Piperonyl Butoxide: Friend or hidden foe?

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Synergists and the role of Piperonyl Butoxide

Synergists are chemicals that in theory lack pesticidal properties but which can increase such pesticidal properties of another substance when prepared as a formulation[1]. Piperonyl butoxide (PBO) is a semisynthetic chemical used as a main ingredient in pesticide mixtures and is used for various agricultural and domestic applications. The ratio of PBO to other compounds of a pesticide mixture is usually in higher concentrations compared to the main active ingredient. PBO's mode of action focuses on the inhibition of cytochrome P450 enzymes[2]. From the addition of PBO, the amount of the main active pesticidal ingredient needed is reduced, but the effectiveness of the toxic effect is achieved to the same level besides the reduction, by inhibiting metabolic enzyme systems, P450s and esterases, inside insects[3,4].

Regulation classification and labeling of Piperonyl Butoxide

Synergists on the EU level, are approved for use in plant protection products when they pass criteria based on Regulation (EC) No 1107/2009. Synergists are substances or preparations which, while showing no or only weak activity, can enhance the activity of the active substance(s) in a plant protection product. PBO is under the non-exhaustive list of safeners and synergists contained in plant protection products authorized for placing on the market in Member States on the date of entry into force of Commission Regulation (EU) No 2024/1487 since 19 July 2024[5].

PBO is a biocidal active substance according to Regulation (EU) No 528/2012 approved by the European Commission in product type 18 that includes insecticides, acaricides and products to control other arthropods. PBO is applied in amenity areas and woodlands (outdoor) against adult mosquitoes and public buildings (indoor) against flying insects (adult mosquitoes and houseflies). PBO is always used in insecticidal formulations in combination with other insecticides mainly belonging to pyrethrins and synthetic pyrethroids. A debate has been established regarding the efficacy of PBO, and whether it may be considered as an active substance or a synergist. It was concluded at EU level that PBO is an active biocide.

PBO's harmonized classification and labelling (ATP18) approved by the European Union in 2022, recognizes this substance as very toxic to aquatic life with long lasting effects, and regarding human health, PBO causes serious eye irritation and may cause respiratory irritation[6].

For PBO to be classified and regulated, several detailed toxicity assessments by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), have been conducted[7-9]. Furthermore, the European Food Safety Authority (EFSA) conducted in 2019 an official control on pesticide residues in food, where PBO was on the list of searched residues, and compliance levels evaluated in regard to national MRLs (Maximum Residue Levels) resulted in 164 samples containing PBO above the LOQ for 82.3% of them[10].

Toxicity of Piperonyl Butoxide

As PBO is mainly used as a synergist to increase the toxic impact of pesticide formulations, the individual toxicity of PBO has recently been of interest in toxicity studies. PBO has been shown to have negative toxic effects such as hepatocellular necrosis and cytotoxicity as well as effects on exploratory behavior, sex-related modulation, development, birth, various sperm functions, embryo development, and male fertility[11-17].

A cardiotoxic effect of PBO alone, could be possible if the exposure was to a formulation not containing any other active ingredient besides PBO (at a high concentration level as well), to exclude a co-exposure effect via the inhalation and dermal route[9,18].

In a 79-week bioassay, PBO significantly affected the

frequency of hepatocellular adenoma in male CD-1 mice and female mice[19].

Furthermore, PBO can cause DNA damage in Chinese hamster ovary cells[20], cytotoxicity in fish lymphocytes[21], and inhibition of the Hedgehog signaling pathway[11]. Agency bodies such as the World Health Organization (WHO) evaluated the human health hazard and risk assessment for PBO-treated nets, where the assessment of the available safety information indicated that a PBO-insecticide treated net (ITN) with a concentration of 1000 mg/m^2 (fabric weight 40 g/m^2 and 25 g/kg net) and a WRI (Wash Resistance Index) of 90% is regarded safe for its intended use as a vector control product. It was noted that these selected values were not intended to put a limit on the possible concentration of PBO in an ITN, and that selected values must not represent the maximum concentration of PBO at which the assessed risks may become unacceptable[22]. The United States Environmental Protection Agency (EPA) classified PBO as a Group C type chemical, indicating that it is a possible human carcinogen based on limited evidence of carcinogenicity in animals[23]. The International Agency for Research on Cancer (IARC) reported that PBO is 'not classifiable as to its carcinogenicity to humans' according to the National Pesticide Information Center (NPIC)[23]. In regard to synergistic effects of PBO, studies have shown a possible link in increasing the toxicity of PBO[24-26].

In New Zealand rabbits, oxidative stress markers suggested that PBO and the co-exposed groups (with cypermethrin) of that study, induced oxidative stress as well as induction of telomerase activity[27]. Similarly, PBO and co-exposed groups (with cypermethrin) caused liver and kidney inflammation and induced genotoxicity, and their combinations resulted in a significant increase of micronuclei frequencies justified by the addition of each compound's toxic effect. Additionally, a statistically significant increase of micronuclei and binucleated cells with micronucleus were estimated, suggesting a cumulative stressful effect that culminates in DNA damage[28].

Conclusion

In essence, PBO's 'hidden' nature stems from the underestimation of its exposure. While considered a simple enhancer, it is used in a wide range of everyday home indoor and outdoor products.

The majority of home pesticide products are formulated with synthetic pyrethrins and PBO together. Hence, there is a possible parallel exposure to PBO every time home pesticide products are used. Indeed, PBO toxicity in humans has not been clarified to the fullest, as most toxicity studies are on rats and only a few on humans[29]. The EPA indicated though a greater risk for moderate and major respiratory symptoms by products containing pyrethrins together with PBO, than by pyrethrins alone[9, 30]. However, due to the limitations of such studies, it is difficult to assess the true underlying risk. It is crucial to develop methods to assess interactions between pesticides and biocides, particularly in mixture scenarios.

Concepts like the RLRS (real-life risk simulation) are needed to bridge these knowledge gaps by integrating multiple chemical exposures and cumulative effects, given PBO's use in chemical mixtures[31].

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CONFLICTS OF INTEREST
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