in young adult rats from mildly zinc deficient dams. *Physiol Behav* 37:451–458
62. Jhamandas K, Boegman R, Beninger R, Flesher S (1998) Role of zinc in blockade of excitotoxic action of quinolinic acid by picolinic acid. *Amino Acids* 14:257–261
63. Seal C, Heaton F (1985) Effect of dietary picolinic acid on the metabolism of exogenous and endogenous zinc in the rat. *J Nutr* 115:986–993
64. Krieger I, Statter M (1987) Tryptophan deficiency and picolinic acid: effect on zinc metabolism and clinical manifestations of pellagra. *Am J Clin Nutr* 46:511–517
65. R. T. Institute, Project report on [<sup>14</sup>C] chromium picolinate monohydrate: disposition and metabolism in rats and mice, submitted to the National Institute of Environmental Sciences, National Institutes of Health, (February 28, 2002)