

UPDATE TO STATEMENT ON THE REVIEW OF TOXICOLOGY LITERATURE ON THE USE OF TOPICAL INSECT REPELLENT DIETHYL-m-TOLUAMIDE (DEET)

Introduction

1. The COT previously assessed the safety of DEET in 2003 and at that time made a recommendation that the literature on DEET should be regularly reviewed. New information was obtained through an extensive literature search and by contacting HSE who are currently participating in a regulatory review under the Biocides Product Directive (BPD).
2. During their assessment, members looked at neurotoxicity studies, combined use of sunscreen and DEET, results from post-market monitoring in the UK and USA and further epidemiology/intervention studies. The outcome of this discussion was generally reassuring. However the neurotoxicity studies were found to have potential methodological problems and the results were difficult to interpret. Therefore the Committee recommended that repeat studies be carried out to clarify these issues. Members requested further information on the toxicokinetics of DEET and sunscreen to provide further reassurance on the safety of their combined use.

Background

3. The COT was asked by the Department of Health to review the available toxicology data on the insect repellent N,N-diethyl-m-toluamide, commonly known as DEET, as part of the strategy being developed by the Chief Medical Officer for England, Professor Sir Liam Donaldson on combating the potential for West Nile Virus (WNV) infection (see paragraph 4). The COT agreed a statement on DEET in 2002 and also agreed to keep DEET under review (<http://www.advisorybodies.doh.gov.uk/pdfs/deetstatement.pdf>). This update to the statement incorporates toxicology information published since 2002, information on biomonitoring of DEET in the UK and all other available data on DEET that has been made available since the original statement was published.
4. Insect repellents are used to prevent nuisance bites from mosquitoes (as well as ticks, biting flies and mites) and may aid in lowering disease transmission from these pests e.g. malaria and West Nile Virus (WNV). N,N-Diethyl-m-toluamide is the most widely used and best studied insect repellent currently available to the general public. DEET has been used world-wide for 40 years. It has been reported to give the best duration of protection and broad-spectrum effectiveness

of topically applied insect repellents and is recommended by the United States Centre for Disease Control in helping to prevent infection with WNV.

5. DEET is marketed in the United Kingdom in a variety of formulations and concentrations including aerosol and pump-spray products intended for application to skin as well as for treating clothing. Liquid, cream, lotion and stick products enable direct skin application. The concentration in these products varies according to formulation type, between 10-95 %. There are no data on the pattern of usage in the UK but a wide range of products is freely available over the counter or via the internet.

6. The Department of Health (DH) has published a strategy for combating the possibility of WNV infection. The strategy is intended to provide advice to the general public and to Environmental Health Departments. <http://www.dh.gov.uk/assetRoot/04/08/33/33/04083333.pdf>

Summary of Recommendations made in the DEET statement in 2002

- Information on exposure should be made publicly available
- Additional animal studies are required to verify the neuropathological effects seen in repeat dosing dermal studies of DEET in rats
- The Department of Health should undertake further monitoring for reports of adverse effects associated with exposure to DEET
- Consideration should be given to undertaking epidemiological studies
- Industry should seek to attain a consistent approach to labelling through voluntary action

Rational for Update Review

7. The objective of this review is to provide an update on the request for additional data requested by the COT in 2002. In this context information from adverse health surveillance schemes has been collated and reviewed. Additional toxicological information from the published literature has been reviewed, in particular a number of absorption studies on DEET following concurrent application of DEET and sunscreen. In addition comments on the risk assessment submitted by the DEET Joint Venture Group (DJV) as part of the regulatory review of DEET under the Biocides Product Directive (BPD) were sought from the COT. Exposure assessment was considered in the 2002 review and is only briefly referred to in this updated statement.

Regulatory control of insect repellents

8. At the present time there is no requirement for DEET-based insect repellents for topical application to human skin to be authorised under a regulatory scheme

within the U.K. Topically applied insect repellents are regulated under the Biocides Products Directive (BPD)(98/8/EC introduced 14th May 2000) enacted in U.K legislation by the Biocide Products Regulations 2001 (which came into force on 6th April 2001). Topically applied insect repellents for human skin are not considered as pesticides or as medicines. There are 23 categories of biocide product listed under 98/8/EC. Insect repellents are included in category 19: (Repellents and Attractants). A centralised review scheme for existing biocides products was set up by the European Union. Members were informed that DEET is currently being considered as part of this review scheme under the Biocide Products Directive. It is only once the review has been completed that individual products containing DEET will require authorisation in the UK. The Committee was also made aware that it would be possible that the COT updated statement could be forwarded to the rapporteur Member State (Sweden). The U.K Competent Authority is the HSE (Biocides and Pesticides Unit).

9. The available products would also have to conform to labelling requirements as established by the Chemicals (Hazards Information and Packaging for Supply) Regulations 2002 (CHIP) which enact EU Directives on Dangerous Substances and Preparations [76/548/EEC]. The COT was also informed that the EU review would provide information on usage and would also allow for consistent labelling to be applied to DEET products.

Summary of Additional Toxicology Information received since 2002

Metabolism studies in animals and humans

10. A number of publications regarding the transdermal absorption of DEET following concurrent application with sunscreen preparations are available. Generous and frequent application of sunscreens is recommended to minimize skin damage due to sun exposure. On the other hand, repellents are recommended for application on an 'as needed' basis. Concurrent application of commercially available repellent and sunscreen products resulted in significant percutaneous permeation of the repellent DEET and the sunscreen oxybenzone across mouse or piglet skin, *in vitro* (Gu et al., 2005; Gu et al., 2004 and Ross et al., 2004) and in an *in vivo* animal study (Kasichayanula et al., 2005).

11. Data from Gu et al. (2005) indicated that to minimize the transdermal absorption of active ingredients arising from the concurrent application of repellent and sunscreen products, sunscreens should be applied first to saturate the skin surface. Physically mixing these products prior to, or during application was not recommended as this could increase transdermal penetration of the active ingredients. These studies demonstrated that the permeability of DEET across mouse or piglet skin, *in vitro*, lead to increased DEET penetration but this was dependent on formulation type, application amount and the application sequence. In an *in vivo* animal study in nine week old piglets a slight enhancement of percutaneous penetration and systemic absorption of DEET and oxybenzone was observed when repellent and sunscreen preparations were used concurrently (Kasichayanula et al., 2005). Measurement of skin penetration rate and extent of a topical preparation was performed by tape stripping (Kasichayanula et al., 2005).

12. COT members considered the absorption of DEET when used concurrently with sunscreen. The committee was reassured by the *in vivo* study in pigs, which had shown only a slight enhancement in the absorption of DEET on concurrent application with sunscreen compared with DEET applied alone (Kasichayanula et al., 2005). Members agreed that if there was any effect of sunscreen on the absorption of DEET, this could reduce the DEET margin of safety following co-exposure with sunscreen. The committee concluded, in view of the differences between the *in vitro* and *in vivo* absorption studies, additional studies to investigate the effect of sunscreen on DEET absorption in human volunteers would be helpful to provide reassurance with regard to the risk assessment based on data from pigs.

Toxicology Studies in animals

Subchronic Neurotoxicity

13. The Committee considered additional neurotoxicity studies from Abou-Donia and colleagues (Abdel-Rahman et al., 2002, 2004a 2004b). These studies added

to papers from this group, already reviewed by the Committee in 2002. These papers suggested that DEET applied dermally at 40 mg/kg/day for periods of 28-60 days can result in adverse effects on sensorimotor performance and histopathological changes in the CNS. The majority of these studies investigated the combined effect of DEET, permethrin and pyridostigmine bromide on sensorimotor and neuropathological effects on the brain.

14. Abdel-Rahman et al. (2004a) investigated the neurological effects induced by DEET, malathion and permethrin alone or in combination in adult rats. Groups of 10 male Sprague-Dawley rats received dermal doses of DEET at 40 mg/kg bw/day for 7 days a week for 30 days (in 70 % ethanol). Animals treated with DEET (40 mg/kg bw/day) exhibited significant sensorimotor impairment compared to controls, which was reflected in inclined plane performance, forepaw grip time, beam-walk scores, and beam walk time when assessed after 30 days of daily exposure. Treatment with DEET alone at 40 mg/kg/d did not cause any significant changes in plasma BChE activity compared to control. However, treatment with DEET caused a significant increase in AChE activity in the cortex and the cerebellum of the brain. The authors found no change in AChE activity in the brainstem following treatment with DEET and reported significant reduction in the density of healthy or surviving neurons in the dentate gyrus, the CA1 and CA3 subfields of the hippocampal formation, the midbrain, the brainstem and cerebellum. The authors contended that a significant number of degenerating neurons were documented in these brain regions.

15. Abdel-Rahman et al. (2002) and a follow-up study in 2004b, investigated the effects of a combined exposure to restraint stress and low dose of pyridostigmine bromide (PB, 1.3 mg/kg bw/day, orally), permethrin (0.13 mg/kg bw/day, dermally) and DEET (40 mg/kg bw/day, dermally) in adult male rats, exposed daily for 28 days. Exposure to chemicals and stress produced blood brain barrier disruption and neuronal cell death in the cingulate cortex, dentate gyrus, thalamus and hypothalamus. Other regions of the brain such as the cerebellum, the cerebral cortex and the hippocampus demonstrated some neuronal cell death but did not exhibit blood brain barrier disruption. There was also decreased AChE activity in the forebrain, midbrain, brainstem and cerebellum and decreased m2-AChR binding in the midbrain and cerebellum. In contrast, in animals exposed to stress or chemicals alone, the above indices were mostly comparable to those of animals exposed to vehicles alone. The authors concluded that combined exposure to stress and low doses of the chemicals pyridostigmine bromide, permethrin and DEET leads to significant brain injury.

16. Inconsistent outcomes between studies were observed during neurobehavioural testing depending on the duration of treatment with DEET. Abdel Rahman et al. (2004a) reported that animals treated with DEET (40 mg/kg bw/day) exhibited significant sensorimotor impairment compared to controls, which was reflected in inclined plane performance, forepaw grip time, beam-walk scores, and beam walk time when assessed after 30 days of daily exposure. Abou-Donia *et al.* (2001a) reported significant effects on beam-walking, beam-

walking time and grip strength when DEET was tested at 4, 40 and 400 mg/kg bw/day DEET for 60 days. These changes were not reproduced in another study, carried out by the same group, in which a dose of 40 mg/kg bw/day was administered for 45 days (Abou-Donia *et al.* 2001b). As discussed at COT previously, the results from a study by Schoenig *et al.* (1993) differ from these findings. Schoenig *et al.* (1993) observed neurobehavioural changes due to DEET but only at a higher dose, when rats were administered undiluted DEET at dose levels of 50, 200, or 500 mg/kg bw/day by gavage. The two measures of neurotoxicity evaluated by Schoenig *et al.* were functional observational battery (FOB) and motor activity measurements.

17. Different results were also observed for the effects of DEET on acetylcholinesterase (AChE) activity in the different brain regions in the available studies (Abdel-Rahman *et al.*, 2004 and Abou-Donia *et al.*, 2001b). This might have been due to differences in the duration of treatment with DEET with regard to effects on AChE. In a 30 day study (Abdel-Rahman *et al.*, 2004a), treatment with DEET caused a significant increase in AChE activity in the cortex of the brain but had little or no effect on activity in the midbrain, brainstem, cerebellum in rats. However, in a 45 day study (Abou-Donia *et al.*, 2001b) treatment with DEET caused a significant increase in brainstem AChE activity but had little or no effect on AChE activity in the cortex, midbrain or cerebellum in rats.

18. Members commented that the neuronal effects attributed to DEET in some of the studies might be due to artefacts such as the "dark cell" artefact caused by incorrect handling of the brain tissue after the death of the animal. Members expressed concern that the reported eosinophilic degeneration of neurons might reflect a basophilic post mortem change. However if there were significant microglial and astrocytic reaction to neuronal damage, it was more likely that the observed lesions occurred in-life.

19. Members agreed that Professor Abou-Donia should be asked to comment on the neuronal effects caused by DEET reported in these studies by his group. In the absence of reply, the Committee agreed that it was not possible to draw definitive conclusions on the evidence reported by Abou-Donia and colleagues and that there was a need for independent verification of these subchronic dermal neurotoxicity studies in rats using the dermal route of administration to evaluate the significance of the published findings for human health. The Committee reaffirmed its opinion reached in 2002 that there were considerable uncertainties regarding the studies published by Abou-Donia and colleagues. The Committee concluded that, in view of the potential methodological problems with these studies and difficulties in assessing the reported neuropathological and neurobehavioural effects, additional repeat studies to verify the results obtained represented the most appropriate course of action to take. Overall, it was not considered appropriate to use the data from these studies for risk assessment. This was consistent with the conclusions reached in 2002. The committee were aware of pre-publication experimental results of microglial reactions in the same tissues that showed neuronal cell death by Professor

Abou-Donia but commented that no weight could be attributed to this information until it was available in a peer reviewed publication.

Toxicology Evidence from Human Case reports in the UK:

20. In order to follow up the recommendation to undertake further monitoring for reports on adverse effects associated with exposure to DEET, the DH Toxicology Unit obtained data on any reports concerning DEET from the Hospital Accident Surveillance Scheme, the Hospital Episode Statistics and information from the National Poisons Information Service Centres from 1st Jan 2002 to 31st July 2005. Data were also obtained from the Royal Society for the Prevention of Accidents (ROSPA) from 1993 to 2001. In total there were reports of 35 individuals exposed to DEET and evidence to demonstrate potential for localised effects (skin/eye irritation). There were no reports of severe CNS toxicity in children (23 reports of minor adverse effects in children). The Committee was reassured that the effects were relatively minor and did not include any cases with overt neurotoxicity. The small number of cases when compared to the estimated high usage of DEET was also reassuring, but it was agreed that there were no precise data for the U.K. in this regard. It was noted that definitive data on exposure would be included in the review being undertaken under the Biocides Products Directive (98/8/EC).

21. Following a request from the COT secretariat, the DJV submitted a poster presentation on post-market biomonitoring data on DEET from the US. The National Registry of Human Exposure to DEET (DEET registry) was operated from 1995 to 2001. It was devised to better understand the role of DEET in more serious medical events. The DEET registry was a voluntary effort by 14 companies that either produce DEET and/or market formulated consumer insect repellents. The presentation indicated that there were over 5 billion applications of DEET during the 7 year span of the Registry and the authors found the overall risk from DEET of clinically significant adverse events to be very low.

Epidemiology Studies

22. When the first review of DEET by COT was undertaken in 2002, the COT commented that no published epidemiological studies of DEET exposure and adverse effects were available. Clinical investigation studies from McGready et al., (2001) and Menon and Brown (2002) have since become available in the literature.

23. McGready et al. (2001) undertook a study investigating the safety of DEET applied daily during the second and third trimesters of pregnancy in a group of Thai women as part of a double-blind, randomized, therapeutic trial of insect repellents for the prevention of malaria in pregnancy. The study received approval from the Ethical Review Committee of the Faculty of Tropical Medicine of Mahidol University, the Central Scientific Ethical Committee of Denmark, and the Karen Refugee Committee. Subjects were randomly allocated to receive a

daily target dose of either DEET and thanaka, a local cosmetic (1.7 g of DEET and 3.2 g of thanaka) or thanaka alone (3.2 g of thanaka) until delivery. Women were instructed to apply the treatment daily after the evening shower to the exposed areas of the arms and legs. Apart from the sensation of skin warming with application of DEET, no significant adverse effects for the mother or the fetus following daily use of DEET were observed. Survival, growth, and neurological development in infants followed from birth up to one year of age did not differ from infants whose mother received thanaka alone. Whilst the authors concluded that the results of their study indicate little risk of DEET accumulating in the foetus and that DEET (20 %) is safe to use in later pregnancy, the committee did not agree with this conclusion. The committee concluded that the study did not provide any information on the accumulation of DEET in the foetus and showed only that the risk of any adverse outcome in pregnancy was low, under the conditions of the study.

24. Menon and Brown (2002) conducted a cross-sectional survey on the use patterns of repellents on children and the associated effects in Maryland campgrounds in 2002. The research protocol was approved by the University of Maryland Institutional Review Board, and all parents of participants gave informed consent. The study yielded 301 respondents (numbers of non-respondents not indicated). DEET was the active ingredient used by most families. In only two instances (one case of eye irritation through direct contact and one case of skin rash), were possible adverse reactions observed by the parent within 24 hours of application of a repellent. In both cases, the repellent contained DEET.

25. Members stated that the available human studies were difficult to interpret but felt reassured that no serious effects were observed in these studies following exposure to DEET.

Risk assessment based on animal studies

26. The Committee was aware that the DEET Joint Venture Group (DJV) had proposed that risk assessment of DEET should be undertaken on the basis of a comparison of Area Under the Curve (AUC) of DEET between dermal application in humans at the 75th percentile exposure (1.5 g/day for males and 1.0 g/day for females for the European population) and the NOAELs from subchronic dermal toxicity studies conducted with rats and mini-pigs. This approach is different to the approach outlined previously by the DJV that risk assessment could be undertaken on the basis of peak blood levels (Schoenig and Osimitz, 2001). The committee felt that in the absence of direct evidence to support the use of the AUC, it was prudent to use peak blood levels for the risk assessment of DEET since an end point of acute neurotoxicity had been demonstrated (in oral studies in rats and dogs). The NOAEL in dogs of an oral dose of 75 mg/kg bw was agreed by the committee in 2002 to be appropriate for use in the risk assessment and this was concurred by the present Committee.

27. Conclusions from the COT risk assessment of DEET made in 2002 were that a risk assessment should be undertaken on the basis of a comparison of peak plasma levels of DEET between dermal application in humans at the 95th percentile exposure (i.e 3 g DEET/day in adult females and 4 g DEET/day in adult males) and the NOAELs for neurotoxicity in rats and dogs. Quantitative comparison of the peak plasma levels of DEET showed that levels were 33x higher in dogs and 16-34x higher in rats given oral doses compared to dermal administration to humans. Members noted that there was no good marker of effect to evaluate dose response for neurotoxicity but agreed that the approach of using peak plasma levels of DEET was pragmatic and acceptable. However, members noted that, although toxicokinetic data were available from the studies in sensitive animal species and for humans, an uncertainty factor was still required for interspecies variability to take into account potential differences in toxicodynamics. It was also noted that the number of human volunteers was small so an uncertainty factor would be required to take into account inter-human variation. The committee felt it was not possible, based on the data at the time, to determine the appropriate Uncertainty Factor to use in risk assessment but that it was likely to be between 10 and 100.

28. The risk assessment of combined use of DEET and sunscreen (oxybenzone) was complicated. The DJV had proposed the use of AUC kinetic data from piglets and toxicological data from the micro-piglet to provide consistency of species. The kinetic AUC data from piglets was then compared to AUC data from DEET exposure alone for humans at maximum predicted use levels (no data are available for co-exposure of humans to DEET and oxybenzone). The kinetic data for humans was adjusted to take account of differences in US and UK body weights and likely maximum use. A margin of safety (MOS) assessment compared the AUC blood level for piglet dermal exposure at the NOAEL and human dermal exposure based on blood level data adjusted for all UK adults was presented by the DJV. The DJV noted that there were many assumptions and uncertainties in this approach but in their view the MOS values were acceptable (see table 1).

Table 1: Data from the DJV. Calculated Margins of safety (MOS) for AUC blood level comparisons of piglet dermal exposure at a NOAEL and human dermal exposure based on blood level data adjusted for UK adults

Time (hours)	MOS (AUC)	
	DEET and oxybenzone	DEET alone
8	751	676
24	536	433

29. The committee commented that combined data on DEET and oxybenzone in animals might not be completely appropriate for humans and noted that there were no relevant data for combined exposure to sunscreen and oxybenzone available for humans.

COT Discussion

30. The COT was aware of data to update its 2002 review of DEET. This particularly related to post-market monitoring and risk assessment of combined use of DEET and sunscreen. The COT was reassured with regard to the data on the likely acute CNS effects in children and considered no further follow up of data was required.

31. With regard to the risk assessment of DEET, the Committee concluded that the most appropriate approach for DEET alone was a conservative one using peak blood levels. With regard to the use of DEET and sunscreen the available approach suggested by the DJV needed additional human data on the toxicokinetics of DEET following combined use with sunscreen and data on repeated exposure in humans. The COT agreed these data requests should be forwarded to the UK regulatory authorities (HSE) and the rapporteur for the BPD review when it became available.

Conclusions

The Committee agreed the following conclusions.

Regulatory control of insect repellents

32. The Committee was aware that DEET was currently being considered as part of a review scheme under the Biocide Products Directive and that it would be possible that the COT updated statement could be forwarded to the rapporteur Member State.

Animal toxicity data

33. Additional evidence for neurotoxicity and neuropathological lesions following repeated dermal application of DEET to rats at comparatively low dose levels have been published since the 2002 review. The Committee concluded in 2002 and again in 2006 that, in view of the potential methodological problems with these studies, and difficulties in assessing the results, additional repeat neuropathology studies were important in order to adequately assess the claimed effects. Members felt that industry should be asked to consider commissioning appropriate research. However the balance of evidence suggested that it was not appropriate to use the data from these studies for risk assessment until further clarification of the studies is obtained.

Risk Assessment

34. The Committee concluded that the most appropriate approach for risk assessment of DEET alone was a conservative one using peak plasma levels of DEET in experimental animals at the NOAEL and in humans at the 95th percentile of exposure and this is in agreement with the conclusions reached by the Committee in 2002. Further studies on the toxicokinetics following combined exposure to DEET and sunscreen in humans were considered desirable in order to confirm the risk assessment which had been submitted. The Committee requested that this information be made available to the appropriate regulatory agencies, once the studies have been completed.

Evidence in humans

35. The Committee was reassured by the results of post-market monitoring of DEET for reports of adverse effects associated with exposure to DEET, Human case reports, collated from information provided by the National Poisons Information Service Centres (NPIS), the Hospital Episode Statistics (HES) and the Hospital Accident Surveillance Scheme (HASS), indicated that the effects seen following exposure to DEET were relatively minor and did not include any cases with overt neurotoxicity. The available information from the US was also reassuring and suggested that any acute adverse effects following normal use were very rare.

36. Since the 2002 review, two epidemiological/intervention studies of DEET exposure have been published. The Committee agreed that these studies were difficult to interpret but felt reassured that no serious effects were observed in the subjects following exposure to DEET.

37. The Committee noted the ongoing regulatory review under the BPD and agreed that future consideration of DEET should be undertaken by the appropriate regulatory agencies.

COT/06/12 Statement

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