2917 Atrazine and Its Metabolites: Aromatase mRNA and *Ex Vivo* Phosphodiesterase Activity

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Atrazine (ATR) and its mono-dealkylated (DIA, DEA) but not its dide-alkylated (DACT) or hydroxy (hydroxy ATR, ammeline) metabolites induced aromatase activity in *in vitro* cell lines, which may be mediated via cyclic AMP. We assessed the time- and concentration-dependent effects of ATR and its metabolites on aromatase mRNA expression and inhibition of phosphodiesterase (PDE) activity. Exposure to 10 μM ATR for 2 hr induced ~2-fold increase in aromatase mRNA. Four to 24 hr exposure resulted in ~3-fold increase, and following 72 hr, mRNA levels were 2-fold higher than baseline. A transient increase in aromatase mRNA following 24 hr exposure to 1 μM ATR was observed. ATR metabolites induced modest responses at the highest concentration. Ammeline and hydroxyATR were largely without effects. DACT also had no effects. In *ex vivo*, ATR inhibited PDE activity at concentrations near or above its solubility limits with an IC50 of 39.2 μM. ATR was less effective than 3-isobutyl-1-methylxanthine, a broad PDE inhibitor with an IC50 of 1.75 μM. Metabolites of ATR did not inhibit PDE. However, DACT acted as a competitive inhibitor of ATR. Addition of 500 nM DACT shifted the IC50 of ATR from 39.2 μM to 492.3 μM. These data indicate that high concentrations of ATR cause an induction of aromatase mRNA in *in vitro* steroidogenic cell systems via an inhibition of PDE as shown in *ex vivo* assays. Pharmacokinetic data indicate that ATR is rapidly metabolized *in vivo* to DACT. Steroidogenic cell lines typically used to evaluate aromatase activity are not metabolically competent and thus would not represent what would happen in an animal or human. Using a PBPK model for ATR, it was determined that exposure of humans to the maximum contamination level (3 ppb) of ATR in drinking water would result in tissue concentrations of ATR equivalents of ~ 0.2 pM. In rats, 60 mg ATR/kg BW/day for 4 days results in a peak concentration of 1 μM ATR, which had no effect on aromatase activity or expression *in vitro*. ATR and its mono-dealkylated metabolites are rapidly metabolized after ingestion and thus would not persist long enough to have an effect on aromatase activity *in vivo*. Considering both exposure and pharmacokinetics provides a scientifically defensible interpretation of the lack of relevance of high concentration *in vitro* data and a more appropriate *in vitro* to *in vivo* extrapolation.

2918 Organophosphate-Induced Neuroinflammation, with and without Corticosterone Pretreatment, Is Not Due to Acetylcholinesterase Inhibition

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Organophosphates (OP) are used worldwide in a variety of settings. Irreversible acetylcholinesterase inhibitors (AChEIs) are used as insecticides (chlorpyrifos (CPF)) and nerve agents (sarin, or surrogate diisopropyl fluorophosphate (DFP)). Reversible AChEIs have been used as a prophylactic as protection from nerve agent and pharmacologically for treatment of myasthenia gravis (pyridostigmine bromide (PB)) and glaucoma (physostigmine (PHY)). Exposure to AChEIs increases acetylcholinergic transmission resulting in salivation, lacrimation, urination, defecation (SLUD) symptoms, muscle rigidity, seizures, paralysis of the heart, and death. Lesser known effects of exposure to some AChEIs (e.g., DFP), paradoxically, include increases in levels of proinflammatory cytokines in the brain, effects exacerbated by chronic (4-7 days) pretreatment with the stress hormone, corticosterone (CORT). Here, we examined neuroinflammatory and AChE effects following peripheral exposure to different reversible and irreversible AChEIs [those that do and do not cross the blood brain barrier (BBB)] with and without CORT pretreatment in male C57BL/6J mice. Using qPCR, we found that the CNS acting irreversible compounds (DFP and CPF) and reversible compound physostigmine (PHY) produced altered levels of proinflammatory cytokines compared to saline treated controls, and further, that pretreatment with CORT augments these brain effects. Administration of the non-BBB crossing reversible AChEI, PB, alone, or following CORT pretreatment produces few inflammatory effects in the brain. Furthermore, irreversible (DFP and CPF) and reversible (PHY) CNS-acting AChEIs all produce decreases in AChE activity levels. Similar effects were not seen following PB exposure, consistent with a lack of brain penetrance. The protection against AChE activity loss and augmentation of neuroinflammation following pretreatment with CORT and subsequent exposure to AChEIs suggests that the neuroinflammation is not a product of AChEIs effect on cholinesterase activity in the brain. Instead, neuroinflammation seen following the exposure to CNS acting AChE inhibiting OPs, with and without CORT, likely is a result of organophosphorylation of yet to be identified protein targets in addition to ACHE.

2919 Jatropha Phorbol Ester Enhanced Serum Amylase Activity in Mice, Leading to Weight Loss and Death

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Purpose: Jatropha curcas attracts rising attention as a biodiesel feedstock in the world. However, Jatropha contains various toxic components, generating concerns about its health effects. One of toxic components is potential tumor promoter, phorbol esters. Toxicity of Jatropha phorbol esters has not been fully described. In this study, transdermal toxicity of main component of Jatropha phorbol esters was assessed in mice. Experimental procedures: One of the Jatropha phorbol esters, 12-deoxy-16-hydroxyphorbol-4′-[12′,14′-butadienyl]-6′-[16′,18′,20′-nonatrienyl]-bicyclo [3.1.0] hexane-(13-O)-2′-[carboxylate]-(16-O)-3′-[8′-butenoic-10′] ate (DHPB) (2.5 μg), was applied onto skin of mice. As a positive control, 12-O-Tetradecanoylphorbol 13-acetate (PMA (TPA)) (2.5 μg) a known tumor promoter was applied. Transformation activity of test compound was assayed using Bhas42 cells. All mice were sacrificed at 4 weeks from the beginning of the study, and hematological tests and serum biochemical examination were carried out. Results and discussion: 2.5 μg of DHPB led to decreased eating and weight loss during 4 weeks observation. There were no such symptoms in the mice treated with the same dose of PMA. White blood cells and red blood cells were decreased in DHPB group, and cell differentiation test revealed that lymphocytes were also decreased. Serum biochemical examination showed that marked increase in amylase activity and change in several biochemical parameters, but there was no significant change in lipase activity. Conclusions: These results suggested that the acute toxicity of Jatropha phorbol esters characterized by anorexia may be attributable to the increase of serum amylase activity derived from pancreas or salivary gland. This study was supported by JICA/JST, SATREPS (Science and Technology Research Partnership for Sustainable Development), JAPAN.

2920 Chronic Toxicity of Phorbol Ester, Crude Oil, and Biodiesel Fuel from *Jatropha curcas*

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Purpose: *Jatropha curcas* attracts rising attention as a biodiesel feedstock in the world. However, Jatropha contains various toxic components, generating concerns about its health effects. One of toxic components is potential tumor promoter, phorbol esters. We previously examined the acute toxicity of Jatropha phorbol ester. However, chronic toxicity has not been fully described. In this study, chronic toxicity of Jatropha crude oil including Jatropha phorbol ester and biodiesel fuel (BDF), which was made from crude oil by alkaline-methanol treatment were evaluated in mice. Experimental Procedures: 12.5–200 mg of Jatropha crude oil or BDF were applied onto skin of mice twice a week after single application of tumor initiator. As a positive control, 12-O-Tetradecanoylphorbol 13-acetate (PMA), a known tumor promoter, was applied. All survived mice were sacrificed at the 60th week of the experiment, and hematological test and serum biochemical examination were carried out. Results: Jatropha crude oil contained phorbol esters differently from BDF which phorbol ester levels were below detection limit. Despite of the absence of phorbol esters, BDF had tumor-promoting activity. LD50 for chronic administration was 2.0 μg/animal/application for PMA, 50 - 100 mg/animal for crude oil, 100 mg/animal for BDF in 60-week observation. Papilloma was developed in all mice of the BDF-200 mg group but all mice died before papilloma was developed in the crude oil-200 mg group, suggesting that acute toxicity of crude oil was stronger than that of BDF. In addition, safety margin of crude oil was suggested to be narrower than that of BDF. Among all survived mice, there were no significant difference of serum biochemical parameters between the BDF and crude oil groups, but were considerable individual variability within each group. Abnormal findings were observed in white blood cell count and its differential cell count in the crude oil and BDF groups. Conclusions: Chronic exposure to Jatropha crude oil and BDF caused development of papilloma in mice as well as PMA, suggesting that Jatropha phorbol ester contained in Jatropha crude oil and unknown component of BDF developed papilloma. Crude oil has stronger acute toxicity and narrower safety margin than those of BDF, suggesting that the strict regulation in handling of both Jatropha products. This study was supported by JICA/JST, SATREPS (Science and Technology Research Partnership for Sustainable Development), JAPAN.

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Preface

This issue is devoted to the abstracts of the presentations for the Continuing Education courses and scientific sessions of the 55th Annual Meeting of the Society of Toxicology, held at the New Orleans Ernest N. Morial Convention Center, March 13–17, 2016.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 603.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 629.

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