**Background**

Piperonyl butoxide, PBO (CAS RN: 51-03-6) is an insecticide synergist co-applied with pyrethroids and pyrethrin-like pesticides. The toxicity of PBO was extensively investigated in animal studies and liver was identified as its main target organ. The severity and type of hepatotoxic effects depend on the duration of exposure to PBO.

- **Short-term (or lower dosage):** Mild changes, including liver steatosis and enlargement (hepatocellular hypertrophy) observed in dogs [1]. Our previous research proposed the glial side chain of PBO to be responsible for the proapoptotic mode of action upon short-term exposure due to PXR/PPAR receptor agonism [2].

- **Long-term (or higher dosage):** More severe effects, including neoplasia and liver cancer observed in mice [3]. The mode of action remains unclear, but a range of experiments, e.g. bacterial mutagenicity, in vitro Chromosomal Aberrations (v/iCA), show no genotoxic activity of PBO [4,5].

Structurally, PBO is a derivative of safrole, a weak genotoxic hepatocarcinogen in mice and rats, exerting its toxicity through metabolic bioactivation. Oxidation of allyl side chain and oxidation of methylphenylnaphthyl group with subsequent cleavage leading to catechol formation [6].

**Objective**

- Investigating the role of different metabolic pathways in hepatotoxic effects elucidated by PBO.

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**Methods**

- The major metabolites and biotransformation pathways of PBO and safrole (selected as a reference compound) were generated with the ChemTunes BioPath, Metabolism Prediction Online Service, developed by Molecular Networks GmbH.

- The genetic toxicity (bacterial mutagenicity, iv/vca, in vivo Micronucleus Assay (iv/vMNI) and carcinogenicity (rat and mouse tumorigenicity) of parent compounds and their metabolites was predicted using ChemTunes Studio, a software platform providing a unique knowledge base of in vitro and in vivo toxicity data for the evaluation and assessment of chemical compounds, developed by Molecular Networks GmbH.

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**Conclusions**

- Metabolic profiling is applied for investigating chemically-induced liver toxicity

- On the basis of the predicted metabolites (catechols and guaiacols) of PBO that result from the opening of the methylenedioxyphenyl ring can (respectively): (1) undergo further oxidation to form reactive o-quinone derivatives; (2) form reactive quinone methides, that may covalently bind to proteins [8,9].

- The bioactivation pathway, involving dealkylation and 2-electron oxidation was predicted for safrole (Fig. 2) [9]. This mechanistic model elucidates the differences between PBO and safrole: Guaiacol-related metabolites predicted as positive in iv/vCA model and PBO and safrole: Guaiacol-related metabolites predicted as uncertain in Mouse Tumor model.

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**ChemTunes Toxicity Prediction of Parent Compounds**

- **SAFROLE (Reference compound)**
  - Negative in bacterial mutagenicity, iv/vCA and iv/vMNI models and positive in iv/vCA model. This result is in agreement with experimental data and confirms genetic toxicity of safrole.
  - Positive in carcinogenicity-related Mouse and Rat models. This result is in agreement with available experimental data.

- **ChemTunes BioPath Metabolism Prediction**

  - Generated known biotransformation pathways and metabolites of PBO:
    - O-dealkylation → Ring opening → Catechol and Guaiacol derivatives
    - O-dealkylation (aliphatic):
      - Side glycol chain modifications
  
  - No genotoxic activity of metabolites resulting from glycol chain biotransformations

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**ChemTunes Toxicity Prediction of BioPath Metabolites**

- O-dealkylation of methylenedioxy ring leads to its opening and catechol and guaiacol derivatives formation:
  - PBO: No genotoxic activity predicted for guaiacol-related metabolites
  - Safrole: Guaiacol-related metabolites predicted as positive in iv/vCA model
  - PBO and Safrole: Guaiacol-related metabolites predicted as uncertain in Mouse Tumor model

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**REFERENCES**

[1] Altamira LLC, Columbus, OH 43235, USA; Molecular Networks GmbH, 91052 Erlangen, Germany; Institute of Biophysics and Biomedical Engineering, ‘Acad G. Bonchev’, bl. 21, 1113 Sofia, Bulgaria; Liverpool John Moores University, Liverpool L3 5UZ, UK


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**Fig. 1. In silico predictions of PBO and Safrole: ChemTunes Studio toxicity predictions for parent compounds (A-B) and selected metabolites (E-F) and ChemTunes BioPath biotransformation pathways and metabolites generation (C-D)**

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**Fig. 2. Proposed safrole bioactivation pathway**

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**Catechols and guaiacols resulting from the opening of the methylenedioxyphenyl ring can (respectively): (1) undergo further oxidation to form reactive o-quinone derivatives; (2) form reactive quinone methides, that may covalently bind to proteins [8,9].**

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**Conclusion**

- Metabolic profiling is applied for investigating chemically-induced liver toxicity

- On the basis of the predicted metabolites (catechols and guaiacols) of PBO that result from the opening of the methylenedioxyphenyl ring can (respectively): (1) undergo further oxidation to form reactive o-quinone derivatives; (2) form reactive quinone methides, that may covalently bind to proteins [8,9].

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