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Comparison of the Usefulness of the Mexican Axolotl (*Ambystoma mexicanum*) and the Clawed Toad (*Xenopus laevis*) in Toxicological Biossays

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In the framework of national legislations and international agreements on water pollution abatement, several procedures and philosophies for the use of bioassays as a guide for determining safe concentrations of toxicants for aquatic life have been proposed. It is generally accepted that it is essential to determine the toxicity of certain compounds to several elements of the aquatic food chain in different life cycle stages to determine the most sensitive groups of organisms from which data safe concentrations can be derived. Thus, to obtain the most meaningful results, different types of organisms should be included into the laboratory screening tests. In this respect more attention should be paid to the amphibians, as this group of higher water organisms is intermediate between water and terrestrial life forms, occupying an important niche in aquatic ecosystems in the successive completely different life cycle stages. The objective of this paper is to compare the usefulness of a representative of the Urodela (Ambystoma mexicanum) and of the Anura (Xenopus laevis) as biological indicators in toxicological bioassays.

MATERIALS AND METHODS

Ambystoma mexicanum. A. mexicanum is placed in the order Urodela and family Ambystomatidae. The mexican axolotl originates from Lakes Chalco and Xochimilco south of Mexico City and is commonly used in embryological, physiological and endocrinological tests (THOMAS 1976) and to a lesser extent in toxicological studies (SLONIM & RAY 1975). The average size of natural spawnings is more than 500 eggs (HUMPHREY 1976). The time from egg-laying to emergence of the larvae ranges from 16 to 20 days at a water temperature of 18°C. The normal stages of the development and the timing of these stages have been well described (SCHRECKENBERG & JACOBSON 1975, HARA & BOTERENBROOD 1977). The animals stay in the fully aquatic neotenic larval form, being able to breed while still retaining larval characteristics as external gills and a caudal fin. In the literature several procedures have been described to induce out-of-season spawning: (a) methods, in which sexually mature animals are stimulated by a cold temperature shock to mate and to release spermatophores by the male followed by insemination of the female (VAN OOSTRUM 1978); (b) methods, in which the female is injected intraperitoneally with LH(Luteinizing Hormone) and subsequently is exposed with a male to a slight cold temperature shock (KETTERER & FORBES 1972); (c) methods involving injection

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of the female with FSH (Follicle Stimulating Hormone) either at the time the male and female are placed together or a short time previously (NEWROCK & BROTHERS 1973); (d) methods in which spermatophores and eggs are obtained by hormonal stimulation of both sexes, followed by artificial fertilization (TROTTIER & ARMSTRONG 1975); (e) methods, sacrificing the male to obtain sperm (FRANKHAUSER 1967) or collecting seminal fluid from the vas deferens of the male (BRUNST 1955), followed by artificial semination. As none of these methods shows a clear advantage as compared to the others, the following procedures were applied to come to a less time consuming but satisfactorial culture method :

a. Cold temperature shock : Both sexes were exposed to a slight increase of water temperature (20 --→ 23°C) during 24 h, followed by a sudden fall in water temperature (23 --→ 10-12°C). After a certain period of time, varying from 1 day to two weeks, the water temperature was gradually increased to 20°C.
b. Hormonal stimulation of the female : Females were injected intraperitoneally with different amount of gonadotrophic hormones (Pregnyl(R), Organon 75 I.U., 400 I.U. and 800 I.U.) and subsequently placed with untreated males.
c. Hormonal stimulation of the male : Males were injected intraperitoneally with 75 I.U. or 400 I.U. of gonadotrophic hormones (Pregnyl(R), Organon) and subsequently brought together with untreated females.
d. Hormonal stimulation of both sexes, involving injection with 75 I.U. of the animals on the same day, or injection of the males with 300 I.U. on two successive days and injection of the females with 600 I.U. Pregnyl(R) on the second day.

For this study 3-year-old animals were used, obtained from the University of Utrecht, The Netherlands.

Xenopus laevis. X. laevis belongs to the order Anura and family Pipidae and originates from South Africa. X. laevis has become a common laboratory animal since the detection of its suitability for pregnancy tests and other hormonal reactions and has been used in toxicity studies as well (GREENHOUSE 1976). Natural spawning amounts to thousands of eggs, whereas the embryological and the larval development takes about 4 and 50 days, respectively, at a temperature of 22 _ 24°C. The stage criteria in the development have been well described (NIEUWKOOP & FABER 1975).
Spawnings were obtained by injecting the male twice with 300 I.U. Pregnyl(R) on two successive days and the females once with 600 I.U. Pregnyl(R) on the second day. The injections have been made into the dorsal lymph sac, piercing the skin of the thigh and the septum between the lymph sacs of the thigh and the back (OCHSÉ 1948).

Toxicity tests. To compare the susceptibility of A.mexicanum and X.laevis to toxicants, the 48 h LC_{50} was determined for 15 chemical compounds, which have been used in comparative studies before (CANTON & ADEMA 1978). For this purpose groups of 10 animals of each species, 3 to 4 weeks after hatching, were exposed for two

days to different concentrations of the test compounds in 1-L standardized medium (Dutch Standard Water [1]) in covered glass basins. The compounds were added to the water dissolved in distilled water or, if necessary, in acetone, ensuring that no toxic concentrations of acetone were used. The temperature was kept at 20 ± 1°C and the basins were illuminated following a circadic rhythm. During the experiments the organisms were not fed. The 48 h LC_{50} values were calculated using the method of LITCHFIELD & WILCOXON (1949).

RESULTS AND DISCUSSION

In spite of repeating the techniques for artificial spawning of A.mexicanum 2 to 12 times, none of the methods followed resulted in fertilized eggs or even in any form of sexual behaviour. Although some spawnings occurred, these spawnings are probably the result of spontaneous mating as may be concluded from the length of time between treatments and corresponding spawnings (1-2 months versus 1-2 days normally) and from the fact that some spawnings were obtained from untreated animals.
The average size of these spawnings was about 250 eggs with 90 % fertilization. The biological effect of Pregnyl(R), containing human choriongonadotrophine, corresponds to that of luteinizing hormone.
Therefore the results of the techniques involving hormonal stimulation should be comparable to those of KETTERER & FORBES (1972), who treated 80 % of the females with 50 µg LH successfully, resulting in spawning on an average of 167 eggs with 82 % fertilization. Literature data on F.S.H.-induced spawnings are varying considerably; on one hand 9 spawnings averaged 81 eggs per spawning with about 50 % fertilization (TROTTIER & ARMSTRONG 1975), on the other hand 36 spawnings averaged 593 eggs per spawning without statement of the percentage fertilization (HUMPRHEY 1976).

In comparison with A. mexicanum, the hormonal induction of the spawning in X.laevis was more successful; more than 60 % of treatments resulted in spawnings of more than a thousand eggs with more than 90 % fertilization.

In table 1 the short term toxicity of 15 different compounds to A. mexicanum and X. laevis is summarized.

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1) 1.36 mmol Ca^{2+}, 0.73 mmol Mg^{2+}, 1.19 mmol Na^{+}, 0.20 mmol K^{+}, 2.72 mmol Cl^{-}, 1.39 mmol HCO_3^{-} and 0.73 mmol SO_4^{2-} per litre distilled water

TABLE 1

48 h LC_{50} values (mg/L) of 15 chemical compounds to A.mexicanum and X.laevis, based on the amount of the compounds added to the water

Test compound	A.mexicanum	A.laevis
n-Propanol	4000	4000
n-Heptanol	52	44
Acetone	20,000	24,000
Ethyl acetate	150	180
Ethyl propionate	54	56
Trichloroethylene	48	45
Allylamine	1.8	5.0
Benzene	370	190
o-Cresol	40	38
Salicylaldehyde	7.0	7.7
Pentachlorophenol	0.30	0.26
Pyridine	950	1,400
Aniline	440	560
Hg(II)chloride	0.4	0.1
Cd nitrate	1.3	32

Assuming that the 48 h LC_{50} can be considered merely as an estimate of the toxicity of a test compound, only 4 compounds resulted in deviating LC_{50}-values, based on a difference of more than a factor 1,78($\sqrt[4]{10}$). X.laevis showed to be more sensitive to Hg(II)chloride and benzene, whereas A.mexicanum was more susceptibly to Cd nitrate and allylamine. As the classification of chemicals with regard to short term toxicity to water organisms is based on classes with a factor 10 difference (CANTON & SLOOFF 1979), only for one compound (Cd nitrate) a difference of one class is obtained. Although no long term toxicity studies have been carried out with A.mexicanum in our laboratory, there are indications that the performance will be more difficult compared with X.laevis as cannibalism will occur among the axolotls interfering with the test results. In addition long terms experiments with A.mexicanum will be relatively time-consuming, as the axolotl needs living food throughout their development whereas the clawed toad is fed with a suspension of nettle powder during the larval development. Since both organisms showed a similar sensitivity to chemical compounds, there is a preference for the use of X.laevis in toxicity studies as it is much easier to handle and to culture than A. mexicanum.

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