Effects of a herbicide formulation, Tordon 75D® and its individual components on the oxidative functions of mitochondria

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Introduction

Toxicological investigations usually examine the effects that are caused by single chemicals. However, most chemical products, such as pesticides, are formulations consisting of several chemicals. One of the situations that first raised concern about exposure to chemical mixtures, particularly herbicide formulations, was the Vietnam war. Australian, New Zealand and American servicemen, as well as a significant proportion of the Vietnamese population were repeatedly exposed to a variety of herbicide mixtures during the war. The possible long-term health effects associated with this exposure is still under investigation (IOM, 1996).

The second most used herbicide during this period was the formulation given the code name Agent White. This consisted of the active components 2,4-D and picloram (present as the triisopropanolamine salts) and a number of other components including solvents and a proprietary surfactant. A herbicide currently used in Australia, Tordon 75D®, is similar in terms of its active components to the herbicide Agent White. As part of an investigation into the toxicological properties of Agent White we have examined Tordon 75D® both as a complete formulation and as well as its individual chemicals. To do this we used an *in vitro* screening assay, the submitochondrial partical assay (SMP) (Blondin et al., 1989; Knobeloch *et al.,* 1990).

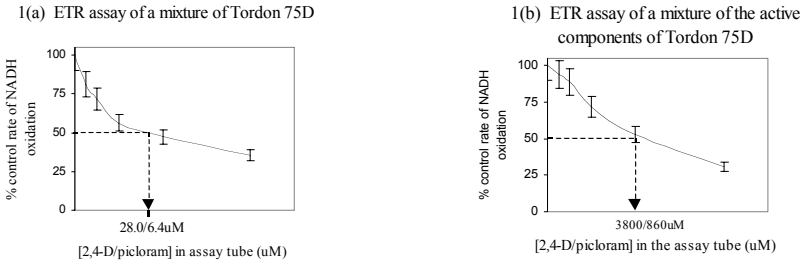
SMPs consist of inverted, vesicular portions of the inner mitochondrial membrane which retain the capacity to perform the integrated oxidative functions of intact mitochondria. The current study was initiated to test the hypothesis that the toxicity of Tordon 75D® is greater than the toxicity of the sum of its active components.

Methods

Submitochondrial particles (SMPs) were purchased from Biorenewal Technologies Inc., Madison. The enzyme system of the SMP assayed was the electron transfer reaction (ETR). A modified spectrophotometric method of Blondin *et al.,* (1989) measured the rate of NADH oxidation in the ETR assay at 340nm. Decreases in reaction rates of chemical exposed SMPs compared to the unexposed SMPs (control) indicated the inhibitory and therefore toxic effects of the added chemicals. EC_{50} values were calculated from the dose response curve by extrapolating the concentration of the chemical at which the rate of NADH oxidation were reduced by 50%. The herbicide, Tordon 75D® was purchased from DowAgroSciences, Australia.

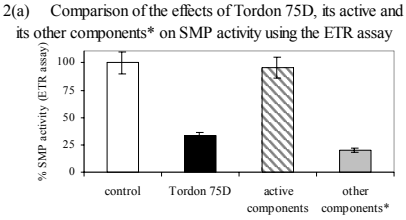
Results

Tordon 75D® formulation inhibited the ETR functions of the SMPs by 50% (EC_{50}) at a concentration of 0.002% (v/v) which corresponds to 28.0µM 2,4-D, 6.4 µM clpioram and the 'other components'* (Fig 1a).



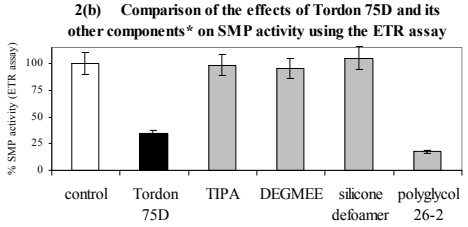
When 2,4-D and picloram were tested in the same ratio as in Tordon 75D® but in the absence of the 'other components'* the EC_{50} was 3,800µM for 2,4-D and 865 µM for picloram (Fig. 1b). Therefore, the SMP toxicity was 136 times lower in the absence of the the 'other components'*.

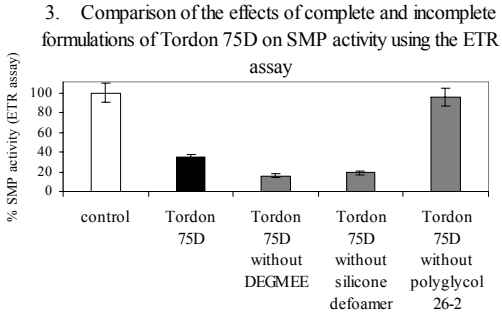
To further investigate the inhibition of SMP activity by Tordon 75D®, the 'other components'*, (the solvents: triisopropanolamine (TIPA) and diethyleneglycolmono-ethylether (DEGMEE), a silicone defoamer and the proprietary surfactant, polyglycol 26-2) were assayed at equivalent concentrations to those present in Tordon 75D®. The Tordon 75D® concentration chosen was 0.005% (v/v), which caused more than 50% inhibition in the ETR assay. Firstly, the effect of a mixture of all the 'other components'* was compared to a mixture of the two active components. The results showed that the inhibition of SMP activity by the Tordon 75D® formulation was caused by the 'other components'* with the active components, 2,4-D and picloram, causing no inhibition (Fig. 2a).



The 'other components'* were then assayed individually. Only the proprietary surfactant caused inhibition at the tested concentration (Fig 2b). Various combinations of the active and 'other components'* of Tordon 75D® were also tested. The results presented in Fig. 3 indicate that SMP inhibition occurred whenever the proprietary surfactant was included in the combinations.

'other components'* = all components of Tordon 75D® excluding the active components: i.e. the solvents, triisopropanolamine and diethyleneglycolmonoethylether, a silicone defoamer and a proprietary surfactant, polyglycol 26-2.





Discussion

The results indicate that the effect of Tordon 75D® on oxidative functions of the SMPs was primarily due to the presence of a proprietary surfactant in the formulation. The active components 2,4-D and picloram had relatively little effect at the EC_{50} of Tordon 75D® formulation. These results are in general agreement with studies by Argese *et al.*, (1994) who showed a variety of different surfactants

disrupt SMP activity. Their studies suggest that it is the hydrophobic moiety of the compound that plays a significant role in determining the extent of SMP inhibition. It is possible the hydrophobic part of the surfactant molecule interacts and therefore disrupts the phospholipid bilayer of the inner mitochondrial membrane. Alternatively, the surfactant may exert a direct toxic effect on a specific mitochondrial enzyme and/or other molecule. Since surfactants have the capability of damaging biological membranes, and thereby increasing their permeability, a mechanism of non-specific membrane damage appears more likely.

The SMP assay has been shown to compare well with the toxicological response of whole organisms, such as fishes and invertebrates (Argese *et al*., 1994; Betterman *et al*., 1996) and vertebrate *in vivo* assays (Degli-Esposti *et al.*, 1996), however, it is important to note that this assay as well as other sub-cellular and cellular *in vitro* tests cannot account for metabolic, pharmacokinetic or pharmacodynamic processes that affect a chemical or chemical mixtures *in vivo*. Hence, these aspects including biotransformation products need to be considered when using data collected from bioassays.

The results of this study (Oakes and Pollak, 1999) highlight the importance of providing toxicity data for complete formulations rather than just for the active components. The presence of surfactants in herbicide mixtures may significantly alter their toxicological profile. If only the active components are tested significant toxicity may be underestimated. It is not known at this stage if surfactants per se or their interactions with active components in Tordon 75D® or Agent White, cause adverse effects in exposed humans. This would require information on human blood levels and metabolic profiles of the surfactant. The complete details of all the components of Agent White, or herbicide formulations generally, are not publicly available.

Acknowledgements
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The authors wish to thank Dow AgroSciences for their cooperation in making the individual components of Tordon 75D® and formulation details available. As Tordon 75D® is currently a commercial product, the complete formulation details and the CAS number of the proprietary surfactant (polyglycol 26-2) are not available for public disclosure.

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