Bull. Environm. Contam. Toxicol. 25, 69-74 (1980)

Effect of Petroleum Oil, Pesticides, PCBs and Other Environmental Contaminants on the Hatchability of *Artemia salina* Dry Eggs

Katsuyoshi Kuwabara, Akio Nakamura, and Takashi Kashimoto
Laboratory of Food Chemistry, Osaka Prefectural Institute of Public Health, 3-69, Nakamichi 1-Chome, Higashinari-ku, Osaka 537, Japan

Artemia salina LEACH is well-known as the brine shrimp used as tropical fish food and its dry eggs are easily obtained at a pet-shop at any season. One or two days after placing the dry eggs into salt water, the nauplius larvae of Artemia begins to hatch. In this paper, the methodology on a hatchability test for environmental contaminants and their effects on the dry egg hatchabilities are presented.

METHODS

Fig.1 shows Artemia dry eggs, their development in water and a larva. The dry egg, 200 μ hollow round, has a weight value of 1 mg corresponding to ca. 300 dry eggs, and becomes a full sphere after absorbing water. After that, emergence can occur, but this step with a thin hatching membrane is not the hatching stage. The final appearance shown in the figure is a hatching step. These procedure will be achieved as mentioned in Table 1 using a temperature-controlled tank.

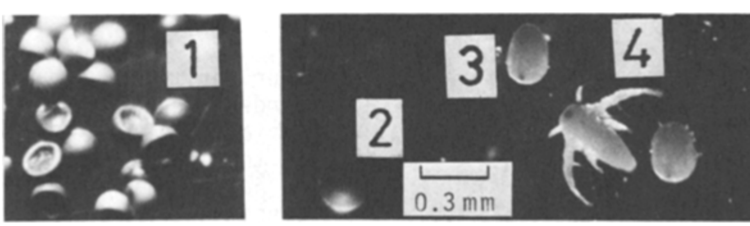

Fig.1. Microscopic examination; 1: Dry egg (cyst), 2: Spherical hydrated egg, 3: Larva of emergence stage, 4: Nauplius larva of hatching stage (× 30) .

After 48 h incubation, hatching larvae in each test tube are fixed by Bouin solution (picric acid, containing 15 % water /40 % formaldehyde aqueous solution /acetic acid = 75 / 25 / 5 (v / v / v)) and the number of hatching larvae is counted with a stereoscopic microscope. A hatching number per mg dry egg in each tube is consequently calculated. At the final step, the hatching number per mg dry egg in the test tube containing the solution of an assayed compound is compared with that of

0007-4861/80/0025-0069 $01.20

TABLE 1. The Standard Method of Hatchability Test Using Artemia Eggs

Water	: 5 mL of 2 % aqueous NaCl (pH 7 - 8)
Artemia eggs	: 2 - 3 mg in each 9-mL test tube
Organic solvent	: Acetone or DMSO 100 µL per tube
Temperature	: 27 °C
Incubation time	: 48 h
Prevention of vaporization	: 1 % Tween 80 in the 5 mL of 2 % aqueous NaCl

the control. After these processes, a relative hatchability for the compound can be obtained. In addition, Tween 80, a surface active agent is available for prevention of vaporization of lipophilic compounds. As a result of the experiment using PCB (Kanechlor^R 500) when it was added initially at 10 ppm (50 µg / 5 mL), 90 % of the PCB remained in the tube after 48 h incubation (KUWABARA *et al.* 1979).

Materials and apparatus: *Artemia salina*, brine shrimp eggs of San Francisco Bay Brand (Japan Pet Drugs Co.); Acetone and DMSO (Dimethyl sulfoxide), pesticide analysis grade; NaCl, special grade; and Tween 80 produced by Wako Pure Chemical Industries, Ltd., Japan. Temperature-controlled tank.

RESULTS AND DISCUSSION

Fig.2 shows the relation of hatching number per mg dry egg and time course in the controlled experiment under the condition listed in Table 1. After 48 h incubation, deviation of the hatching number per mg dry egg results in less than ±10 %. Moreover, the Tween 80 has no influence on the hatchability, so even lipophilic compounds can be assayed. Consequently, this method is considered to be available as one biological test for environmental contaminants.

Fig.3 indicates the result of an effect on the hatchability of petroleum heavy oils. At the both 100 and 500 ppm, the toxicity of A, B and C oil to Artemia is observed. And this order of the toxicity is almost equal to that to chicken embryos and that to killifish (NAKAMURA & KASHIMOTO 1979). It is, therefore, suggested for less volatile heavy oil to have a more toxic effect on animals.

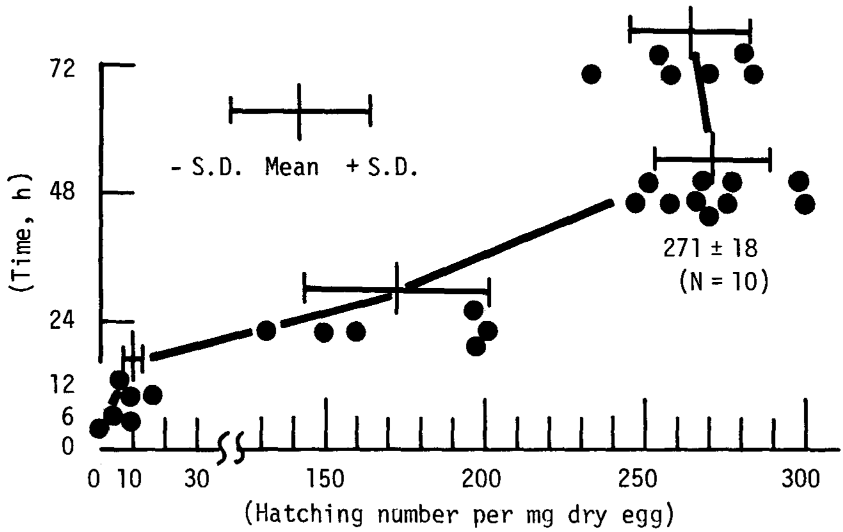

Fig.2. Time Course of Hatchability of Artemia Dry Eggs in 2 % aqueous NaCl.

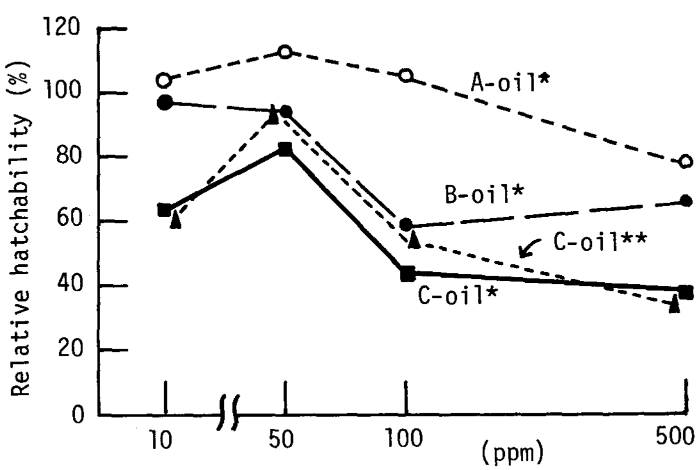

Fig.3. Hatchability of Artemia Eggs Exposed to Heavy Oil in 2 % aqueous NaCl containing 1 % Tween 80.
*Koa Oil Co. ; **Mitsubishi Oil Co.

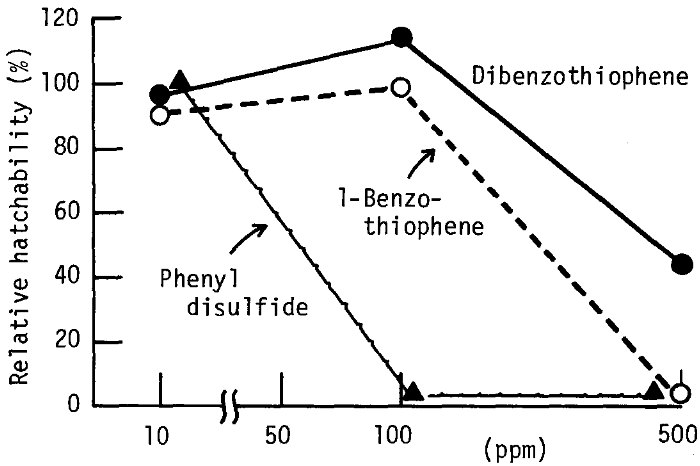

Fig.4. Hatchability of Artemia Eggs Exposed to Sulfur Compounds in 2 % aqueous NaCl containing 1 % Tween 80.

Fig.4 depicts the effect of sulfur-containing materials. Dibenzothiophene and 1-Benzothiophene are compounds derived from heavy oils (NAKAMURA & KASHIMOTO 1978). In the 500 ppm, both compounds have serious influence on the Artemia hatching, though their toxicity is less than that of another sulfur-containing compound, phenyl disulfide.

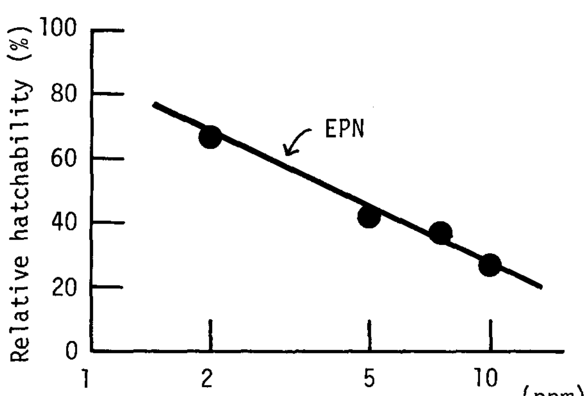

Fig.5. Hatchability of Artemia Eggs Exposed to EPN in 2 % aqueous NaCl cotaining 1 % Tween 80.

In the next case, the organophosphorus pesticide, EPN (ethyl *p*-nitrophenyl phenylphosphonothionate) is examined. As shown in Fig.5, even at 2 ppm, the hatchability is inhibited. Several other organophosphorus pesticides indicated in Table 2, however, have no influence upon the Artemia hatchability.

Although PCB (polychlorobiphenyl) and PCQ (polychloroquaterphenyl) are noted in the "Yusho" accident and PCQ in particular have caused serious problems under some circumstances (MIYATA *et al.* 1978), they make no difference in the hatchabilities.

Table 2 lists about forty environmental contaminants that were investigated; and these materials had no effect on the hatchability of Artemia dry eggs at 10 ppm in the aqueous system by the standard method mentioned above. Whereas most compounds are quite toxic and some of them even have a carcinogenicity, the Artemia dry eggs have been found to hatch well in a living state. However, in other studies on the mortality test using Artemia larvae, Aflatoxin B-1, for instance, has the

TABLE 2. Compounds Having No Difference in Hatchability from the Control at 10 ppm Using the Artemia Egg Test

P-Pesticide	Sn-Pesticide	Dibenzothiophene
DDVP	Tricyclohexyl-tin hydroxide	1-Benzothiophene
Diazinon		Phenyl disulfide
Dimethoate	Carbamate pesticide	Carbazole
Parathion	Carbaryl	A-Oil (Koa Oil Co.)
Disyston		B-Oil (Koa Oil Co.)
Fenitrothion	Feed additive for chicken	3,4-Benzopyrene
Phenthoate		
Malathion	Amprolium	N-Nitrosodimethylamine
Cl-Pesticide	Robenidine hydrochloride	Piperidine
p,p'-DDT		Aflatoxin B-1
Dieldrin	Nitrofurazone	
β-BHC	Caprylohydroxamic acid	Chlorhexidine dihydrochloride
Captan		
PCB	Carbadox	
Kanechlor 300	Decoquinate	
Kanechlor 400	Pyrimethamine	Sterigmatocystin (on 5 ppm)
Kanechlor 500	Zoalene	
Kanechlor 600		Lipid sol. toxin(s) of scallop (on 0.08 mouse unit / mL)
PCQ derived from the used thermotransfer medium		

LC 50 value (Median Lethal Cocentration) of 1.4 ppm (YAMAMOTO *et al.* 1974). Additionally, in the studies on methodology of a bioassay for heavy metals (OKUNO & MORI 1967), the Artemia hatchability is affected by lower level of them compared to that of the compounds in Table 2. *Artemia salina* is considered not to be sensitive at a hatching stage and to such lipophilic compounds in the Table. The test method reported here would nevertheless bring on simple and quick results for comparison of toxicity among subject materials.

REFERENCES

KUWABARA, K., A. NAKAMURA, & T. KASHIMOTO: Proc. Osaka Pref. Inst. Publ. Health, Ed. Food Sanitation (in Japanese) 10, 77 (1979).

MIYATA, H., T. KASHIMOTO, & N. KUNITA: J. Food Hyg. Soc. Japan 19, 364 (1978).

NAKAMURA, A., & T. KASHIMOTO: Bull. Environm. Contam. Toxicol. 20, 248 (1978).

NAKAMURA, A., & T. KASHIMOTO: J. Food Hyg. Soc. Japan (in Japanese) 20, 161 (1979).

OKUNO, T., & K. MORI: Proc. Osaka Pref. Inst. Publ. Health (in Japanese) 5, 70 (1967).

YAMAMOTO, K., H. TSUBOUCHI, S. MORIYAMA, & Y. SAKABE: J. Food Hyg. Soc. Japan (in Japanese) 15, 11 (1974).