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In vitro neurotoxic hazard characterization of different tricresyl phosphate (TCP) isomers and mixtures

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ABSTRACT

Exposure to tricresyl phosphates (TCPs), via for example contaminated cabin air, has been associated with health effects including the so-called aerotoxic syndrome. While TCP neurotoxicity is mainly attributed to *ortho*-isomers like tri-*ortho*-cresyl phosphate (To*C*P), recent exposure and risk assessments indicate that To*C*P levels in cabin air are very low. However, the neurotoxic potential of non-*ortho* TCP isomers and TCP mixtures is largely unknown. We therefore measured effects of exposure (up to 48 h) to different TCP isomers, mixtures and the metabolite of To*C*P (CBDP: cresyl saligenin phosphate) on cell viability and mitochondrial activity, spontaneous neuronal electrical activity, and neurite outgrowth in primary rat cortical neurons.

The results demonstrate that exposure to TCPs (24–48 h, up to 10 μM) increases mitochondrial activity, without affecting cell viability. Effects of acute TCP exposure (30 min) on neuronal electrical activity are limited. However, electrical activity is markedly decreased for the majority of TCPs (10 μM) following 48 h exposure. Additional preliminary data indicate that exposure to TCPs (48 h, 10 μM) did not affect the number of neurites per cell or average neurite length, except for T*m*CP and the analytical TCP mixture (Sigma) that induced a reduction of average neurite length.

The combined neurotoxicity data demonstrate that the different TCPs, including To*C*P, are roughly equipotent and a clear structure-activity relation is not apparent for the studied endpoints. The no-observed-effect-concentrations (1 μM) are well above current exposure levels indicating limited neurotoxic health risk, although exposures may have been higher in the past. Moreover, prolonged and/or repeated exposure to TCPs may exacerbate the observed neurotoxic effects, which argues for additional research.

1. Introduction

Tricresyl phosphate (TCP) is an organophosphate (OP) used as plasticizer, flame retardant, and oil additive to reduce wear and tear of the engine. TCP consists of one phosphate with three cresyl groups (methylphenyl) at the single bonded oxygen molecules (see Fig. 1 in de Ree et al. (2014) for schematic illustration). These cresyl groups can exist in three different forms: *ortho*,*meta* and *para*, determined by the position of methyl on the phenyl. TCP is thus a mixture consisting of different TCP isoforms of which in particular the *ortho*-isomer (tri-*ortho*-cresyl phosphate; To*C*P) is neurotoxic (Aldridge, 1954; Henschler, 1958).

To*C*P can be metabolized to cresyl saligenin phosphate (2-(*ortho*-cresyl)-4*H*-1,2,3-benzodioxaphosphoran-2-one; CBDP) by multiple cytochrome P450 subtypes (Reinen et al., 2015). CBDP inhibits acetylcholine esterase (AChE) and butyrylcholinesterase (BuChE), leading to an excess of the neurotransmitter acetylcholine and subsequent cholinergic syndrome. In addition, CBDP can inhibit neuropathy target esterase (NTE), resulting in organophosphate-induced delayed neuropathy (OPIDN) (Honorato de Oliveira et al., 2002; Padilla and Veronesi, 1985; Barrett and Oehme, 1994; Carrington and Abou-Donia, 1988; Carletti et al., 2013).

Human occupational exposure to To*C*P, for example via contaminated cabin air in aircraft, has received particular attention as it has been proposed to result in neurological complaints such as the so-called aerotoxic syndrome (Winder et al., 2002; Ross, 2008; Furlong, 2011; Liyasova et al., 2011; Abou-Donia et al., 2013). As a consequence of its neurotoxicity and the proposed association

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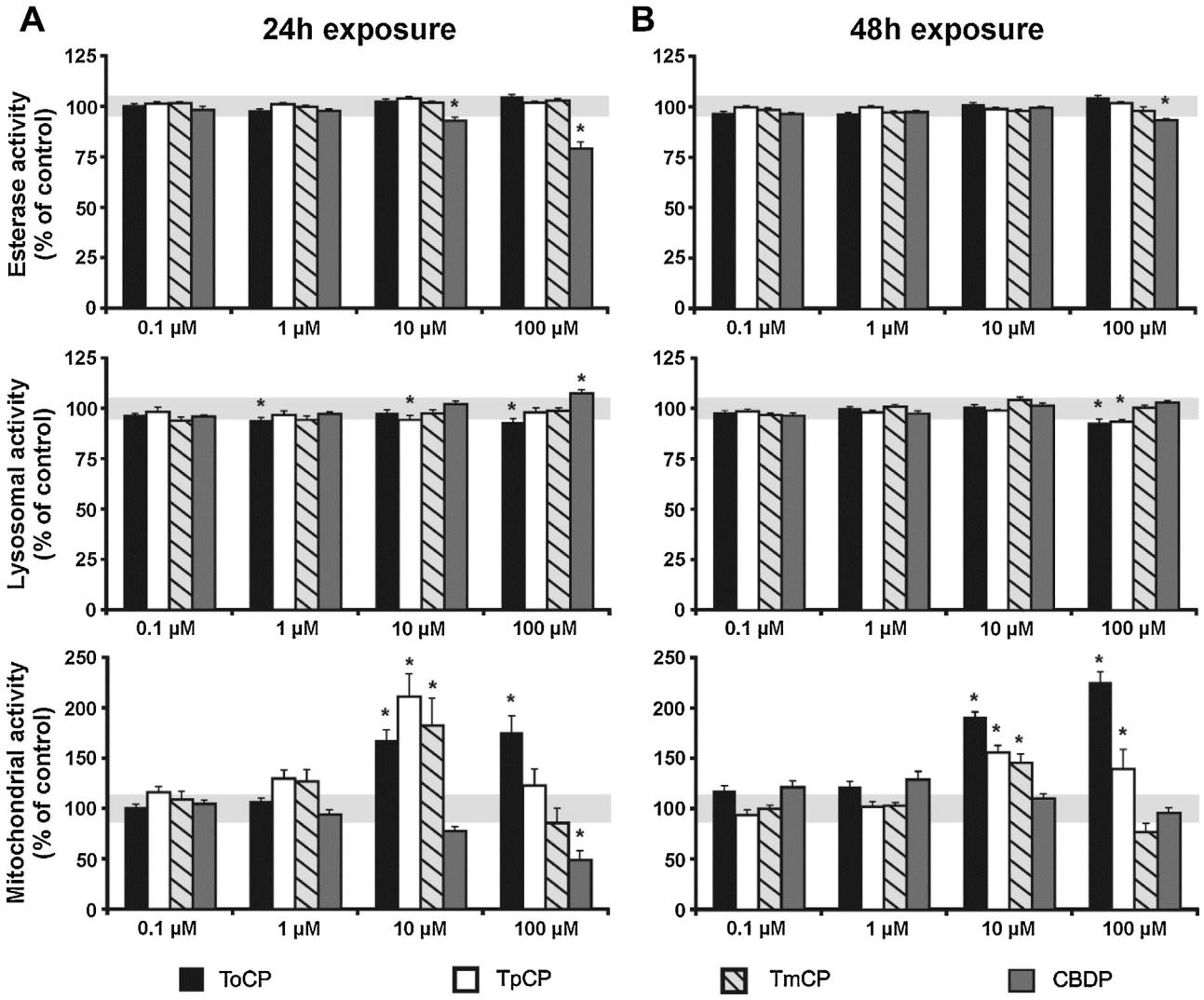

Fig. 1. Effects on cell viability following 24 h (A) or 48 h (B) exposure to different TCP isomers and CBDP. Effects on cell viability were assessed using esterase activity (CFDA assay, top), lysosomal activity (neutral red assay, middle) and mitochondrial activity (alamar blue assay bottom) normalized to DMSO controls (ACN controls for CBDP). Data are expressed as mean ± SEM of 15–44 wells (*n*) per condition from 4–10 plates (*N*) derived from at least 3 independent dissections (cultures). Gray-shaded areas indicate the minimal relevant effect size (MES) derived from the average standard deviation of control cells, whereas an asterisk indicates a statistically significant ($p < 0.05$) effect that exceeds the MES.

with aerotoxic syndrome, the commercial use of ToCP has been strongly reduced over the last decades and ToCP now constitutes no more than 2% of the commercial TCP blends used in aircraft engine oil (SAE, 2005; DeNola et al., 2008, Table 1). Consequently, ToCP exposure in cabin air is nowadays below the limits for analytical detection (Houtzager et al., 2013; Schindler et al., 2013; but see also Cranfield, 2011; Murawski and Michaelis, 2011). Despite ongoing debate (de Boer et al., 2015), it is thus unlikely that exposure to ToCP is responsible for reported health complaints, such as the alleged aerotoxic syndrome (de Ree et al., 2014).

While the neurotoxicity of ToCP is relatively well-studied and mainly attributed to the inhibition of NTE, AChE and BuChE by its metabolite CBDP, it is possible that ToCP or its metabolite affect yet unknown cellular targets as observed previously for among others OP-induced acute and subchronic inhibition of voltage-gated calcium channels (VGCC; Meijer et al., 2014a; Meijer et al., 2014b; Meijer et al., 2015). Interestingly, ToCP was indeed recently shown to inhibit VGCC as well as glutamatergic calcium signaling in mouse embryonic neurons following 1–6 days of exposure (Hausherr et al., 2014). However, the neurotoxic potential of

Table 1
Isomer composition of the used analytical and commercial TCP mixtures.

	m-*m*-*m*-TCP	*p*-*p*-*p*-TCP	*o*-*o*-*o*-TCP	*m*-*m*-*p*-TCP	*m*-*p*-*p*-TCP	Other
TCP (Sigma)	22%	5%	2%	41%	25%	5%
TCP (TCI)	42%	31%	1–2%	n.p.	n.p.	25%[a]
Disflamoll TKP-P (Lanxess)	33%	3%	0%	44%	20%	0%
Durad 125 (Chemtura)	13%	11%	0.3%	39%	36%	0.7%
Kronitex TCP-S (Chemtura)	30%	3%	1.5%	40%	18%	7.5%

n.p. not provided by the manufacturer.
[a] likely mainly *m*-*m*-*p*-TCP and *m*-*p*-*p*-TCP as these were not specified by the manufacturer, but purity is reported to be >99%.

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