Mini-review

Is DEET a dangerous neurotoxicant?

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Abstract

Controversies surrounding the safety of *N,N*-diethyl-*meta*-toluamide (DEET) when used as an insect repellent are centered around conflicting findings in the scientific literature and inaccurate reporting in the public media. Lethal cases of DEET poisoning are few, and usually due to deliberate or other overdoses that ignore product label instructions. Deleterious effects of DEET typically involve skin reactions and even when encephalopathies, such as seizures, occur they often abate without sequelae. Recent mode-of-action studies prove it has little direct effect on acetylcholinesterase, and have identified G protein-coupled receptors as a site of action deserving of further investigation. Studies with pregnant women found that DEET had no effect on the developing fetus from proper use and its continued deployment as a repellent is endorsed by both the Centers for Disease Control and Prevention and the Environmental Protection Agency, with specific recommendations of how it should be used on children.
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1 INTRODUCTION

The insect repellent *N,N*-diethyl-*meta*-toluamide (DEET) is considered to be the 'gold-standard' for insect repellents and has been widely used since the early 1950s.[1] There were an estimated eight billion discrete applications of this compound as of 2009 and roughly 200 million people use it for bite protection every year.[2] Recently, questions have arisen regarding the human safety profile of DEET and public platforms of discourse continue to have conflicting reports regarding its potential toxicity. Examples are readily available from an internet search, where the safety of DEET is questioned,[2–4] and in one particular instance, a BBC article reported that 'DEET works in the same way as paralysing nerve gases used in warfare.'[5] The basis for this claim was apparently a paper published by Corbel *et al.*[6]; implicating acetylcholinesterase (AChE) inhibition as a neurotoxic mechanism of action for DEET. However, other studies concluded that DEET is a poor anticholinesterase, active only at high (mM) concentrations.[7,8] Finally, many popular press articles cite statements from experts insisting that DEET is safe to use if applied according to labeled directions,[2–4] but conflicting claims are sometimes reported within the same article.

This situation has led to misunderstanding of the risks involved in DEET usage and probably reduced its deployment as a protective measure for blocking arthropod transmission of pathogens. For instance, an anonymous survey of pregnant women in the southern United States was conducted during the Zika virus epidemic, and found that 50% of the pregnant women questioned would not use an insect repellent due to concerns about use during pregnancy.[9] Yet, a randomized controlled trial conducted among 897 pregnant women who were given 1.7 g of DEET per night throughout the second and third trimesters resulted in no significant differences in birthweight, newborn anthropometrics, newborn neurologic exams, or developmental milestones in the first year of life between control and treatment groups.[10] These data indicate that the risk of DEET accumulating in the fetus is low and that DEET is safe to use in pregnancy, at least during the second

and third trimesters. The U.S. Centers for Disease Control and Prevention recommends the use of DEET or other repellents by pregnant women for protection against mosquitoes and ticks,[11] and its use is also supported by the U.S. Environmental Protection Agency (EPA).[12]

Considering these issues, the goal of this review is to highlight what is known regarding the epidemiology of DEET use, its safety profile, and includes a discussion of the current understanding of the mechanism(s) of DEET neuro- and cardiac toxicity, with particular emphasis on AChE and cholinergic nerve pathways. A comprehensive review of the literature will not be attempted, especially concerning the pathophysiology of DEET exposure in animal models, which was the focus of a recent review.[13] Instead, we rely on previous reviews, research articles, summaries, and case studies, as appropriate.

2 OVERVIEW OF HUMAN POISONINGS BY DEET

Although the overwhelming majority of DEET applications have led to safe and efficacious repellency of insects, nine documented incidents over the past 60 years have reported death after DEET intoxication; four from intentional ingestion of a concentrated DEET solution and five related to high concentrations of dermal exposure.[14] Documented cases of oral DEET lethality resulted from intentional ingestion of large amounts of concentrated (47% to

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95%) DEET. Patients that died displayed similar symptomology and presented with seizures, hypotension, coma, dilated pupils, and bradycardia, whereas another patient shared these symptoms, but presented with tachycardia.[14] Although intentional and accidental exposure to large amounts of DEET has resulted in death, it has been noted that additional substances were also present, and thus death cannot be attributed solely to DEET itself. For instance, postmortem analysis performed after intentional ingestion of 50 mL of 95% DEET and 5% related toluamides also found excessive amounts of prescription phenothiazine antipsychotic medications (chlorpromazine and hydralazine) along with ~ 1 mmol L^{-1} of DEET in the blood.[14] Similarly, a second patient was analyzed post mortem and found to have a blood alcohol level of 130 mg dL^{-1}, the presence of cannabinoids, and a DEET concentration of 1.2 mmol L^{-1} in the blood.[14] These additional substances convolute identification of the active principle responsible for death and thus, the overall contribution of DEET exposure.

Although case reports of toxicity or clinical symptomology stemming from DEET exposure are rare considering the number of applications, it has been shown to have various sublethal effects after topical exposure, including effects on skin, neuropathologies, and action on the cardiovascular system. Dermatitis is the most common side effect of DEET exposure, and can result in a burning sensation, skin eruption, erythema (redness), pruritis (itching), blisters, and local necrosis.[14,15] Encephalopathies observed from either chronic or acute exposures to DEET include tremors, coma, hypertonia, and seizures.[14,15] The cardiovascular system is also a major target affected by DEET in humans, typically resulting in hypotension and bradycardia.[14,15] Our conclusion from reviewing the available literature is that in the vast majority of cases, discontinuing the use of DEET and given supportive care, the signs and symptoms subside and the patient fully recovers.

The majority of manifestations of clinical signs of DEET intoxication have occurred in pediatric exposures, including rare cases of death.[16] Of five deaths associated with dermal exposure to DEET, three occurred in children.[14] Death was preceded over several days by various encephalopathies and patients had a history of multiple topical applications of DEET, in contravention of label directions.[14] Interestingly, an interaction between DEET and the alcohol found in commercial formulations has been recognized as a possible mechanism that could increase toxic effects in children.[17] Out of an abundance of caution about the possible toxicity of DEET to children, the U.S. government has issued the following recommendations for its use. If the child is less than 2 years old, a treated netting is suggested over strollers, etc. instead of direct application.[11] In older children: (i) do not let them apply DEET themselves; (ii) keep it off their faces; (iii) preferably use on clothing (test apply to a small piece of fabric to assure color fastness), as opposed to dermal application; and (iv) use sparingly.[11,12]

3 TOXICOLOGY AND MODE OF ACTION STUDIES

3.1 Lethal dose studies and symptomology
There have been a number of different animal studies aiming to quantify lethal exposures of DEET and reported similar LD_{50} values, as recently reviewed.[13] An oral LD_{50} of 3664 mg kg^{-1} was determined in male Sprague–Dawley rats that were administered technical grade DEET, comparable with the value of 3564 mg kg^{-1} obtained for Wistar rats.[13] This LD_{50} value places DEET in EPA acute toxicity category 3, which is the same category as NaCl table salt ($LD_{50} = 3000$ mg kg^{-1}).[18] Lethal doses in rats caused intoxication

marked by lachrymation, chromodacryorrhea, depression, prostration, tremors, and asphyxial convulsions.[19] A dermal LD_{50} value in the rabbit (3167 mg kg^{-1}) was similar to those observed orally in rats.[13] Skin reactions in rabbits included erythema, desquamation, and scarring.[19] Generally, the signs of intoxication and pathophysiology seen in experimental animals treated with toxic doses of DEET mirror those documented in human poisoning cases. A better understanding of DEET toxicology requires identifying sites and modes of action that might underlie these effects.

3.2 Acetylcholinesterase as a target for DEET
Corbel et al. concluded that an anticholinesterase action of DEET might engender neurotoxic risk to humans from its use as an insect repellent.[6] Some of the evidence for this conclusion was indirect, consisting of electrophysiological analysis of DEET action on cholinergic synaptic preparations from insects and mammals. In these experiments, 0.5 µmol L^{-1} DEET had biphasic effects on the excitatory postsynaptic potential amplitude of the cockroach cercal nerve–giant fiber synapse and 500 µmol L^{-1} DEET augmented the end-plate potential at the mouse hemi-diaphragm preparation.[6] However, their enzyme kinetic experiments suggested a DEET potency for interacting with enzyme–substrate catalysis to be in the millimolar range and that it acted as a reversible inhibitor.[6] Indeed, direct biochemical measurements of the potency for inhibiting human AChE activity by DEET gave IC_{50} values of 21.7 and 12 mmol L^{-1}.[7,8] Consistent with Corbel et al.,[6] Wille et al.[7] also characterized DEET as a weak and reversible inhibitor of AChE, and importantly, this mechanism is different from the irreversible inhibition of AChE induced by chemical warfare agents, as claimed for DEET.[5] Both Wille et al.[7] and Swale et al.[8] concluded that any deleterious human effects from DEET exposure cannot be attributed to AChE blockage. To address this point, one case study showed that the blood serum concentration of a patient that died after ingesting DEET was ~ 500 µmol L^{-1},[20] which is similar to the concentration that affected the mouse hemi-diaphragm preparation,[6] but > 20-fold lower than the in vitro IC_{50} of DEET to human AChE.[7,8] These results indicate the patient mortality was not due to AChE inhibition.

3.3 G protein-coupled receptor targets of DEET
DEET was found to activate the firefly light organ, a finding that was supported by data from the Sf21 cell line, where DEET application activated calcium-dependent fluorescence and this action was blocked by phentolamine, an established octopaminergic receptor antagonist in insects.[8] Similarly, DEET excited the Musca domestica larval central nervous system in a phentolamine-sensitive manner that was essentially indistinguishable from that of octopamine itself.[8] The extent to which DEET might activate octopamine or other biogenic amine receptors in other tissues or species remains unknown. In the free-living nematode Caenorhabditis elegans, DEET was found to activate an orphan G protein-coupled receptor (GPCR) (str-217) that was responsible for mediating DEET repellency in this organism.[21] Another recent study found that DEET was an allosteric modulator of muscarinic acetylcholine receptors, another possible GPCR target for DEET, and it was hypothesized that such an action might potentiate the effects of carbamate insecticides in insects.[22] Further, DEET may modulate M_3 receptors in endothelial cells to affect angiogenesis, including proliferation, migration and adhesion.[23] How predictive these studies might be for any in vivo toxicological effect of DEET to human users requires further study.

To our knowledge, direct measurement of DEET effects on mammalian aminergic receptors have not been performed. However, one paper confirmed hypotension and bradycardia following sublethal intraperitoneal doses of DEET (56–225 mg kg^{-1}) to rats or dogs.[24] It was also observed that the drop in blood pressure caused by systemic acetylcholine administration to rats was reduced by DEET, but responses to epinephrine, norepinephrine and histamine were unchanged.[24] Further study of DEET effects on aminergic neurotransmitters and their associated transporters and neurochemistry are warranted, as it might lead to a better understanding of its hypotensive, cardiac, or other effects in humans.

3.4 Effects on ion channels

A novel finding is that DEET can interact with voltage-sensitive ion channels.[8] Patch clamp studies of rat cortical neuron sodium and potassium currents found IC$_{50}$ values for inhibition of 683 and 114 µmol L^{-1}, respectively. For reference, lidocaine and benzocaine are known sodium channel analgesics and inhibit Na$^+$ currents in rat sensory neurons with a potency of 1.3 and 1.9 mmol L^{-1}, respectively.[25] An action on these channels might be related to the burning sensation that DEET causes on skin[14] or numbness of mucous membranes.[8] We are unaware of additional research into this site of action.

4 SUMMARY AND CONCLUSIONS

Although there is evidence that DEET poses some minor risk from human use as a repellent, two large analyses[16,26] of US Poison Center data have reviewed over 29 000 human exposures and concluded that DEET presents little to no risk when applied according to product labels. Further, although exposure of humans to DEET has been associated with some negative, albeit minor health effects, it should be noted that considering the millions of DEET applications per year, the number of reports that document serious health effects after proper use of DEET represent an extremely small cohort. The continued and proper use of DEET is imperative for human health considering: (i) the re-emergence of highly infectious mosquito transmitted pathogens that have deleterious consequences to adult, child, and fetal health; (ii) that DEET remains a very efficacious and economical insect repellent; and (iii) there remains a lack of evidence supporting consistent negative health effects resulting from the use of DEET.

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