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# Prescreening teratogenic potential of chlorinated drinking water disinfection by-products by using *Hydra* regeneration assay

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**Abstract**—Practicability of method for the *Hydra* regeneration assay on the prescreening teratogenic potential of chlorinated drinking water disinfection by-products was studied through both the assays of toxicity of adult *Hydra* (T) and inhibition of the growth of regeneration *Hydra* (I) by using chloroform, dichloromethane and chloroacetic acid. The results showed that $T_{50}/I_{50}$ ratios of chloroform and chloroacetic acid were 2.77 and 6.16 respectively, with teratogenic potential. $T_{50}/I_{50}$ ratio of dichloromethane was 1.69, with weaker teratogenic potential. These experimental results indicated preliminarily that the *Hydra* regeneration assay has certainly applied value as a prescreening assay for developmental toxicity.

**Keywords**: *Hydra* regeneration assay; chlorinated disinfection; chloroform; dichloromethane; chloroacetic acid.

The chlorinated disinfection of drinking water has been applied for over seventy years for control water-borne infectious deseases(Fowle, 1986). In the early 1970s, an organic pollution of drinking water resources was regarded widespread. Thereafter, the mutagenicities of a chlorinated drinking water disinfection by-products and an organic extracts of drinking water were studied in a number of countries, but no data of the teratogenic potential of the both chemicals are available (Smith, 1986; Fu, 1990). We studied teratogenicities of several the chlorinated drinking water disinfection by-products by attempting to use *Hydra* regeneration assay.

## 1 Materials and methods

### 1.1 Materials

Experimental animal: *Hydra* pseudologactis was collected from pool in Hefei suburb. The pure strain was obtained by an asexual budding reproduction for many years.

Chloroform(density 1.489g/ml), dichloromethane (density 1.325 g/ml) and chloroacetic acid were selected as the chlorinated drinking water disinfection by-products. Colchicine(Fluka Co.) and sodium pyruvate were applied as positive and negative control respectively (Ji, 1996). Except noted, the reagents are all analysis pure.

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### 1.2 Procedure

After experimental *Hydra* was prohibited to feed for 24 hours, the adult hydras of unanimous size were divided random into two groups. The head part (hypostome and tentacles) and tail part (peduncle with bud and basis disk) were cut out in a group, and was retained digestive region (*Hydra* of regeneration phase). The *Hydra* of regeneration phase and another group intact adult polyps(*Hydra* of toxicity phase) were placed random into disinfected culture dishes with the medium of different concentration reagents respectively. Observations were recorded over a 72 hours exposure period, during which the medium and test compound were renewed at 24 hours intervals. The medium consists of 1 mmol calcium chloride dihydrate, 0.458 mmol TES ≪N-[Tris-(hydroxymethyl) methyl]-2-aminoethane sulfonic acid≫, and 0.012 mmol EDTA, and 150mg Amikacin sulfate (a Amp containing 200 mg Amikacin sulfate, made in Tianfeng Pharmacy Factory, Shanghai, China) was added into medium in every liter (Mayura, 1991). At the 72th hour of exposure period, the morphological characteristics of each polyp and regenerator were scored using previously system described (Wilby, 1986; Ji, 1996). The mean score for each concentration was calculated and plotted. The concentration 50% toxic to polyps($T_{50}$) and 50% inhibitory to regeneration ($I_{50}$) were estimated from the graphs as those giving mean scores of 5. The $T_{50}/I_{50}$ ratios were calculated.

The experiments were divided into three sections. Section I determined preliminarily whole-log concentration of toxic and inhibitory effects on polyps and regenerators respectively by exposing *Hydra* to solutions ranging from $10^{-3}$ mg/L to $10^{3}$mg/L. Section II divided into 5 or 7 dosage groups in the range of confirmed concentration of the section I, according to equivalent distance grade. Section III repeated the confirmed concentration of the section II or further divided meticulously dosage groups as testing and verifying the result in front of the section. Each section sets up a control group of reagent blank. In section I and II, each dosage and control group placed in 5 polyps or regenerators respectively, in section III, experimental individual of each dosage group increased to ten.

## 2 Results

The toxicity/inhibition of regeneration ratio (the $T_{50}/I_{50}$) was calculated from the concentrations in each phase at which a mean score of 5 was recorded after a 72 hours incubation, and their teratogenic potential judgment shown in Table 1.

**Table 1 The results of *Hydra* regeneration assay and its judgement of teratogenicity**

| Compounds | Effective concentration | | | Teratogenic potential judgement |
|---|---|---|---|---|
| | $T_{50}$ | $I_{50}$ | $T_{50}/I_{50}$ ratio | |
| | mg/L | | | |
| Colchicine | 95.0 | 35.0 | 2.71 | + |
| Chloroform | 205.0 | 75.0 | 2.77 | + |
| Dichloromethane | 245.0 | 145.0 | 1.69 | ± |
| Chloroacetic acid | 955.0 | 155.0 | 6.16 | + |
| Sodium pyruvate | 2500.0 | 2450.0 | 1.02 | − |

Notes: + Positive teratogenesis; − negative teratogenesis; ± weaker teratogenesis, i.e. there is probably teratogenesis when concentration of the compound rendered adult polyp poisoning

## 3 Discussion

*Hydra* digestive region regeneration assay to test teratogenic potential of chemicals was used earliest by Wilby(Wilby, 1986), though its regenerative ability had been known earlier on. *Hydra* regeneration is cell redifferentiation, development, and reorganized processes which injured body (due to be cut out) becomes intact. In this stage, its development is similarly the cell differentiation and the organogenesis of mammalian fetus. Thus, teratogenic potential of chemicals can be predicted through chemicals affected *Hydra* regeneration. In order to avoid this effect may be *Hydra* cell poisoning rather than only teratogenesis, experiment matched relevant concentration which compound affected intact adult *Hydra* for the control. Teratogenesis of the chemicals was estimated through $T_{50}/I_{50}$ ratios were calculated. On the basis of Wilby, values between 1.2 and 2 indicate that the toxicity of the test substance is of the same order in both adult and regenerating *Hydra*, whereas value $>2$ indicate low adult, high regenerating *Hydra* toxicity, but values below 1.2 indicate that teratogenesis is negative.

In this experiment, two chlorinated drinking water disinfection by-products, chloroform and chloroacetic acid $T_{50}/I_{50}$ ratios were 2.77 and 6.16 respectively, according to Wilby, they have high regenerating *Hydra* toxicity, with teratogenicity, but dichloromethane $T_{50}/I_{50}$ ratio 1.69 ($2>\text{value}>1.2$) is of the same order in both adult and regenerating *Hydra*, without or with weaker teratogenicity, i. e. it exhibits embryonic effect only at maternally toxic levels. The results of chloroform and chloroacetic acid are identical with the experimental results of mammalian teratogenic test and *Hydra* "artificial embryos" assay(Smith, 1986; Fu, 1990).

*Hydra* digestive region regeneration assay is simplier and more conveniently, and more quickly than regeneration assay of *Hydra* from regregated cells, i. e. "artificial embryos" methods, moreover, the judgment of result applied middle effective concentration as those giving mean scores of 5, therefore, on numerical value, it is more steady than lowest effective concentration ratio value (adult/developmental, A/D ration) by Johnson(Mayura, 1991).

These experimental results indicated preliminarily that the *Hydra* digestive region regeneration assay has certainly applied value as a prescreening assay for developmental toxicity.

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