Appendix III

The abstracts of peer-reviewed published research presented in this section mostly deal with the estrogenic and general **endocrine disrupting properties of Sumithrin** (chemically known as phenothrin). A few studies in this section also deal with the chemicals activity as a genotoxic agent. The various chemical and biological assays used in these studies clearly demonstrate the chemicals ability to induce developmental aberrations and abnormal 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.

1: J Reprod Dev. 2004 Apr;50(2):245-55. Related Articles, Links

J-STAGE

Assessing estrogenic activity of pyrethroid insecticides using in vitro combination assays.

Kim IY, Shin JH, Kim HS, Lee SJ, Kang IH, Kim TS, Moon HJ, Choi KS, Moon A, Han SY.

Endocrine Toxicology Division, National Institute of Toxicological Research, Korea Food and Drug Administration, Seoul, Korea.

Pyrethroid insecticides are among the most commonly used classes of insecticides worldwide, but their endocrine disrupting activities remain unclear. Therefore, in the present study, we examined the estrogenic activities of pyrethroid insecticides in E-screen and competition binding assays. In addition, we measured estrogen receptor (ER) protein and pS2 mRNA levels in human breast cancer cells (MCF-7 BUS) to clarify the mechanism of their estrogenicity. Seven pyrethroid insecticides (bioallethrine, cypermethrin, deltamethrin, fenvalerate, permethrin, sumithrin, and tetramethrin) were tested because of their worldwide usage. In addition, 17beta-estradiol was tested as a positive control. As expected, 17beta-estradiol significantly increased MCF-7 BUS cell proliferation at concentrations of 10(-11) M and above. Of the pyrethroid insecticides tested, only sumithrin increased MCF-7 BUS cell proliferation in a dose-dependent manner; the maximum induction of cell proliferation was observed at a dose of 10(-5) M. In the anti-estrogenic activity test, bioallethrin, fenvalerate, and permethrin significantly inhibited 17beta-estradiol-induced MCF-7 BUS cell proliferation at 10(-6) M, a concentration comparable to the effective dose (10(-9) M) of ICI 182,780, a pure ER antagonist. However, none of the pyrethroid insecticides competitively inhibited the binding of [(3)H]estradiol to rat uterus ERs in competition binding assays. Both 17beta-estradiol (10(-10) M) and sumithrin (10(-5) M) decreased the levels of cytosolic ERalpha and ERbeta protein expression significantly as compared with the vehicle control. In addition, 17beta-estradiol (10(-10) M) increased pS2 mRNA expression markedly, and sumithrin significantly increased pS2 mRNA levels in a dose-dependent manner. The other six compounds tested in the present study did not affect ER protein levels or pS2 mRNA levels. These results suggest that certain pyrethroid insecticides may be

considered to be estrogen-like chemicals that act through pathways other than direct ER binding, and may function as endocrine modulators in both wildlife and humans.

PMID: 15118252 [PubMed - indexed for MEDLINE]

PMID: 15118252 [PubMed - indexed for MEDLINE]

2: Mutagenesis. 2004 Mar;19(2):85-90. Related Articles, Links

Evaluation of in vivo genotoxicity of cypermethrin in Drosophila melanogaster using the alkaline Comet assay.

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The single cell gel electrophoresis (SCGE) assay, also known as the Comet assay, is one of the most promising genotoxicity tests developed in recent years to measure and analyse DNA damage in single cells. The present study was undertaken to assess the in vivo genotoxicity of the synthetic pyrethroid cypermethrin in brain ganglia and anterior mid gut of Drosophila melanogaster. Freshly emerged first instar larvae (22 +/- 2 h) were placed in different concentrations of cypermethrin (0.0004, 0.0008, 0.002, 0.2 and 0.5 p.p.m.) mixed in standard Drosophila food and allowed to grow. At 96 +/- 2 h, brain ganglia and anterior midgut from control and treated larvae were dissected out, single cell suspensions were prepared and a Comet assay was performed. Our results revealed a significant dose-dependent increase in DNA damage in the cells of brain ganglia and anterior midgut of D.melanogaster exposed to cypermethrin as compared with controls ($P < 0.05$ at 0.002 p.p.m.; $P < 0.001$ at 0.2 and 0.5 p.p.m.). The present study shows in vivo genotoxicity of cypermethrin even at very low concentrations, which proves D.melanogaster as a model for in vivo genotoxicity assessment using the Comet assay.

PMID: 14981154 [PubMed - indexed for MEDLINE]

3: Endocr Pract. 2003 Sep-Oct;9(5):370-5. Related Articles, Links

Epidemic of gynecomastia among haitian refugees: exposure to an environmental antiandrogen.

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Breast development

OBJECTIVE: To investigate an observed epidemic of gynecomastia among Haitian refugees in US detention centers in 1981 and 1982. METHODS: All identifiable environmental exposures were investigated for estrogenic and antiandrogenic activity. RESULTS: A high incidence of gynecomastia was observed among Haitian refugees in five detention centers in the United States. Of 284 men screened, 20 (from 18 to 53 years old) demonstrated new-onset gynecomastia (Tanner stages 2 to

5) in June 1982. The mean onset of gynecomastia was 130 +/- 12 days after arrival in the United States. Other symptoms included loss of libido (in all 20 patients) and decreased beard growth (in 10). Plasma concentrations of luteinizing hormone, follicle-stimulating hormone, prolactin, testosterone, and estradiol were not significantly different from those in 20 age-matched control subjects. Environmental substances, including tap water and the delousing agents Kwell shampoo and R&C Spray (applied to bedding and clothing), were tested for estrogenicity and androgenicity. None of these substances bound to cytosol estrogen receptors. The delousing agents were assayed for androgen binding by using genital skin fibroblasts. R&C Spray competed equally with testosterone for androgen-binding sites. Phenothrin, the "multi-cide" component of R&C Spray, reproduced this competitive binding result. When tested for antiandrogenic effects on prostate growth by using immature male rats treated with testosterone-filled Silastic capsules, phenothrin antagonized androgen action, as demonstrated by decreased prostate weights. CONCLUSION: The antiandrogenic activity of phenothrin may explain this unusual epidemic of gynecomastia.

Phenothrin = Sumithrin

PMID: 14583418 [PubMed - indexed for MEDLINE]

4: Environ Int. 2002 Nov;28(5):429-32. Related Articles, Links

Effects of pyrethroid insecticides and estrogen on WNT10B proto-oncogene expression.

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Breast cancer is a serious illness affecting approximately one in nine women in the United States. Although an actual cause for breast cancer is unknown, genetic and environmental factors have been associated with its onset. Elevated levels of estrogen and heightened expression of the WNT10B proto-oncogene have been implicated in the development of human malignant breast tumors because they enhance the proliferation of mammary tissue. Two pyrethroid insecticides, sumithrin and fenvalerate, have been shown to mimic estrogenic activity in MCF-7 human breast carcinoma cells by inducing pS2 expression whereas two other pyrethroids, permethrin and d-trans allethrin do not have the same capability. To investigate if estrogen and these four pyrethroid insecticides could affect the expression of a gene related to mammary gland development, WNT10B expression in pyrethroid-treated MCF-7 cells was examined. MCF-7 cells under normal growth conditions do not express WNT10B. Reverse-transcriptase polymerase chain reaction (RT-PCR), nested PCR and Southern hybridization were employed to detect WNT10B expression. As controls, cells were treated with either ethanol, corn oil, or Vista LPA solvent. When compared to the solvent-treated controls, sumithrin, fenvalerate and estrogen treated MCF-7 cells all had increased levels of WNT10B expression. The non-estrogenic pyrethroids, d-trans allethrin and permethrin, demonstrated a similar elevation of WNT10B expression at a lower concentration, but not at the higher concentration. The results suggest that pyrethroid insecticides and estrogen can enhance the expression of the WNT10B proto-oncogene. However, since both

the estrogenic and non-estrogenic substances amplified Wnt10B expression, the mechanism likely involves multiple distinct pathways.

PMID: 12437293 [PubMed - indexed for MEDLINE]

5: Environ Health Perspect. 1999 Mar;107(3):173-7. Related Articles, Links

Estrogenic potential of certain pyrethroid compounds in the MCF-7 human breast carcinoma cell line.

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Estrogens, whether natural or synthetic, clearly influence reproductive development, senescence, and carcinogenesis. Pyrethroid insecticides are now the most widely used agents for indoor pest control, providing potential for human exposure. Using the MCF-7 human breast carcinoma cell line, we studied the estrogenic potential of several synthetic pyrethroid compounds in vitro using pS2 mRNA levels as the end point. We tested sumithrin, fenvalerate, d-trans allethrin, and permethrin. Nanomolar concentrations of either sumithrin or fenvalerate were sufficient to increase pS2 expression slightly above basal levels. At micromolar concentrations, these two pyrethroid compounds induced pS2 expression to levels comparable to those elicited by 10 nM 17ss-estradiol (fivefold). The estrogenic activity of sumithrin was abolished with co-treatment with an antiestrogen (ICI 164,384), whereas estrogenic activity of fenvalerate was not significantly diminished with antiestrogen co-treatment. In addition, both sumithrin and fenvalerate were able to induce cell proliferation of MCF-7 cells in a dose-response fashion. Neither permethrin nor d-trans allethrin affected pS2 expression. Permethrin had a noticeable effect on cell proliferation at 100 microM, whereas d-trans allethrin slightly induced MCF-7 cell proliferation at 10 microM, but was toxic at higher concentrations. Overall, our studies imply that each pyrethroid compound is unique in its ability to influence several cellular pathways. These findings suggest that pyrethroids should be considered to be hormone disruptors, and their potential to affect endocrine function in humans and wildlife should be investigated.

PMID: 10064545 [PubMed - indexed for MEDLINE]

Items 1 - 3 of 3 One page.

1: Arch Environ Contam Toxicol. 2005 Feb;48(2):251-9. Epub 2005 Jan 11.

SpringerLink FULL TEXT ARTICLE

A comparison of the toxicity of synergized and technical formulations of permethrin, sumithrin, and resmethrin to trout.

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Synthetic pyrethroids often have synergists added to improve effectiveness, yet decisions regarding the use of these pesticides are often based upon toxicity tests using technical material without the synergist, piperonyl butoxide. We conducted toxicity tests with brook trout (Salvelinus fontinalis) and brown trout (Salmo trutta) to compare the toxicity of synergized and technical formulations of permethrin, sumithrin, and resmethrin. We found a significant increase in toxicity in the synergized permethrin formulation using traditional 24, 48, and 96-h tests, relative to tests with the technical formulation. However, there was little difference in toxicity between synergized and technical sumithrin until 48 h had elapsed. Many test fish were strongly intoxicated by either formulation of permethrin or sumithrin, but the synergized formulations of both chemicals affected fish at lower concentrations. Intoxication was potentially severe enough to reduce the survival of these fish in the wild. Following short (6-h) exposures, we also found a larger difference in the number of fish that died or became intoxicated between the synergized and technical formulations of permethrin and sumithrin. Finally, we tested the ability of exposed fish to swim against a current. Fish exposed for 6 h to synergized permethrin and resmethrin had far less swimming stamina than those exposed to technical formulations. We found no difference in the effect on swimming between the synergized and technical formulation of sumithrin. In general, the synergized formulations of these chemicals appeared to cause a faster response than the technical formulations. This response increases the lethal and sublethal impacts of the insecticides. We also found that sumithrin was the least toxic of the three pyrethroids. Since the maximum application rate of sumithrin is half that of the