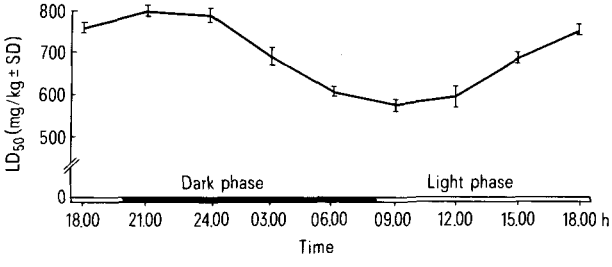
chamber equipped to provide 250 foot candles of cool white flourescent light for a minimum of 3 weeks before use in this study. The mice were housed in clear plastic cages in groups of 8 animals. Feed and water were supplied ad libitum. The photoperiod was automatically-timed (12-h dark/12-h light) with the light period from 08.00 h to 20.00 h. Injections of diazepam in 0.9% saline (Hoffmann-La Roche) were made i.p. every 3 h over a 24-h period and LD_{50}-value (mg/kg) for each time was determined by the method of Litchfield and Wilcoxon[12]. For each hour 18 animals were used.

Results and discussion. Our data are presented in the figure. The maximum acute toxicity of diazepam occurred at 09.00 h during the light phase of the photoperiod while the minimum acute toxicity of the compound was at 21.00 h during the dark phase of the photoperiod. This study demonstrates for the first time the chronotoxicity of diazepam, a CNS depressant drug of the benzodiazepine group, in that the toxicity of the compound varies with the time of administration. The effectiveness and/or toxicity of CNS drugs might be related to endogenous levels of brain biogenic amines and other neurotransmitters during different times of the day since it has been shown[8-11] that some CNS drugs can alter the circadian patterns of brain biogenic amines. It will, therefore, be interesting to investigate in a future study the effect of diazepam on the circadian patterns of brain biogenic amines.



Chronotoxicity of diazepam in mice. The maximum toxicity is at 1 h after the onset of the light phase (09.00 h). The minimum toxicity is around 21.00 h which is 1 h after the onset of the dark phase.

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Total atresia of the ovaries of *Tilapia leucosticta* (Cichlidae) after intoxication with the insecticide Lebaycid®

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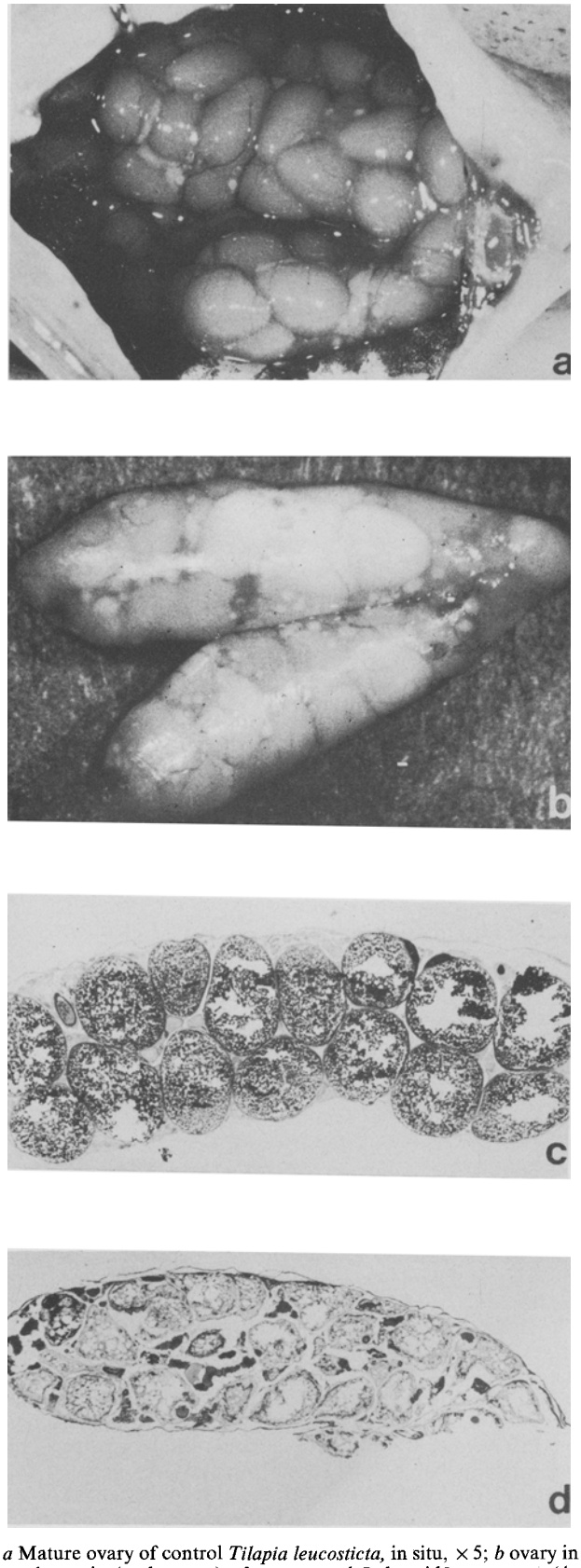
Summary. Intoxication with sublethal Lebaycid® concentrations led to total atresia of the ovaries in 90% of the treated specimens of *Tilapia leucosticta.* The gonads were filled with atretic follicles and mature eggs were never found. In regeneration trials, intoxicated fish proved to be unable to spawn for at least 9 weeks.

The organophosphorus insecticide Lebaycid® (Fenthion) is being employed in the combat against Anopheles-, Culex- and Simulium-larvae in many tropical freshwaters[2-6]. The preparation is introduced repeatedly into the water and thereby it also effects non-target organisms especially fish, which are an important source of protein for human nutrition in many countries[7,8]. The highly toxic effects of Lebaycid on the spawn and different developing stages of Cichlid fishes have already been shown[9]. The aim of this investigation was to examine to what extent the periodical output of sublethal concentrations of Lebaycid affects the ovaries and reproductive activity of *Tilapia leucosticta.*

Materials and methods. Within a period of 14 days 20 individually marked females of *Tilapia leucosticta* (overall length 8.6± 1.2 cm, total weight 9.6± 3.8 g) were treated 5 times with sublethal 'pesticide-shots', each lasting 24 h. The shots were always interrupted by a freshwater-phase of 2 days duration. In each experiment 4 females were put in an aerated glass aquarium with a capacity of 10 l (adaptation period 1 day). In the 1st series, 8 females were exposed to a Lebaycid concentration of 7 ppm during each pesticide-shot. To make sure that the ovaries were in a comparable state at the beginning of the treatment, in a 2nd series, the gonads were visually controlled. The peritoneal cavity of anaesthetized females was opened by an incision in the ventral line under the Zeiss Dissecting Microscope. Through the transparent ovarian sack the different oocyte stages could be recognized and photographed. Afterwards the edges of the wound were approximated with catgut. 9–14 days after the operation, 8 females with maturing ovaries were exposed to the pesticide programme. In this 2nd series the Lebaycid concentration was reduced to 4 ppm. In a 3rd series, the possible stimulating influence of males on the ovarian cycle was taken into account. 4 females (visual control of the ovary) together with one male were treated with Lebaycid. 20 females (12 with ovaries visually examined) served as controls. After the experiments, the fish were killed and their total weight and overall length determined. The ovaries were dissected, measured and weighed. The usual histological methods (fixation in Bouin, Azan-staining) were employed. In the 3rd series, half of the



a Mature ovary of control *Tilapia leucosticta,* in situ, × 5; *b* ovary in total atresia (early stage) after repeated Lebaycid® treatment (4–7 ppm), dissected, × 5; *c* longitudinal section through a mature ovary of control. Azan-staining, × 7; *d* longitudinal section through a ovary of repeatedly intoxicated *Tilapia* (7 ppm), totally filled with atretic follicles (late stage). Azan-staining, × 7.

ovary was macerated[10] and the released oocytes counted and measured.

Results and discussion. In all series the relative ovary weight (ratio body weight to ovary weight) was higher in the females exposed to Lebaycid, whereas the gonosomatic index (GSI = ovary weight as a percentage of total weight) was lower than that of the controls. (GSI in the 1st series: Lebaycid-♀♀ 1.9 ± 1.0%; controls 6.7 ± 1.9%; 2nd series: Lebaycid-♀♀ 2.9 ± 1.6%; controls 4.3 ± 1.3%; 3rd series: Lebaycid-♀♀ 2.0 ± 0.3%; controls 3.5 ± 1.2%). In the 1st and 3rd series the values differed significantly on the 1% and 5% level respectively; in the 2nd only on the 10% level. 90% of the ovaries of the intoxicated fish were in total atresia: the whole gonad was filled with degenerating eggs (figure, b, d). Ovaries in an advanced stage of degeneration always had the lowest GSI. In the remaining 10% of the Lebaycid-treated females only immature oocytes up to the early yolk formation stage (oocytes in phase I to IV[11]) had developed. The degeneration of oocytes in the late yolk formation stage and of ripe eggs (oocytes in phase V and VI) was already macroscopically recognizable. The egg cells became white and took on irregular contours, and the yolk mass 'flowed off'. After the histological examinations follicular atresia could be described as follows: it began with the hypertrophy of the follicle epithelial cells, which immigrated into the ooplasma, devoured the yolk mass and finally filled the entire atretic follicle. Exceptionally, corpora atretica could be found in the ovaries of controls. However, their number was significantly lower than in the Lebaycid-treated fish. In the 1st series, 100% of the ovaries of the controls belonged to the mature ovary type (tightly filled with oocytes in phase V and VI, figure, a, c). Also the visually controlled ovaries of the untreated fish had usually developed into fully ripe ovaries. On the contrary, mature eggs (oocytes in phase VI), ready for ovulation were never found in Lebaycid-exposed females. In the intoxicated fish, also, unripe oocytes (phase III and IV) underwent resorption, which occurred in none of the controls.

In the 1st series (highest Lebaycid concentration), haematomae were observed in the ovaries of 2 of the treated females, which appeared in the histological picture as atretic follicles with large accumulations of erythrocytes.

The experiments show that the insecticide Lebaycid® not only damages the spawn of *Tilapia leucosticta*[9], but also causes the resorption of mature eggs in the ovary. Whereas follicular atresia may occur spontaneously[12], in these trials, the Lebaycid treatment always induced total atresia of the ovaries. Regeneration trials give evidence that females exposed to Lebaycid are unable to spawn at least for a period of 9 weeks (spawning period of controls: 35 ± 4 days).

1 This work was undertaken with the aid of Prof. Dr E. Kulzer.
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