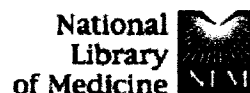


Appendix II

The abstracts of peer-reviewed published research presented in this section mostly deal with the **genotoxicity of piperonyl butoxide**, the synergant used in Anvil 2+2. The various different biochemical assays used in these studies clearly demonstrate the chemicals ability to induce mutations and/or modify normal patterns of gene expression and natural biochemical pathways.

To aid the layperson who scans these abstracts, I have placed brackets around the most pertinent text or, in some cases underlined specific findings.



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1: Carcinogenesis. 1983;4(3):291-5.

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The morphological transformation of Syrian hamster embryo cells by chemicals reportedly nonmutagenic to Salmonella typhimurium.

Amacher DE, Zelljadt I.

Nine chemicals classified as presumptive carcinogens on the basis of chronic rodent bioassays and one suspected human carcinogen which are reportedly not mutagenic to Salmonella typhimurium tester strains were tested for the ability to produce morphological transformation in Syrian hamster embryo cells in the absence of any exogenous source of metabolic activation. Acetamide, benzene, carbon tetrachloride, L-ethionine, monuron, piperonyl butoxide and trichloroethylene all induced positive morphological transformation in the presence of at least one of two medium and serum combinations as did the positive controls, ethyl methanesulfonate and benzo[a]pyrene. The remaining three chemicals, griseofulvin, isoniazid and trypan blue, did not induce morphological transformation under these same test conditions suggesting that they differ from the other seven chemicals in mechanism of action, target specificity or species susceptibility. Our results for seven of those ten selected chemicals in the clonal transformation assay using Syrian hamster embryo cells differ from their reported activity in the S. typhimurium point mutation assay. On the basis of this small sample, the Syrian hamster embryo transformation assay was a better predictor of the reported rodent bioassay results.

PMID: 6339095 [PubMed - indexed for MEDLINE]

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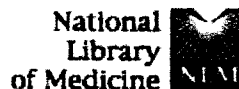
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1: Mutat Res. 1995 Aug;344(1-2):27-30.

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Piperonyl butoxide mutagenicity in human RSa cells.

Suzuki H, Suzuki N.

Department of Toxicology, Tokyo Metropolitan Research Laboratory of Public Health, Japan.

Piperonyl butoxide (PB) is used as a pesticide synergist and food additive. Its chemically induced mutagenicity was found in cultured human RSa cells by determination of ouabain-resistant (Ouar) phenotypic mutation, with the highest frequency at the concentration of 0.2 microgram/ml. Moreover, K-ras codon 12 mutations in genomic DNA, analyzed by polymerase chain reaction (PCR) and differential dot-blot hybridization using digoxigenin-labeled probes, were detected in RSa cells 6 days after exposure to PB (0.03-0.40 microgram/ml).

PMID: 7565889 [PubMed - indexed for MEDLINE]

2: Toxicol Ind Health. 1995 Mar-Apr;11(2):175-84.

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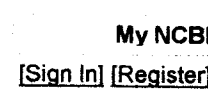
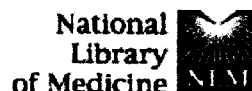
Developmental toxicity study of piperonyl butoxide in CD rats.

Tanaka T, Fujitani T, Takahashi O, Oishi S, Yoneyama M.

Department of Toxicology, Tokyo Metropolitan Research Laboratory of Public Health, Japan.

Piperonyl butoxide was administered to pregnant rats by gavage at a level of 0 (control), 630, 1065, and 1800 mg/kg bw on days 11-12 of gestation. The animals were killed on day 20 of gestation. Average maternal body weight gain (gestational days 11-20) was significantly reduced in the 1065 and 1800 mg/kg bw groups. Total resorption rate was significantly increased in the 1800 mg/kg bw group and those effects were significantly dose-related. The average fetal body weight of each sex was significantly reduced in the 1065 and 1800 mg/kg bw groups. External limb deformity (oligodactyly, syndactyly, and polydactyly) was significantly increased in the 1065 and 1800 mg/kg bw groups in a dose-related manner. The dose levels of

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1: Mutat Res. 1996 Jul 5;368(3-4):249-60.

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Cytogenetic effects of piperonyl butoxide and safrole in CHO-K1 cells.

Tayama S.

Department of Toxicology, Tokyo Metropolitan Research Laboratory of Public Health, Japan.

Recently, hepatocarcinogenicity in rats and mice was reported with regard to the methylenedioxyphenyl compound, piperonyl butoxide (PB), which is used as a synergist for pyrethrins and related insecticides. Induction of sister-chromatid exchanges (SCEs) and chromosomal aberrations (CAs) due to PB were investigated using CHO-K1 cells with or without rat liver S9 fraction (S9); at the same time, the effects of safrole (SF), a methylenedioxyphenyl compound and a weak hepatocarcinogen, were also examined. PB (0.25 and 0.3 mM) and SF (0.8 mM) caused a slight but significant increase in SCEs followed by a cell-cycle delay in the 3-h treatment without S9. In the presence of S9 (4.5%), the cytotoxicity of PB or SF was weakened greatly or slightly, the top dose capable of cell division was raised to 0.6 mM (2-fold) or 1 mM, respectively. PB with S9 induced SCE at doses of 0.4 and 0.5 mM, and caused endoreduplications (ERDs, 7%) at a dose of 0.6 mM, while SF caused a dose-related significant increase in SCE at all doses used (0.4-1 mM) with S9. Genotoxicity of the metabolites of PB or SF was cleared by changing the dose of S9 (1.5-9%) while holding the dose of each chemical constant. In the case of SF (0.6 mM), induction of SCE, ERD and cell-cycle delay intensified almost in a dose-effect relationship, and CAs and a high level of ERD (14%) were caused by a 9% dose of S9. The concentration of unchanged SF in the incubated medium was certainly in inverse proportion to the dose of S9. This strongly suggests that the metabolites of SF are genotoxic. In the case of PB (0.3 mM), no positive responses were produced in the cultures, even with a high level of S9, though the amount of unchanged PB left in the incubated medium was very slight. This indicates that the metabolites of PB may not be genotoxic. In conclusion, PB and SF are possible to somewhat induce SCE at high dose(s) in the absence of S9, and the genotoxic effects of SF are more intensified in the presence of S9 than in its absence, while PB is probably no genotoxic in the presence of sufficient metabolic activation.

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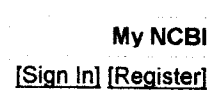
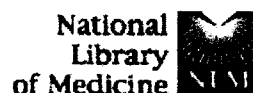
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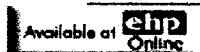
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1: Environ Health Perspect. 2002 May;110(5):507-14.

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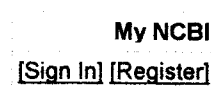
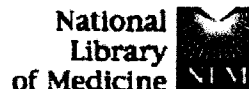


Residential pesticide use during pregnancy among a cohort of urban minority women.

Whyatt RM, Camann DE, Kinney PL, Reyes A, Ramirez J, Dietrich J, Diaz D, Holmes D, Perera FP.

Columbia Center for Children's Environmental Health, Mailman School of Public Health, Columbia University, New York, New York 10032, USA.

Residential pesticide use is widespread in the United States. However, data are limited specific to use among minority populations. Nor are data available on the extent of pesticide exposure resulting from residential use during pregnancy. We have gathered questionnaire data on pesticide use in the home during pregnancy from 316 African-American and Dominican women residing in northern Manhattan and the South Bronx. Additionally, 72 women underwent personal air monitoring for 48 hr during their third trimester of pregnancy to determine exposure levels to 21 pesticides (19 insecticides and 2 fungicides). Of the women questioned, 266 of 314 (85%) reported that pest control measures were used in the home during pregnancy; 111 of 314 (35%) reported that their homes were sprayed by an exterminator, and of those, 45% said the spraying was done more than once per month. Most ($\geq 90\%$) of the pesticide was used for cockroach control. Use of pest control measures increased significantly with the level of housing disrepair reported. Of the women monitored, all (100%) had detectable levels of three insecticides: the organophosphates diazinon (range, 2.0-6,010 ng/m³) and chlorpyrifos (range, 0.7-193 ng/m³) and the carbamate propoxur (range, 3.8-1,380 ng/m³), as well as the fungicide o-phenylphenol (range, 5.7-743 ng/m³). We also frequently detected the following four insecticides (47-83% of samples) but at lower concentrations: the pyrethroid trans-permethrin, piperonyl butoxide (an indicator of exposure to pyrethrins), and the organochlorines 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane and chlordane. Thirty percent of the women had detectable levels of all eight pesticides. Exposures were generally higher among African Americans than among Dominicans. We



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1: Inflamm Res. 2003 Apr;52(4):154-63.

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Pyrethroid insecticides influence the signal transduction in T helper lymphocytes from atopic and nonatopic subjects.

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Diel F, Horr B, Borck H, Irman-Florjanc T.

Institut für Umwelt und Gesundheit, University of Applied Sciences FH Fulda, Marquardstrasse 35, 36039 Fulda, Germany. friedhelm.diel@t-online.de

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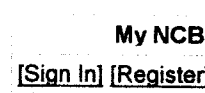
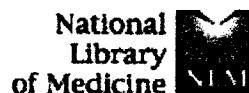
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OBJECTIVE AND DESIGN: Pyrethroids are claimed to have a low human toxicity with some neuro- and immunotoxicity. The objective of this study was to investigate the immunotoxicological properties of six commercially used pyrethroids, including natural pyrethrum and synergist piperonyl-butoxide (PBO). **MATERIAL AND METHODS:** PHA-stimulated cultures of T-helper lymphocytes and blood basophil incubates from nonatopic and atopic patients (IgE > 1000 IU) provided cytokine and histamine determination. Western blot analysis was used for the measurement of Th2-specific signal transducer and activator of transcription-6 (STAT6). Pyrethroids and xenobiotics were added 4 h post-plating. **RESULTS:** We demonstrated that interferon-gamma (IFN-gamma) production and expression was correlated with lymphocyte proliferation, however, interleukin-4 (IL-4) was down-regulated at the end of the 3 day culture. Atopics showed significantly higher IL-4 activity than nonatopics. Pyrethroids inhibited IFN-gamma and IL-4 in both groups at around 10(-5) M. Only fenvalerate and S-bioallethrin combined with 10-fold PBO in the atopic-enriched blood basophil incubates caused a weak but significant increase in histamine release. Histamine acted bidirectionally on STAT6, but pyrethroids inhibited the intracellular Th2-specific STAT6 more effectively in atopics than in nonatopics. **CONCLUSION:** It can be suggested that pyrethroids inhibit signal transduction in human lymphocytes ex vivo, and do not act via lymphocyte-influencing histamine release.

Publication Types:

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PMID: 12755381 [PubMed - indexed for MEDLINE]



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1: Food Addit Contam. 2003 Mar;20(3):207-14.

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Reproductive and neurobehavioural effects of piperonyl butoxide administered to mice in the diet.

Tanaka T.

Department of Toxicology, Tokyo Metropolitan Research Laboratory of Public Health, 3-24-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan. tanaka@tokyo-eiken.go.jp

Piperonyl butoxide was given in the diet to mice to provide levels of 0 (control), 0.01, 0.03 and 0.09% from 5 weeks of age of the F(0) generation to 9 weeks of age of the F(1) generation, and selected reproductive and neurobehavioural parameters were measured. There were no adverse effects of piperonyl butoxide on either litter size, litter weight or sex ratio at birth. The average body weight of male offspring was significantly increased in the middle-dose group at post-natal days 4 and 7 during lactation. That of female offspring was significantly increased in the middle-dose group at post-natal days 7 and 14 during lactation. In behavioural developmental parameters, surface righting at post-natal day 7 was significantly delayed in the higher-dose groups in male offspring, and those effects were significantly dose related (p < 0.01). Olfactory orientation at post-natal day 14 was significantly depressed in the higher-dose groups in male offspring, and those effects were significantly dose related (p < 0.01). For movement activity of exploratory behaviour at 9 weeks of age of the F(1) generation, the total distance of males was significantly increased in the higher-dose groups, and those effects showed a dose-related manner (p < 0.01). Average distance and speed were significantly increased in the high-dose group, and those effects showed a dose-related manner (p < 0.01 in each). The dose levels of piperonyl butoxide in the present study produced some adverse effects in reproductive and neurobehavioural parameters in mice.

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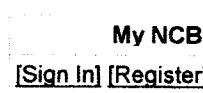
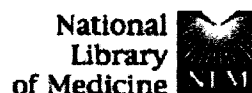
1: Int Immunopharmacol. 2005 Feb;5(2):263-70.

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FULL-TEXT ARTICLE**Influence of pyrethroids and piperonyl butoxide on the Ca(2+)-ATPase activity of rat brain synaptosomes and leukocyte membranes.****Grosman N, Diel F.**Department of Pharmacology, The Panum Institute, University of Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N, Denmark. fing@farmakol.ku.dk

Pyrethroids are widely used insecticides of low acute toxicity in mammals but the consequences of long-term exposure are of concern. Their insecticidal action is related to neurotoxicity and, in addition, there are indications of mammalian immunotoxicity. In order to clarify structure-activity relationships of the membrane interactions of pyrethroids, the present study compared the influence of selected pyrethroids, i.e. permethrin and the more water soluble esbiol (S-bioallethrin), both type I, and cyfluthrin, type II, on the Ca(2+)-ATPase activity of rat brain synaptosomes and peritoneal leukocyte membranes. The pyrethroids were tested alone as well as mixed with the enhancing substance piperonyl butoxide (PBO) at concentration ratios of 1:5 and 1:10. At the highest concentration tested, permethrin (10 microM) alone inhibited the ATPase activity of leukocyte membranes by 20%, whereas the synaptosomes were affected less. Esbiol and cyfluthrin alone did not affect either membrane preparation significantly, whereas PBO (50 microM) alone caused 10-15% inhibition. Mixtures of either pyrethroid with PBO inhibited the ATPase activity of both types of membranes (up to 40% inhibition) in a synergistic manner, which always tended to be supra-additive. With esbiol a true potentiation took place. The synergistic interaction between pyrethroid and PBO was most apparent with mixtures of a concentration ratio of 1:5. The ATPase activity of leukocyte membranes tended to be more susceptible to inhibition than that of synaptosomes. The results are in accordance with the assumption that the mammalian toxicity of pyrethroids can be ascribed to a general disturbance of cell membrane function in neuronal tissue. The results indicate that it may also be the case in the immune apparatus.

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1: Environ Sci Technol. 2003 Oct 15;37(20):4543-53.

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Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust.

Rudel RA, Camann DE, Spengler JD, Korn LR, Brody JG.

Silent Spring Institute, 29 Crafts Street, Newton, Massachusetts 02458, USA. rudel@silentspring.org

Chemicals identified as endocrine-disrupting compounds (EDCs) have widespread consumer uses, yet little is known about indoor exposure. We sampled indoor air and dust in 120 homes, analyzing for 89 organic chemicals identified as EDCs. Fifty-two compounds were detected in air and 66 were detected in dust. These are the first reported measures in residential environments for over 30 of the compounds, including several detected at the highest concentrations. The number of compounds detected per home ranged from 13 to 28 in air and from 6 to 42 in dust. The most abundant compounds in air included phthalates (plasticizers, emulsifiers), o-phenylphenol (disinfectant), 4-nonylphenol (detergent metabolite), and 4-tert-butylphenol (adhesive) with typical concentrations in the range of 50-1500 ng/m3. The penta- and tetrabrominated diphenyl ethers (flame retardants) were frequently detected in dust, and 2,3-dibromo-1-propanol, the carcinogenic intermediate of a flame retardant banned in 1977, was detected in air and dust. Twenty-three pesticides were detected in air and 27 were detected in dust, the most abundant being permethrins and the synergist piperonyl butoxide. The banned pesticides heptachlor, chlordane, methoxychlor, and DDT were also frequently detected, suggesting limited indoor degradation. Detected concentrations exceeded government health-based guidelines for 15 compounds, but no guidelines are available for 28 compounds, and existing guidelines do not consider endocrine effects. This study provides a basis for prioritizing toxicology and exposure research for individual EDCs and mixtures and provides new tools for exposure assessment in health studies.

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1: Chem Biol Interact. 1997 Jun 6;105(1):53-63.

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FULL-TEXT ARTICLE

Regulation of hepatic CYP1A isozymes by piperonyl butoxide and acenaphthylene in the mouse.

Ryu DY, Levi PE, Hodgson E.

Department of Toxicology, North Carolina State University, Raleigh 27695, USA.

The regulation of CYP1A1 and CYP1A2 isozymes by piperonyl butoxide (PBO) and acenaphthylene (ACN) was studied in the liver of male C57BL/6 and DBA/2 mice. These two cytochrome P450 genes are known to be regulated by the aromatic hydrocarbon-responsive receptor (AHR); however, it has been suggested that CYP1A2 is also induced by an AHR-independent mechanism. In this study, PBO induced hepatic CYP1A1 considerably more in C57BL/6 (Ahrb-I) than in DBA/2 (Ahrd) mice. In addition, the superinduction of CYP1A1 in wildtype hepatic cells, which is AHR-dependent, resulted from PBO and cycloheximide treatment of the cells. In other studies in this laboratory using AHR knock-out (AHR^{-/-}) mice, a hybrid of 129/SV and C57BL/6 strains, no induction of CYP1A1 occurred with PBO or ACN. [D.-Y. Ryu, P.E. Levi, P. Fernandez-Salguero, F.J. Gonzalez, E. Hodgson, *Mol. Pharmacol.*, 50 (1996) 443-446.] ACN, however, did not induce CYP1A1 under the experimental conditions used. These results suggest that PBO, but not ACN, induces CYP1A1 through a weak activation of AHR. On the other hand, hepatic CYP1A2 mRNA and hnRNA were induced by PBO in both C57BL/6 and DBA/2 strains, but were not induced by ACN, a strong inducer of CYP1A2 in the B6C3F1 strain. However, both PBO and ACN induced CYP1A2 in AHR^{-/-} mice. It is assumed, therefore, that the transcriptional induction of CYP1A2 by PBO and ACN is AHR-independent. In addition, the induction of CYP1A2 by ACN depends upon the strain of mice. Immunohistochemical studies for CYP1A1/CYP1A2 apoproteins showed that PBO induced CYP1A1/CYP1A2 around the central veins as did 3-methylcholanthrene (3-MC). The induction of CYP1A1/CYP1A2 by ACN, however, was not observed, consistent with the northern blot results.

PMID: 9233375 [PubMed - indexed for MEDLINE]

2: Toxicol Ind Health. 1995 Mar-Apr;11(2):175-84.

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