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# COMMENTS ON THE PURPORTED GENERATION OF FORMALDEHYDE AND ADDUCT FORMATION FROM THE SWEETENER ASPARTAME

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Abstract. A recent paper by Trocho *et al.* (1) describes experiments meant to show that formaldehyde adducts are formed when rats are administered the sweetener aspartame. These authors assume that the methanol carbon of aspartame generates formaldehyde which then forms adducts with protein, DNA, and RNA. Doses employed range widely. In this letter, studies which have been published previously and which were not cited by these authors are reviewed in order to put into perspective the disposition of methanol and formaldehyde in monkeys and humans, species relevant to the toxicity of methanol and its toxic metabolite, formic acid. © 1999 Elsevier Science Inc.

*Key Words:* aspartamine, formaldehyde, adduct formation, protein, DNA, RNA

## Comments

A recent paper published in LIFE SCIENCES, **"Formaldehyde Derived from Dietary Aspartame Binds to Tissue Components *In Vivo,*"** by Trocho *et al.* (1) requires comment. The authors conclude, based on their data, that "aspartame consumption may constitute a hazard because of its contribution to the formation of formaldehyde adducts." In their studies, acute doses of $^{14}$C-aspartame (20 mg/kg) were administered to normal and cirrhotic rats, with and without 10 day pretreatment with oral aspartame (200 mg/kg). A low level of radioactivity was observed in protein, DNA and RNA isolated from various tissues, especially liver. The authors assume that the label was derived directly from formaldehyde and that adducts of formaldehyde with protein, DNA and RNA resulted. It should be noted that the authors did not employ $^{14}$C-labeled methanol (at any dose) or $^{14}$C-labeled formaldehyde to support or complement their claims except to show that albumin will react with formaldehyde *in vitro* as is well known. Thus, one must question their interpretation that methanol generated from aspartame leads to adduct formation. Furthermore, the use of rats (or for that matter any animal species other than primates or humans) in studies of methanol disposition must take into account that methanol does not produce toxicity in this species (2). The doses of aspartame that the authors used do not even yield methanol concentrations in blood or tissues above control values (3).

Formaldehyde concentrations in blood or tissues of lower species have never been shown to increase above control levels in blood or tissues even after high doses of methanol (4). Since monkeys are sensitive to methanol, our laboratory studied monkeys and folate-deficient monkeys, which are supersensitive to methanol poisoning (5). Several specific techniques were used to capture and quantify the amount of formaldehyde produced from large doses (3g/kg) of $^{14}$C-methanol in these monkeys and, in addition, to apply these techniques after a dose of $^{14}$C-formaldehyde was administered. Body fluids and a number of tissues (including freeze-clamped liver) were evaluated for changes in formaldehyde over time. Radioactivity in fluids or tissues derived from methanol and captured by the aldehyde dimedon technique or formaldehyde bound to semicarbazide and distilled from acid showed no significant elevations after methanol (3g/kg). Monkeys, normal and folate-deficient, injected with $^{14}$C-formaldehyde were also studied. A half-life of disappearance of formaldehyde from blood of 1.5 minutes was observed in both animals. This result supported data obtained from other laboratories where formaldehyde disappearance from the blood of dogs, cats, rabbits and guinea pigs ranged from 1-2 minutes (6). As expected, high levels of formic acid was found in every monkey where methanol or formaldehyde was administered. Formate is the methanol metabolite that is responsible for the metabolic acidosis and the ocular toxicity (7) of methanol. These direct studies of methanol in monkeys at large doses cast considerable doubt on the proposal that formaldehyde adducts are being formed from extremely low doses of methanol or aspartame.

Formic acid accumulation is responsible for the toxicity of formaldehyde. Eells *et al.* (8) studied a case of human formaldehyde intoxication and observed a rapid and massive formation of formic acid within minutes after ingestion which led to a blood pH of 6.9 thirty minutes after ingestion of a fatal dose of formaldehyde. The half-life of formaldehyde disappearance from the blood was about 1.5 minutes in this patient. A similar oral and lethal dose in rats led to the rapid accumulation of formate and metabolic acidosis (8).

The metabolism and disposition of methanol in rats, monkeys and, presumably humans, has been reviewed recently (7). In spite of the difference in the disposition of methanol and its metabolite formate in higher mammals compared to lower species, many investigators continue to use lower species in deriving information from methanol, be it from methanol itself or from methanol derived from aspartame (1). Methanol, like ethanol, oxidation is catalyzed in monkeys and humans by hepatic alcohol dehydrogenase(s). Formaldehyde formed from methanol is rapidly converted to formate in reactions involving a specific formaldehyde dehydrogenase and an hepatic thiolase (9,10). In this reaction, the formaldehyde carbon is converted to S-hydroxymethylglutathione which serves as substrate along with NAD for the formaldehyde dehydrogenase. The product of the reaction is S-formylglutathione. This product is then rapidly converted by the thiolase to formate. Formate enters the folate biochemical pathway and the one carbon moiety, through a series of reductions, flows to DNA, RNA, proteins, amino acids and phospholipids in reactions catalyzed by numerous enzymes of the pathway. The final step is considered to be S-adenosylmethionine formation, which is the active energy intermediate for methylation reactions.

Formate is also oxidized through 10-formyltetrahydrofolate dehydrogenase (10-FDH) activity to $CO_2$ and tetrahydrofolate (7). This latter reaction is the key to the toxicity of methanol in humans where massive amounts of one carbon units as formate are found. Humans and monkeys are relatively deficient in folate and 10-FDH, features

that account for the accumulation of formic acid in humans and monkeys. Makar and Tephly (11) showed that folate-deficient rats could also be intoxicated with methanol. This was the first demonstration of methanol toxicity in lower animals. These animals displayed metabolic acidosis and formic acidemia. Garner *et al.* (12) and Eells *et al.* (13) have used folate-deficiency or perturbation of the folate pathway by nitrous oxide, respectively, to produce ocular toxicity from methanol in rats.

Aspartame has also been extensively studied. Of particular relevance are studies related to the acute disposition of the aspartame methanol carbon in humans and the long term studies of high dose aspartame in humans. Stegink *et al.* (3) showed that at doses of up to 100 mg/kg of aspartame, methanol blood levels in humans were not significantly elevated above control values. Abuse doses of 100, 150 and 200 mg/kg administered to human subjects by Stegink *et al.* (3) led to slight increases of methanol above control values. Peak blood levels found were predictable based on complete conversion of the aspartame methyl carbon to methanol and immediate distribution to body water. Blood formate levels were not increased above control values in these individuals but, at the 200 mg/kg dose, urinary formate values were significantly elevated.

What about long term chronic high doses of aspartame? Leon *et al.* (14) studied 108 male and female volunteers aged 18-62 years who were administered either 75 mg/kg of aspartame per day, about 25 times the 90th percentile daily consumption of aspartame (15), or placebo for 24 weeks. No significant changes in blood or urine methanol or formate were found. Furthermore, clinical chemical values, amino acid levels and the number of subjects experiencing symptoms were not significantly different between groups. Thus, consumption of aspartame at many times the 90th percentile daily consumption level in humans would not lead to methanol blood or urine levels above control values in humans. Even at experimental doses impossible to obtain from aspartame sweetened foods no toxicity related to the methanol carbon of aspartame has been found.

It is known that the consumption of fruit and certain fruit juices and alcoholic beverages can contribute to body methanol (16). Pectin-containing fruits, fruit juices and brandy have been shown to yield elevated blood levels of methanol which are lower than those obtained by drinking aspartame-containing beverages. To be able to measure an elevation of blood methanol above control one must ingest a dose of about 100 mg/kg of aspartame. This would be the equivalent of one half can of diet beverage (100 mg) per kilogram of body weight, or about 25 cans at one time for a 50 kg person, in order to detect an elevation in blood methanol level.

The finding of a small percentage of radioactive carbon (one per cent) from methanol in protein, DNA or RNA as reported by Trocho *et al.* (1) might be accounted for by consideration of the very rapid flux of the carbon through S-adenosylmethionine or other folate dependent reactions. This flux is very rapid and can be seen when nitrous oxide is administered to rats, a treatment that slows down the methionine synthetase reaction (17). We believe that the normal flux of one carbon moieties whether derived from pectin, aspartame or fruit juices is a physiologic phenomenon and not a toxic event. Methylation of proteins, DNA and RNA is known whereas formaldehyde adduct formation *in vivo* has yet to be proven to be generated from aspartame. Based on the experimental data presented, aspartame appears not to constitute a hazard.

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