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Biophoton emission, free radical and toxicity of benzene to aquatic biosystems*

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Abstract——The observation on biophoton emission, free radical and toxicity of benzene to zebra fish and carp hepatic microsome has demonstrated that there is the corresponding relationship between them by means of $^{60}Co\gamma$ radiolysis. Free radicals play a key role in this relationship. A common photon originates of two biosystems are the excited species including singlet and triplet molecules ($^1O_2$ and $C^*=O$) as well as hydrocyclohexadienyl radical $\cdot OH$ and so on. $\cdot OH$, to a certain extent, directly participates in photon emission and toxicity of benzene to aquatic biosystems. These are the conclusions, but these are also problems. With the solution of the latters, model established in this work could be expected that it will be favorable for the development of photon-toxicology.

Keywords: biophoton emission; free radical; benzene; aquatic biosystems.

1 Introduction

Benzene, being considered as an important organic poison to cause hemo- and myelotoxicity, genetoxicity and carcinogenicity is a common industrial and domestic solvent and also an extensive pollution in air and water (Kalf, 1987). By the data from a variety of hypotheses and techniques, benzene toxicity has been postulated to associate with the catabolism of benzene exposed biosystem or the generation of free radical (Karam, 1989). It involves, in other words, metabolism to ring-hydroxlative derivatives and open-ring metabolites in the different biostructural level of the biosystems, thus interfering a normal function of them (Snyder, 1981; Witz, 1989). Despite of these achievements, the toxicological mechanism of benzene still is a very complicated problem.

In this work we make use of the technique of biophoton emission measurement to study

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the effect of benzene on fish(Wang, 1991; 1992; Ma, 1989) and its hepatic microsome(Cadenas, 1984) via free radicals. Namely, toxicity and its mechanism of benzene to aquatic biosystem is further researched by photon emission from the different emitters, because biophoton emission associates with oxidation metabolism, intoxication among many life processes. At present, so, the photon counting technique has been widely accepted to study toxicity and its mechanism(Slawinska, 1985). The observation of the potential role of free radical in the toxicological process, in particular, is of a pronounced advantage via biophoton emission (Jezowska, 1987). Thus, we apply the benzene-water radiated by $^{60}Co\gamma$ -ray as the medium (Wang, 1988) to study the subject mentioned above. It fulfills the need of this experiment because the medium contain the high reactive species due to ioning and exciting of the radiation (Cadenas, 1984). Prior to this, biophoton emission-toxicity model of benzene exposed fish is established firstly. Photon emission from fish and its hepatic microsome, subsequently, is studied in medium containing various gamma ray-radiolylic products, thus revealing the potential and induced role of the metabolites via free radicals in biophoton emission and toxicity of benzene to aquatic biosystem.

2 Materials and methods

Zebra fish (Brachydanio rerio) and carp (Cyprinus carpio) were purchased from Guangyuan-Aquarium and Wan Quan Zhuang Fishery in Beijing, respectively. The fish were acclimated for at least one week in aerated tap water at 25±1°C prior to the start of the experiment(Wang, 1991; 1992; Ma, 1989).

Benzene, oliver oil, phenylbarbital, calcium chloride, potassium chloride, sucrose, sodium benzoate, dithionate and carbonic oxide were the products(analytical reagent, A. R.) from China. Tris was from Merck(German) and Bio-Rad reagent was from Bio-Rad Co. (USA). Sodium azide was from Sigma Co. (USA).

^{60} Co γ source used in this irradiation is provided by Institute of Biophysics, the Chinese Academy of Sciences. Rate of radiation dose is 130 Gy/min, distance from the object to the ray source is 20 cm and the operation is performed in the room temperature. Radiated time of benzene-water is 30 min.

Induction of P 540 in carp to be acclimated was performed for three days (Q. d.) by intraperitoneal injecting the mixture of phenylbarbitall and oliver oil at the dose of 25. 0mg/kg body weight(Slawinska, 1985; Wang, 1988). Microsome of fish hepatic cell was prepared by the centrifugation in addition of Ca^{2+} ions to its postmitochondrial supernatant liquor(S_9). The P450 content of microsomal fraction was measured at 413 nm by co-difference spectrum. The protein concentration of the microsome was determined by Bio-Rad reagent. The ratio of P450 content from the fish liver tested in this paper was calculated to be 2. 64 nmol/mg protein.

Biophoton emission was measured as previously described (5－7) with model Intelligence Low Level Luminescence Measurement System(Made in China).

3 Results and discussion

The curves in Fig. 1 show the relationship between biophoton emission from zebra fish and carp hepatic microsome in benzene-water medium radiated with and without ^{60}Co γ ray. Four piecewise linear lines demonstrate that each of biophoton emission varies with the increasing benzene dose in two different media. The photon emission from zebra fish in benzene-water is decreased with the increase of benzene dose rapidly before 40μl, subsequently, rised step by step until 220μl and finally tend to decline again. The maximal intensity of benzene induced biophoton emission appears at 220μl, but is a little greater than the initial (0 point) intensity.

Photon emission from zebra fish in benzene-water radiated by ^{60}Co γ ray is declined sharply before 20μl benzene, subsequently, rised until 200μl and finally tend to fall off followed by greatly step down. Besides the intensity at initial dose is the same with that in benzene-water, there exist three maximal intensities to appear at the benzene dose of 60, 100, and 160μl, respectively, and the most among them is at 60μl. It is similar to fish in unradiated medium in the intensity, but is different site at benzene dose to appear the maximum. Namely, the former is at 60μl, but the latter is at 220μl. These distinctive differences obviously imply, that the reactive radiolytic species to generate from benzene-water via ^{60}Co γ ray play a potential role in photon emission process of the living zebra fish.

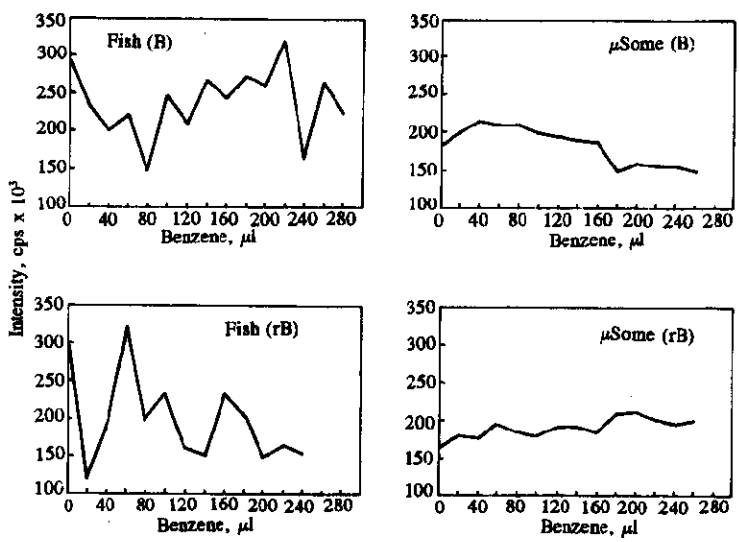


Fig. 1 Relationship between biophoton emission intensity from zebra fish and carp hepatic microsome and benzene dose in benzene-water with and without ^{60}Co γ radiation

B: Unradiated benzene; rB; ^{60}Co γ-radiated benzene

Unlike these, photons emissions from carp hepatic microsome in benzene-water radiated

with and without ^{60}Co γ ray are far lower than that of fish in both media. The light intensity from microsome in unradiated medium is slightly rised before 40μl benzene. Subsequently, decreased smoothly and finally continue decline followed by step down at 160μl. The maximal intensity from hepatic microsome is little, being less 1.5 times than that of fish. In γ-radiated benzene-water, the light intensity from microsome is a little lower than that from one in unradiated medium before 120μl benzene, subsequently, overlap each other of both from 120 to 160μl, but the former intensity is rised at 160μl and finally varies in a low level with the increasing benzene dose. The maximal intensity does not appear in the course of adding benzene. Namely, the intensity from microsome exposed benzene radiolytics is from high to low, on contrary, one from microsome exposed benzene is from low to high. As a result, photon emission from microsome-radiolytic system is a consequence of the enhance of the emitter accumulation, but one from microsome-benzene system is a consequence of the reduction of emitter attenuation. Both possesses an equal progressing period and, followed by the parted point of 160μl benzene dose, each continues to fluctuate with benzene dose. To differ from this, photon emission from fish-radiolytic system is a consequence of immediate radiation of the emitter, merely having a short progressing period, but one from fish-benzene system is a consequence of the emitter accumulation, having a longer progressing period.

Two biophoton emission-time curves in Fig. 2 were measured from zebra fish in ^{60}Co γ- and un-radiated benzene-water, in which the dose of benzene was used to be a singular greater dose of 40μl possibly to lead an acute toxicity of fish, respectively. The former decreases rapidly (around 30%) and the fluctuate with the increasing exposure time. A maximal intensity appears at 120s in the course of benzene exposure. To differ from this, the latter only have a slightly fluctuation with the increase of exposure time. Until 120s, a little increase of photon emission occurs, too. Correspondingly, a harmful symptoms of zebra fish in two media concomitantly to the process of biophoton emission are shown in Table 1. A comparison of two events demonstrates that the occurrence time of all the systemic signs of fish in γ-radiated benzene-water are delayed. More prominent, it is no death of fish even if it exposes for 150s. On contrary, fish exposed in unradiated benzene-water die and, at the same time, have a flash light as fast as the exposure time reach 120s.

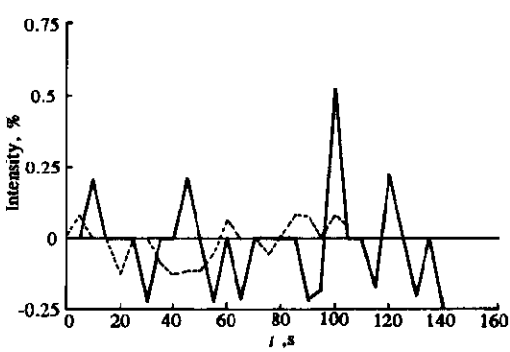


Fig. 2 The time variation of biophoton emission from zebra fish in two benzene-water media
A. unradiated B. ^{60}Co γ radiated

As a result, the fact that benzene to undergo ^{60}Co γ radiation in water leads to decrease of its toxicity and photon emission of zebra fish appears that game ray plays a reduced role not to expect enhanced. In addition, other fact in this experiment testifies that there is an objective relationship between a temporal, strange light emission and death of the living or-

ganism indeed.

Table 1 Time of toxic effect from zebra fish exposed in aqueous medium

Medium	Time, s			
	Uneasy (mania)	Lose of equilibrium sense (convulsion)	Swinning activeness loss (coma)	Death
Benzene	0—20	20—90	90—120	120
^{60}Co γ-benzene	0—50	50—100	100—150	No death

To clarify the relationship between biophoton emission and toxicity described above, two testes were conducted as follows: The emission spectra of fish and microsome in benzene-water radiated with and without ^{60}Co γ-ray were measured. A shown in Fig. 3A, the maximal intensity (relative value 100%) for microsome in γ-radiated benzene-water is around 705 nm and bor both fish and γ-radiated medium are around 475nm. However, both latters have also several higher intensities such as 45%, 82%, 41% and 27%, 81%, 59% at 575, 640 and 705 nm, respectively; the former have merely 7%, 8% and 20% at 475, 575 and 640 nm.

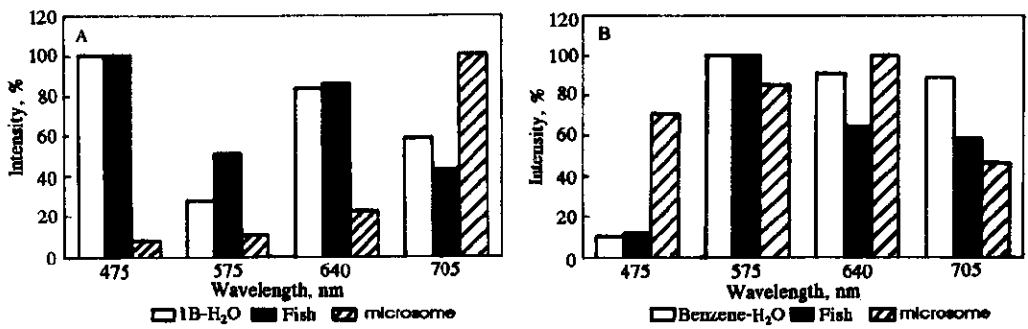


Fig. 3 Emission spectra from zebra fish and carp hepatic microsome in benzene-water
A: ^{60}Co γ-radiated B: Unradiated

The maximal intensity, as shown in Fig. 3B, for microsome in benzene-water is around 640 nm and for both fish and benzene water are around 575 nm, respectively. It is different from the case of γ-radiated medium, however, because microsome in benzene water has also the higher relative intensities of 71%, 85% and 46% at 475, 640 and 705 nm, whereas both fish and benzene-water have 11%, 64%, 58% and 10%, 89% at 475, 640 and 705 nm. As a result, singlet and triplet species as well as hydroxyl radical('OH) are the possible emitters of fish and microsome. Besides, the comparison of the results reveals that the γ-radiolytics of benzene-water make biophoton emission from carp hepatic microsome red-shift, on contrary, the living fish blue-shift. And this distribution of relative intensities are also indicative of a possible reason that the light intensity from fish is stranger than that from microsome.

The results another of the quench test shown in Fig. 4A indicate that photon emission

from fish in benzene-water with and without γ-radiation were quenched at the start of 100s using sodium benzoate(C_6H_5COONa), an effective quencher of ·OH. And the ·OH scavenged effect of the former is a little stronger than the latter. For microsome, almost no quench effect for ·OH occurs. As shown in Fig. 4B, however, the light intensity decreases with the increasing dose of NaN_3, an effective quencher of singlet molecule such as $^1O_2$ is a main emitter from carp hepatic microsome, participating in multienzymatic microsomal system for the hydroxylation of xenobitics, such as benzene, is capable of photon emission induced by phenylbarbital. Although ·OH does not participate in the above process. It participates in the photon emission from the living fish. It is evidence that two biosystem as the donor of biophoton in the different structural level have the own mechanism of photon emission. Moreover the fact that the alteration of fish photon emission induced by ·OH reflects that there is a corresponding relationship between biophoton emission from fish and toxicity of benzene hydroxyl derivatives.

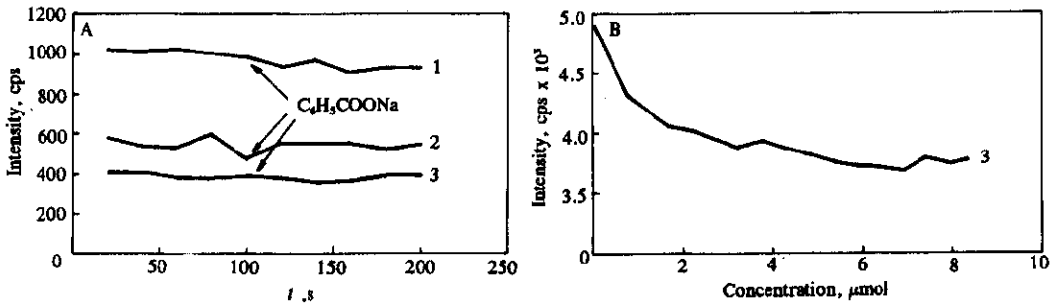


Fig. 4 Quench effect of ·OH and $^1O_2$ by C_6H_5COONa and NaN_3 and $^1O_2$ by C_6H_5COONa and NaN_3
A. C_6H_5COONa B. NaN_3 1. γB-H_2O 2. Fish 3. microsome

The intensity from fish and microsome in γ-radiated benzene-water are in range from 100—135 cps and from 150—250 cps, respectively. As contrasted these with the intensity obtained from benzene-water radiated by the corresponding dose(1.0kGy) in Fig. 5, several phenomena are exhibited. Photon energies to product from benzene-water undergoing ^{60}Co γ radiation are expensed by two biosystems. In other word, both latters as the target absorb a

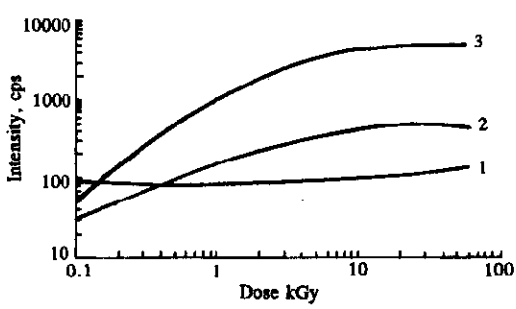


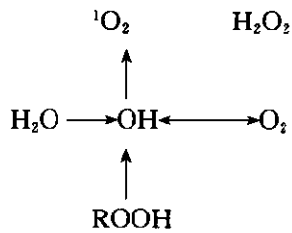
Fig. 5 ^{60}Co γ dose-intensity curve of water benzene and benzene-water
1. H_2O 2. Benzene 3. Benzene-H_2O

part of photon energies via the reactive species in the medium in collision. At the same time they couplet quickly self-reaction through electron transfer, thus forming the emitter from the different biosystems each other. In addition, the shrinking inconsistency of blue- and red-shift described as before is led probably due to the different absorbed energies of two biosystems from γ-radiated medium.

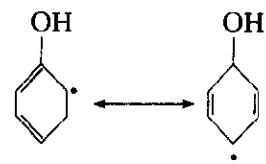
Because this is so, energy is a potential cause to lead four different photon emissive patterns described as before. The living fish to again higher energies from γ-radiolytics burst the emitters enough even at the low benzene dose. Whereas the natural microsome not to supplement NADPH and cofactors have a little burst of the emitters at the higher dose due to the attack of the reactive species with the microsome membrane. In benzene-water, the photon emission from fish and microsome may be associated with the toxic chemicals.

From the time curve, the toxicity of benzene exposed fish in sign of the death and flash light is higher than that of fish exposed ^{60}Co γ-radiated benzene-water, to be indicative of the delay of the acute toxic symptom, survive and no flash light. This implies that the radiolytics from benzene-water pretreated with ^{60}Co γ ray reduce the toxicity and induced photon emission because fish all were died even at 1.0 kGy so that the test can not be conducted. When the immediate radiation of fish in benzene-water by the manner as shown in Fig 5. As to microsome, the observation of biophoton emission and enzymatic activity (to supplement NADPH and cofactors) expressing the toxicity of two media will be described in the future.

From a popular viewpoint, biophoton emission is considered to involve biochemical generation of electronically excited species and their reactive derivatives such as ·OH and so on (Jezowska, 1987; Cadenas, 1984). The radiolytics of water under ^{60}Co γ radiation contain various reactive species as follows (Borrs, 1980):



The γ-radiolytics form benzene in water contain a hydroxycyclohexadienyl radical else and the existence of two mesomeric forms.



In ^{60}Co γ radiated benzene-water, thus, these benzene- and oxygen-containing reactive species, for instance, an excited singlet and triplet molecules such as $^1O_2$ and as well as a hydroxycyclohexadienyl radical ·OH and so on.

Fish is a complex living boimatrix and microsome is a mixed function oxidase-containing membrane of cellular endoplasmic reticulum. When attacking with various reactive species as

above, fish emit more intricate spectra, lead blue-shift of the spectral line and enhance the light intensity due to relaxation from the excited states(the different energy grades) to the ground state via the interaction of free radicals and enzymatic reactions(Snyder, 1981; Cadenas, 1984).

Although no NADHP and co-factors were added for the observation of the natural microsome is the aid in this work, the excited singlet molecules are generated in the low level via microsomal lipid peroxidation and a tiny amounts of triplet state are formed via the self-disproportionation of lipid peroxy radicals by Russells mechanism(Cadenas, 1984), because microsome prepared from carp liver induced by phenylbarbital is of a determined rate of P450 activity (2.64 nmol/mg protein). So the emission of microsome exposed γ- and un-radiated benzene-water in 640 — 705 nm region mainly causes from the transformation of two molecules of singlet specials to two of ground state, being a contribution of the singlet dimor. As to γ-radiolytics make the microsomal emission extended to the longer wavelength, an possible interpretation is the transfer of one molecule of singlet ($^1\sum^+ g$) to one of triplet state ($^3\sum^- g$). In fact, no emission beyond 705 nm was detected because the instrument used was sensitive only up to 705 nm.

The high toxicity of benzene to fish in the poison-induced process is mainly related to catabolism of benzene. As to fish exposed ^{60}Co γ-radiated benzene-water, its reduced toxicity and simultaneously, the rised light intensity is likely relevant to the following events. The reactive species in the medium accepted a high energy photon from ^{60}Co γ ray make trans, trans-muconaldehyde of the ring-opened metabolites of benzene, a six-carbon diene dialdehyde, detoxification, resulting in the production of non-toxic trans, transmuconic acid, which the loss of its electrophilicity leads to lack the ability to act as a direct-acting alkylating agent in involving interaction with the bioconstituents. On the other hand, these high energy species in the medium emit more amounts of photon via self-collision and/or impact to the target of fish.

·OH radicals participate in benzene poisoning and, obviously in photon emissive process of zebra fish via the interaction between active oxygen radicals. This is because that ·OH frequently generate at benzene exposure site in the organism. Thus, the end effects are the enhances of both toxicity and photon emission. However, the fact that photon emission is rised but toxicity is reduced demonstrate that ·OH in γ-radiated medium is controlled and so its amounts are restricted at a defined extent. Regarding the increase of light intensities, it may be the existence of other induced factors.

In summary,there is a dose-response relationship between the living zebra fish and carp hepatic microsome and/or the dose of benzene and, in fact, γ-radiolytics of benzene to be a consequence of the interaction of the reactive species in two media with two targets of aquatic biosystems by using the different fashion and quality.

Except for this similarity, four events of biophoton emission are also of some distinctive differences each other. First, the light intensity of microsome in two media is far less than fish. Second, four events each have of a special variation patterns of intensity-dose. The in-

tensity from microsome exposed ^{60}Co γ-radiolytics and benzene separately veers around to the increase and decrease. Unlike this pattern, the maximal intensities from zebra fish in two media appear at the different points of benzene dose, i. e. , stagger each other off. One in γ-radiated medium is at the lower dose, but the other in unradiated is at the higher dose. Third, each of fish and microsome of the different biostructural level as the target absorbs available amounts of energies from γ-photon breakdown benzene-water in collison. And thus they each form the corresponding emitter which can emit the representive spectra. Last, the emitters for zebra fish include the exited singlet and triplet molecules. Of course, ·OH participants, to a certain extent, in the photon emission from fish, too. Whereas the main emitter for carp hepatic microsome is excited singlet molecule.

The radiolytics from benzene-water pretreated with ^{60}Co γ ray play a reduced role to the toxicity and induced photon emission of benzene.

The achievements of this work preliminarily open the way not only to study the toxicology of chemicals such as benzene but also to research the relationship between biophoton emission, free radical and toxicity. However, every one of some points as above contains one aspect of the problem as well as one aspect of a conclusion.

There is the corresponding relationship between biophoton emission from two different aquatic biosystem such as zebra fish and carp hepatic microsome and toxicity of benzene. The key role playing in the relationship as above is demonstrated to be the excited species, including ·OH, hydroxycyclohexadienyl radical(C_6H_5OH), singlet and triplet such as $^1O_2$, $C^*=O$ and so on, by means of ^{60}Co γ radiolysis. Thus, free radicals are the common photon originate of the objects in the different biostructural level. ·OH as a main energy donor, to a certain extent, directly participates in photon emission and toxicity of fish and microsome induced by benzene and its derivatives. So, free radical is responsible for photon emission and toxicity from the biological objects induced by benzene in water.

These are the conclusion obtained from this experimental results. The following points are the problems that need further to research, for instance, the measurements of various type of free radicals in the medium through the course of observation; the supplement of other physiological index in the observation of fish ; the determination of enzymatic activity concomitantly with measurement of biophoton emission under system by adding NADPH and cofactors to clarify the cause of the low intensity from the natural microsome in two media.

Model established in this experiment, in a word, could be expected that it will be adequate to study biophoton emission, free radicals and toxicity and their relationship under the conditions of the continuous improvement of this model. In addition, the results of this experiment supply some scientific data and technique for the better understanding to aquatic toxicology of benzene and for monitor and control benzene pollution in water environment.

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