NeuroToxicology 29 (2008) 476–477

Contents lists available at ScienceDirect

NeuroToxicology

Letters to the Editor

Developmental neurotoxicity: When research succeeds through inappropriate statistics

Decabromodiphenyl ether (CASRN 1163-19-5; BDE-209) (MW = 959.2) is the primary component of a commercial flame retardant, with a no-adverse-effect-level of at least 1000 mg/kg day in chronic, repeated dose studies in rats and mice, and a no-effect-level of 1000 mg/kg day for maternal and fetal toxicity when administered on gestation days 0–19 to rats (Hardy, 2002; Hardy et al., 2002; U.S. NTP, 1986). Viberg et al. (2008) report that a single dose of BDE-209 (20.1 mg/kg bw) to neonatal mice on postnatal day (PND) 3 alters brain protein expression on PND 10 and "[f]urther strengthen our findings concerning PBDE 209 as a developmental neurotoxicological agent".

Viberg et al. (2008) uses the same methodology as two other papers in NeuroToxicology, Viberg et al. (2007) and Johansson et al. (2008). In all three, the authors base their conclusions on statistics using the individual pup as the experimental unit. Two of the three publications claim that random selection of pups from at least 3 litters has "[t]he same statistical effect and power compared to the use of litter-based studies" (Viberg et al., 2007; Johansson et al., 2008). This is not the accepted norm in developmental studies where controlling for the "litter effect" is a priori (U.S. EPA, 2000; OECD, 2007). Holson et al. (2007) said "[t]reating multiple offspring from the same litter as independent subjects is a fundamental violation of assumptions that can severely inflate alpha levels". Mice are litter-bearing animals, littermates are more alike than non-littermates, and pups from the same litter are not independent of one another (Festing, 2006; Holson et al., 2007).

Viberg et al. (2007, 2008) and Johansson et al. (2008) rely on inappropriate statistics as the basis for the reported treatment-related effects. It is unfortunate that this problem was not recognized during the peer-review process. We agree with Holson et al. (2007) that "[i]gnoring litter effects in the statistical analysis of DNT studies is simply not an acceptable practice".

Disclaimer

Albemarle Corporation is a global specialty chemical manufacturer whose product line includes decabromodiphenyl ether.

References

Festing MR. Design and statistical methods in studies using animal models of development. ILARJ 2006;47:5–14.

Hardy M. The toxicology of the three commercial polybrominated diphenyl oxide (ether) flame retardants. Chemosphere 2002;46:757–77.

Hardy M, Schroeder R, Biesemeier J, Manor O. Prenatal oral (gavage) developmental toxicity study of decabromodiphenyl oxide in rats. Int J Toxicol 2002;21:83–91.

Holson RR, Freshwater L, Maurissen JP, Moser VC, Phang W. Statistical issues and techniques appropriate for developmental neurotoxicity testing. A report from the ILSI Risk Science Institute Expert Panel on neurodevelopmental endpoints. Neurotoxicol Teratol 2007. doi: 10.1016/j.ntt.2007.06/001.

Johansson N, Fredriksson A, Eriksson P. Neonatal exposure to perfluoooactane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. Neurotoxicology 2008;29:160–9.

OECD. Guideline for the testing of chemicals—developmental neurotoxicity study. 2007;426:1–26.

U.S. EPA. Benchmark dose technical guidance document, EPA/630/R-00/001; 2000. p. 1–87.

U.S. NTP. Toxicology and carcinogensis studies of decabromodiphenyl oxide (CAS No. 1163-19-5) in F344/N rats and B6C3F1 mice (feed studies). Technical report series. 1986;309:1–242.

Viberg H, Fredriksson A, Eriksson P. Changes in spontaneous behaviour and altered response to nicotine in the adult rat, after neonatal exposure to the brominated flame retardant, decabrominated diphenyl ether (PBDE 209). Neurotoxicology 2007;28:136–42.

Viberg H, Mundy W, Eriksson P. Neonatal exposure to decabrominated diphenyl ether (PBDE 209) results in changes in BDNF, CaMKII and GAP-43, biochemical substrates of neuronal survival, growth, and synaptogenesis. Neurotoxicology 2008;29:152–9.

Marcia Hardy*
Todd Stedeford
Albemarle Corporation, Health, Safety & Environment, 451 Florida Street, Baton Rouge, LA 70801, United States

*Corresponding author. Tel.: +1 225 388 7616; fax: +1 225 388 7046
E-mail address: marcia_hardy@albemarle.com (M. Hardy)

7 February 2008
Available online 16 February 2008

doi:10.1016/j.neuro.2008.02.002

Potential proconvulsant effects of oral zinc supplementation–A case report

Keywords:
Zinc
Seizure
Epilepsy

Dear Editor,

Based on anecdotal information disseminated on the World Wide Web, there is a popular misconception that individuals with

seizure disorders require oral zinc supplementation. We report a case of an individual who experienced a serious exacerbation of his seizures following oral zinc supplementation.

A 28-year-old male presented with a 16-year history of seizures. His seizures commenced with an epigastric "rising sensation", followed by an unconscious staring spell lasting 1–2 min; occasionally, these staring spells would generalize to tonic–clonic convulsions. His seizures were being treated with phenytoin (300 mg/day) and occurred approximately twice per year. In an attempt to "take control of his health", he collected data about seizures from the World Wide Web and became concerned that a "zinc deficiency" was causing his seizures. Accordingly, he began to take oral zinc supplementation. He reported self-administering 64 mg of elemental zinc per day (*i.e.* 240 mg zinc gluconate orally twice per day); he selected this dose (without guidance from a physician or dietician) based solely upon information he obtained on the World Wide Web. (He was already consuming a "balanced, normal" meat-containing 2100 kcal/day diet, which provided approximately 9–12 mg zinc/day; the recommended daily allowance of zinc for adult males is 10–15 mg.) Two days after starting the zinc supplementation, he experienced six complex partial seizures in 1 day, with two of these seizures generalizing to tonic–clonic convulsions. The following day, he experienced three complex partial seizures. He discontinued the zinc and had no further seizures. One month later, he re-initiated zinc therapy (at the same dose) and once again suffered a seizure recurrence (seven seizures over 3 days), which again abated following discontinuation of the zinc supplement. His electroencephalogram demonstrated a previously documented right frontotemporal epileptiform focus; CT/MRI imaging was normal. There was no change in the serum concentration of his phenytoin, and he was on no other medications (prescribed or over-the-counter).

Multiple studies suggest that altered zinc homeostasis in brain contributes to epileptic seizures (Koh, 2001). Zinc is one of the most abundant transition metals in the brain, with particularly high concentrations in the hippocampus, a region known to play a central role in the pathogenesis of seizures. Within such proconvulsant areas of brain, Zn^{2+} contributes to electrical hyperexcitability by modulating multiple ligand-gated ion channels including glutamate receptors and $GABA_A$ receptors, as well as affecting the voltage-gated ion channel family including K^+, Na^+, and Ca^{2+} channels (Mathie et al., 2006). This proposed proconvulsant mechanistic role for zinc in epilepsy has received *in vivo* study, primarily in animal models (Moreno et al., 2006). These animal studies have demonstrated higher Zn^{2+} concentrations in the brain of epileptic mice than control mice, and have shown that these elevated Zn^{2+} concentrations lead to neuronal excitation and a proconvulsant effect by inhibiting $GABA_A$ receptor function and by stimulating NMDA-mediated glutamatergic activity (Buhl et al., 1996; Hirate et al., 2002; Mathie et al., 2006). Thus, the seizure-inducing influence of Zn^{2+} is complex, but primarily mediated by ligand-gated ion channel proteins.

The complex role of Zn^{2+} in epilepsy is further complicated by speculations concerning anticonvulsant-mediated Zn^{2+} deficiency. However, in a study with 114 epileptic patients and 30 volunteers, Kuzuya et al. (1993) demonstrated that both Zn^{2+} and Cu^{2+} concentrations in epileptic patients receiving chronic anticonvulsant therapy (including phenytoin) differed only slightly from control levels, remaining within the normal range and indicating that there is no need to supplement either of these trace elements in people with seizure disorders.

In conclusion, the exact role of zinc in human epilepsy is incompletely evaluated, and zinc supplementation remains controversial (Moreno et al., 2006). Nevertheless, clinicians should be aware to the possibility that oral zinc supplementation could predispose to an exacerbation of an underlying seizure disorder—a clinically relevant observation in view of the growing problem with uncontrolled overuse of nutraceuticals and supplements in people with neurological disorders.

References

Buhl EH, Otis TS, Mody I. Zinc-induced collapse of augmented inhibition by GABA in a temporal lobe epilepsy model. Science 1996;271:369–73.

Hirate A, Takeda A, Tamano H, Enomoto S, Oku N. Distribution of trace elements in the brain of EL (epilepsy) mice. Epilepsy Res 2002;51:109–16.

Koh JY. Zinc and diseases of the brain. Mol Neurobiol 2001;24:99–106.

Kuzuya T, Hasegawa T, Shimizu K, Nabeshima T. Effect of anti-epileptic drugs on serum zinc and copper concentrations in epileptic patients. Int J Clin Pharmacol Ther Toxicol 1993;31:61–5.

Mathie A, Sutton GL, Clarke CE, Veale EL. Zinc and copper: pharmacological probes and endogenous modulators of neuronal excitability. Pharmacol Ther 2006;111:567–83.

Moreno CB, Gutierrez-Alvarez AM, Gonzalez-Reyes RE. Zinc and epilepsy: is there a causal relationship between them? Rev Neurol 2006;42:754–9.

A. Laine Green
Donald F. Weaver*
Department of Medicine, Dalhousie University, 5849 University Avenue, Halifax, Nova Scotia, Canada B3H 4H7

*Corresponding author. Tel.: +1 902 473 2130
E-mail address: donald.weaver@dal.ca
(D.F. Weaver)

24 January 2008
Available online 7 April 2008

doi:10.1016/j.neuro.2008.03.008