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W. Steiling, J. Kreutz, M. Spanier and W. Sterzel

One of the most relevant toxicological properties of consumer products like cleansing agents or cosmetics is the compatibility to mucous membranes especially to the eye. For the evaluation of these properties, standardized *in vivo* tests are requested by official authorities yet as being valid to estimate the irritation potential of chemicals and being suitable for any risk assessment.

During the last decade HENKEL has established a useful strategy for this evaluation without any animal test. One of the most important part in this strategy is the so-called HET-CAM, the *in vitro* assay on the chorion-allantoic membrane of fertilized chicken eggs.

HET-CAM results of nearly 100 different chemicals, liquids and solids, within a huge scale of different irritating properties and different chemistry have been demonstrated. As it is clearly shown the predicted irritating properties fit very well with the available *in vivo* Draize data.

Very inhomogenous *in vivo* data are taken to be responsible for most of the few underpredicted irritating properties in relation to available *in vivo* data. But a critical point of this *in vitro* assay seems to be the weight of persistency of cornea effects over a time period of 21 days in the *in vivo* classification scheme.

As a result of our experience with the HET-CAM, the following conclusion can be drawn:

- The HET-CAM has been proven to be suitable for routine testing.
- The test results are in accordance with data from animal experiments in consideration of the limitations of each system.
- The HET-CAM has been proven to be valuable for reducing animal studies for the evaluation of irritating properties to the eye.

HENKEL KGaA, TTB-Toxikologie , D-40191 Düsseldorf, Germany

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### CHANGES IN SEXUAL BEHAVIOR OF MALE OFFSPRING AFTER EXPOSURE TO LINDANE DURING LACTATION

P.R. Dalsenter*, A.S. Faqi, J.Webb and I. Chahoud

Sexual development during the prenatal and neonatal period is under hormonal control and is therefore sensitive to exogenous substances with an endocrine effect. It is reported that γ-Hexachlorocyclohexane (lindane) possesses an endocrinological potency. We carried out comprehensive toxicity and toxicokinetics studies with lindane during the lactation period in rats. In this abstract, we present some of the results.

For the toxicity study, a group of lactating dams (n = 9) were treated orally with 6 mg lindane/kg on postnatal day 9, control dams (n = 9) received only the vehicle on the same day. At the age of 130 days, male offspring of both groups were mated (1:1) for 15 minutes with unexposed females in heat. The sexual behavior of male (n = 20/group) rats was then recorded. Likewise, the testosterone level was determined. While only 3 males (15%) of the control group were unable to ejaculate during the 15 minutes of recording, there were 12 males (60%) in the lindane-exposed offspring group. The Testosterone level (ng/ml) of the lindane group was significantly lower when compared to the corresponding control rats (lindane: 2.00 ± 0.92; control: 2.74 ± 1.11). A week after sexual behavior recording, males were mated with control females (1:1), 3 hrs daily for 8 days. All females were feconded and yielded viable fetuses.

For the toxicokinetic study, 6 lactating dams were treated with lindane in the same manner. Twenty four hrs after treatment, the dams and their offspring were sacrified and the concentration (ng/g tissue) of lindane was measured in different organs. The concentration of lindane in the liver of pups (616 ± 229) was one third of the liver concentration of the dams (1788 ± 192). In the testes, the concentration of lindane was 289 ± 33.

We conclude that exposure of rats to lindane during the lactation effects the sexual behavior in adult male offspring by changing the state of libido and by reducing the testosterone level without affecting their fertility.

Institut für Toxikologie und Embryopharmakologie, Freie Universität Berlin
Garystr. 5, 14195 Berlin.

* Scholarship awarded by CNPq- Brasilia, Brazil.

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### EFFECTS OF FLUOROQUINOLONES AND MAGNESIUM DEFICIENCY ON CARTLAGE (IN VIVO AND IN VITRO STUDIES)

Christian Förster[1], Jürgen Vormann[2], Irmela Baumann-Wilschke[1], Marcus Rücker[1] and Ralf Stahlmann[1]

Quinolones induce joint cartilage lesions in juvenile animals which are identical to cartilage lesions observed in $Mg^{2+}$-deficient juvenile rats. The $Mg^{2+}$ threshold concentration leading to cartilage damage is not known. We conducted two independent experimental series in juvenile rats and induced $Mg^{2+}$ deficiency by feeding a $Mg^{2+}$-deficient diet for 9 days starting on day 28 postnatally. The incidence of cartilage lesions (knee-joints) in the first experiment was 100% (7/7) as compared to 14% (3/21) in the second experiment. In series II, $Mg^{2+}$ deficiency was not as pronounced as in series I (plasma concentrations: 37% *vs* 22% of control; bone concentrations: 62% *vs* 48% of control). $Ca^{2+}$ concentrations were not significantly altered in bone.

To further elucidate the effects of $Mg^{2+}$ deficiency or quinolones on cartilage *in vitro* experiments were performed. Limb buds from mouse embryos (day 12) differentiated well in Biggers medium without magnesium. Calcium was shown to be essential: limbs did not grow in $Ca^{2+}$-free medium. When ciprofloxacin or temafloxacin were added (30, 60 and 100 mg/l), slight, concentration-dependent effects were seen with 60 and 100 mg/l (regular Biggers medium). In culture medium without $Mg^{2+}$ growth of limb-buds was completely inhibited with both fluoroquinolones at 100 mg/l. With 60 mg/l retardation was observed in magnesium free culture medium being slightly more pronounced as compared to regular medium. Both fluoroquinolones had no effect on limb bud differentiation at 30 mg/l in both media. Further studies are necessary to investigate the synergistic effects of magnesium deficiency and quinolone-treatment on cartilage to reveal the mechanism of quinolone-induced arthropathy in detail.

***1) Institut für Toxikologie und Embryopharmakologie, 2) Institut für Molekularbiologie und Biochemie, FU Berlin, 14195 Berlin, Germany***

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### PERSISTENT HEMATOLOGICAL CHANGES IN RATS AFTER PRENATAL TREATMENT WITH ACICLOVIR

Susanne Kuschel, Renate Thiel, Georg Golor, Ibrahim Chahoud, Hans-Joachim Merker, Ralf Stahlmann

Aciclovir is a teratogen in rats and causes impaired function of the immune system after prenatal exposure. We studied hematological alterations in rat offspring after treatment on a single day during organogenesis. Rats were treated on day 10 of gestation with s.c. injections of 1 x 50, 3 x 50, 1 x 100 or 3 x 100 mg aciclovir/kg b wt. Nine months postnatally we observed a significant decrease of total leukocyte counts in male and female offspring in the 3 x 100 mg/kg b wt group [male offspring: 7.9 ± 1.8 x 1000/µl vs. 9.4 ± 1.2 x 1000/µl in controls (mean values ± sd; n = 12)]. The ratio of CD4-/CD8-positive leukocytes in peripheral blood as determiend by flow cytometry was significantly increased (t-test; $p < 0.05$) in rats prenatally exposed to one or three injections of 100 mg/kg. [Control: 1.8 ± 0.2; 1 x 100 mg/kg: 2.1 ± 0.3; 3 x 100 mg/kg: 2.4 ± 0.3; male offspring; n = 12 each group]. At 12 months postnatally organ weights were determined. In most aciclovir-treated groups spleen weight was increased, whereas thymus weight was decreased. For example, relative spleen weights (in relation to body weight) in the female offspring were significantly increased in the 1 x 50, 3 x 50, and 3 x 100 mg/kg b wt group. In summary, treatment with aciclovir on a single day of organogenesis induces persistent hematological alterations in rat offspring at doses causing no gross-structural anomalies. In humans no prenatal toxicity has been observed so far with aciclovir under therapeutic conditions, but hematological alterations have not been checked.

Results are part of the doctoral thesis of S. Kuschel to be submitted to the Fachbereich Humanmedizin, FU Berlin

***Institut für Toxikologie und Embryopharmakologie, Fachbereich Humanmedizin, Freie Universität Berlin, 14195 Berlin, Germany***

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