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RESPONSE

We are pleased to respond to Dr. Symms' letter (16) critiquing our paper on *Growth and Behavioral Effects of early postnatal chromium and manganese exposure in Herring Gull (Larus argentatus) chicks* (8). It is always gratifying to know that ones work receives such close scrutiny and thoughtful consideration.

Our paper was concerned primarily with utilizing our established neurobehavioral paradigm (5,6,9) to evaluate possible neurotoxicity of manganese and chromium. The relationship to risk was a secondary issue. Symms' critique focuses largely on the implications of our findings for risk assessment for chromium, an issue we have addressed previously (12).

Among the many points raised, Symms has identified two that are significant. We did inject our birds with chromium nitrate, and Symms rightly asks whether the effects of chromium can be distinguished from those of the nitrate. This is not entirely disingenuous, for we raised the same concern in our studies of lead. In our extensive studies of lead we have used both lead acetate and lead nitrate, and found similar, but not identical dose-response curves. We will pursue the neurotoxicity of chromium with other salts. However, our prior work with lead does not support the suggestion that the nitrate was responsible for our neurobehavioral findings.

Symms questions the use of the intraperitoneal route rather than the oral route. This point has been raised by others, and we have addressed it in some of our previous publications on neurobehavioral toxicity of lead in developing birds. Where controlling dose is important, we have found it unreliable to use daily dosing of food in our avian model. Moreover, we found that the stress of daily gavage dosing interfered with the behavioral testing regime. The single high level dose is a reasonable possibility for Herring Gulls in the wild, since the adults obtain food for their young from a variety of sources including landfills.

We have indeed investigated the effects of a single dose of lead compared to divided doses (6,9), and there are many interesting similarities and differences, that we expect to explore in the future with chromium.

Symms cites the EPA Wildlife Exposure Factors Handbook (17) which states that adult Herring Gulls consume 150 to 320 g/day. Adult Herring Gulls can maintain their weight on 300 g of fish/day, but frequently consume much more than that. Although a 65 g chick on hatching day is likely to consume only 30–50 g in its first day, chicks one week of age, weighing over 150 g, readily consume their own weight daily if fed ad libidum. Obviously they produce much greater excreta, since the amount they can assimilate is limited. Thus growth rate is not a linear function of food intake. The relationship between ingestion rates and intestinal absorption rates for chromium in this species deserves study.

It was reasonable for Symms to cite the EPA document, which, by the way, draws heavily on our past studies of this species, citing 11 of our papers (12% of all papers cited). Two of those papers (2,4) relate specifically to feeding, but rarely is one able to quantify food intake in the field. Extensive field observations will be required to assess the distribution of food ingestion rates for this species at different ages. However, the quantity of food ingested obviously influences the exposure assessment, but is not relevant to the question of whether chromium is neurotoxic in this model.

During the first half of the 20th century, chromium processing factories produced large quantities of chromium-rich waste which was distributed over many sites in northern New Jersey (10). Many of those sites were later developed for residential housing. In discussing the form of chromium in the chromite ore processing residue, Symms may be confusing bioavailability with toxicity. It is true that there was a low concentration of water soluble trivalent chromium compounds in many of the soil samples. This would impact bioavailability, but our work addressed the toxicity of trivalent chromium. Trivalent chromium comprised the majority of the chromium contamination (10). Hexavalent chromium, the carcinogenic species, was also present in exceedingly high concentrations in some areas of Hudson County, New Jersey (10). It passes cell membranes much more readily than the trivalent form, but is readily reduced to trivalent chromium both intracellularly and extracellularly (i.e. in the gasterointestinal tract) (15).

The literature on possible neurotoxic effects of chromium is remarkably sparse. The Agency for Toxic Substances and Disease Registry (ATSDR) published an extensive literature review (1), which has negligible information on the nervous system, and emphasizes the paucity of neurobehavioral testing of dosed animals or exposed humans.

The Heinz and Haseltine (13) study mentioned by Symms, used a different avian model and a different behavioral test—one on which we cannot comment, except to say that different behavioral modalities are affected differently by neurotoxicants. Even lead with its broad spectrum effects, impacts certain modalities more than others, both in animals and humans, and organic and inorganic species had different effects (14). Moreover, the lack of effect in the Heinz and Haseltine study may have been due to the poor intestinal absorption to which Symms alluded.

One study (11) found a decrease in motor activity and balance at a dose of 98 mg/kg/day of sodium chromate (oral 28 day exposure). This is a hexavalent compound. The fact that in our test system trivalent chromium shows some of the same neurodevelopmental effects as lead, is therefore of consequence.

Most of Symms' letter is concerned with risk assessment issues of bioavailability and absorption, which are largely irrelevant to our experimental results, although they are highly relevant to the public health risks around the 150+ contaminated sites in New Jersey (10).

Symms says little about manganese, but this deserves response as well, since judicial decisions have recently cleared the way for petroleum companies to add an organic manganese to gasoline as an antiknock compound. Manganese is a well-known neurotoxin which affects the basal ganglia. We took

the opportunity to see whether we could detect neurodevelopmental effects in our system. At the doses used, manganese tended to affect more behavioral components than chromium.

Symms states that the "acetic acid salt of manganese cannot possibly provide a good representation of native forms of manganese." He is presuming that exposure would be mainly to naturally occurring oxides of manganese in soil. But with the use of manganese as a gasoline additive, this assumption is premature. Again our priority was to provide manganese in an absorbable form which could be taken up by the blood stream from the peritoneal cavity. Even when manganese oxides, ingested with soil, are absorbed, the manganese moves as ions not salts, and the same is true for the injected manganese acetate. In our paper we clearly and redundantly state: "This experiment represents the first in a series of dose-response tests that are required before the actions of these metals can be understood" and "Determining the dose-response relationship for chromium and manganese requires a series of experiments with different doses."

Symms was confused by our choice of words describing when we weighed the birds. We should have been clearer. In the discussion section we stated: "The major methodologic problem with this study is that the behavioral tests were performed from 18–48 days of age (from 16–46 days postinjection). We initially did not weigh or test the birds because we wanted to avoid added stress. We used this protocol because, without previous research on sublethal effects of chromium and manganese, we were unsure of the appropriate dose. However, future studies should examine the immediate effects of chromium and manganese."

"Initially" in this context, meant after the injection. Symms read this to mean that we did not weigh the chicks prior to randomization and injection. Our methods section states that "Chicks were marked with numbered leg bands, and randomly allocated to one of three treatment groups. There were no significant differences in initial weights among chicks." Our abstract says that the groups were matched by age and weight.

To clarify matters: all chicks were weighed prior to injection, at the time of randomization. In this study they were not weighed again until 18 days of age (16 days post-injection).

Overall we believe we were properly cautious in interpreting our results and we concluded that:

"The behavioral deficits we observed in the laboratory relate directly to growth and survival of the chicks in the wild, and suggest that chromium- and manganese-impaired chicks would have lower survival if such exposure were achieved in the wild." This remains a true statement. Whether such exposures are achieved in the wild, requires direct field studies not supposition or assumption.

There are many opportunities for future studies of these two heavy metals in a variety of models. We did not anticipate that chromium would produce a broad range of neurodevelopmental effects.

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Michael Gochfeld

EDITOR'S NOTE: Dr. Michael Gochfeld served as principal investigator on a chromium exposure screening project funded by the New Jersey Department of Health, the protocol and results of which were subject to peer review. He also has testified for patients exposed to chromium.

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