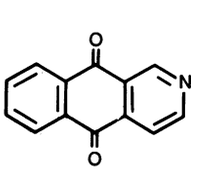
Benzoquinolinediones: Activity as Insect Teratogens

Abstract. *Morphological abnormalities including extra compound eyes, extra heads, and distally duplicated legs were generated in cricket embryos by treating eggs with single doses of either benz*[g]*isoquinoline-5,10-dione or benzo*[h]*quinoline-5,6-dione. Slight structural modifications of the molecules resulted in a loss of teratogenic activity, although embryotoxicity occurred. These potent insect teratogens can be used for analysis of developmental events during embryogenesis.*

Studies of the toxicity of several complex chemical mixtures derived from coal feedstocks established the existence of substances that produce extra body parts in insect embryos (*1*, *2*). Abnormalities occurred after cricket eggs [*Acheta domesticus* (L.)] were exposed to low concentrations of the unidentified chemicals; afflicted embryos had extra compound eyes, extra antennae, and extra heads. Branching of the antennae and distal duplications of the legs sometimes occurred in response to chemical treatment.

Table 1. Embryotoxicity (*9*) and teratogenicity of benz[*g*]isoquinoline-5,10-dione and benzo[*h*]-quinoline-5,6-dione to *A. domesticus* eggs. Individual eggs were treated topically, then were incubated at 31°C in groups of 20 eggs per treatment. The eggs were scored for multiple eyespots on days 8 to 11 of development; hatching typically begins on day 11 for controls. Survival is expressed as mean percent survival ± the standard error of the mean for groups of 20 eggs.

Dose (ng/egg)	Number of eggs	Survival (%)	Embryos with abnormal number of eyes
		Untreated control	
0	720	87 ± 1.5	0
		Dimethyl sulfoxide control	
0	660	82 ± 2.0	0
		Benz[g]*isoquinoline-5,10-dione (1)*	
20	358	21 ± 4.4	2
16	360	34 ± 4.6	2
12	360	48 ± 3.7	6
8	360	60 ± 4.0	2
6	360	75 ± 2.8	13
4	340	70 ± 2.2	17
1	300	66 ± 4.0	17
0.1	300	76 ± 3.6	8
		Benzo[h]*quinoline-5,6-dione (2)*	
125	179	22 ± 5.8	2
62	180	57 ± 8.0	5
31	180	79 ± 3.4	1
15	180	76 ± 3.8	0



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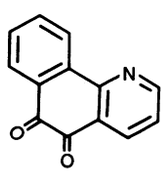

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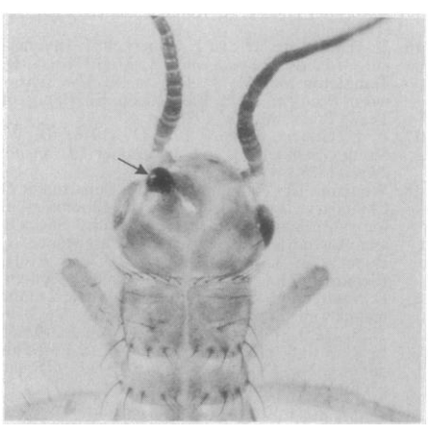

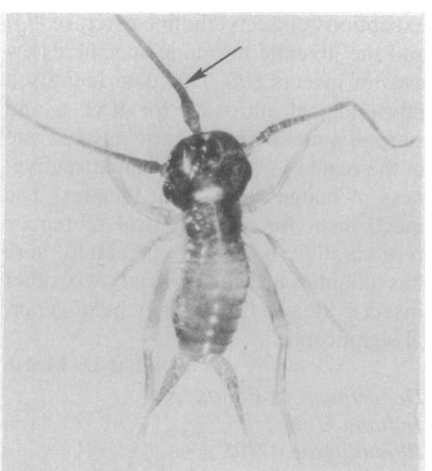

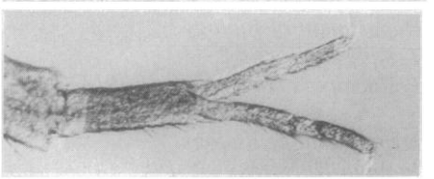

Identification of the biologically active substances in the complex chemical mixtures used in these studies could provide developmental biologists with useful probes for examining the mechanisms by which cells form specific body structures. In addition, chemical identification would facilitate testing the substances on animals in other taxa. Such tests are necessary in establishing the usefulness of the cricket embryo as an invertebrate model system for chemically induced teratogenesis (*3*).

During studies designed to isolate and identify specific chemicals disrupting embryonic development in *A. domesticus*, several azaarenes were synthesized and tested on eggs. Two diones of three-ringed nitrogen-containing heterocyclic molecules, benz[*g*]isoquinoline-5,10-dione (compound **1**) and benzo[*h*]quinoline-5,6-dione (compound **2**) (Fig. 1) (*4*), proved to be teratogenic when applied to eggs soon after oviposition (*5*). Morphological abnormalities in nymphs emerging from treated eggs were similar to those obtained with organic chemicals produced from coal feedstocks, including a chemical impurity present in commercial samples of acridine (*6*). Extra compound eyes (Fig. 2), extra antennae (Fig. 3), extra heads, and legs with distal portions duplicated (Fig. 4) were among the abnormalities produced.

The presence of extra compound eyes served as a convenient, objective criterion for evaluating teratogenicity in ovo, but this index does not indicate the total number of embryos with morphological abnormalities. Nonetheless, the data in Table 1 establish that both **1** and **2** are capable of producing extra compound eyes in crickets. Compounds **1** and **2** also produced other disruptions in embryogenesis, such as highly fragmented yolks, distorted embryos, or well-formed body structures developing in isolation. For example, a treated egg might contain a normal head devoid of both thorax and abdomen. Major abnormalities of this kind were invariably lethal and occurred

Fig. 1 (upper left). Compound **1**, benz[*g*]isoquinoline-5,10-dione; compound **2**, benzo[*h*]-quinoline-5,6-dione. Fig. 2 (lower left). A 1-week-old, three-eyed cricket that was treated during the egg stage with 5 ng of **1**. Fig. 3 (upper right). A cricket nymph with an extra antenna in the middle of the head (indicated by the arrow), a condition that sometimes occurs after exposure of the egg to benzoquinolinediones **1** and **2**. Fig. 4 (lower right). Leg with two tarsi and four tarsal claws from a cricket treated during the egg stage with 5 ng of **1**. A normal leg has one tarsus and two tarsal claws.

with increasing frequency at doses approaching or exceeding the median lethal dose (LD_{50}). Thus, the benzoquinolinediones displayed teratogenic activity over a range of doses. At higher doses the abnormalities were severe and the embryos died, whereas at lower doses of the range, embryos survived to emerge with visible evidence of teratogenicity in the form of extra body structures or distally duplicated appendages. Indeed, the highest percentages of multiple-eyed embryos were seen at doses of **1** producing low mortality (Table 1).

Subsequent isolation and characterization of the impurities in commercial acridine have demonstrated the presence of **1** in the teratogenic mixture and have confirmed the identification by gas chromatography–mass spectrometry and by infrared and ^{13}C nuclear magnetic resonance spectra (*7*). Whether **1** is also responsible for the teratogenic activity of the other organic mixtures tested on cricket embryos is not known at this time.

Bioassays of molecules structurally similar to **1** and **2** illustrate the unique teratogenic properties of the two $C_{13}H_7NO_2$ isomers (molecular weight, 209) and establish the critical contributions of the two keto groups and the nitrogen atom in conferring morphogenetic activity. None of the eight three-ringed aromatic isomers of $C_{13}H_9N$ (molecular weight, 179) produced extra body structures, although embryotoxicity was observed with all of the isomers; this shows that the lack of teratogenicity was not caused by an inability to penetrate the egg (*7*). In addition, benzo[*g*]quinoline-5,10-dione ($C_{13}H_7NO_2$; molecular weight, 209), which differs from **1** only by the position of nitrogen in the ring, lacked teratogenic activity (*8*), further indicating that slight changes in chemical structure resulted in loss of teratogenicity.

Compound **1** proved to be more embryotoxic than **2**. On the basis of the data in Table 1, the LD_{50} for **1** is 13.2 ng per egg (95 percent confidence limits of 12.6 and 14.2 ng per egg), and the LD_{50} of **2** is 85.3 ng per egg (95 percent confidence limits of 74.1 and 98.3 ng per egg) (*9*). Extra compound eyes were not observed in the 1380 control eggs used in this study, nor in any of the 50,781 control eggs examined in earlier studies of chemical teratogenesis in *A. domesticus* (*1*, *2*).

The cricket *A. domesticus* provides numerous advantages as a research animal for embryology. It is easily obtained and reared; the eggs are relatively large, with less than 2 weeks required to complete development; and considerable information is available on embryonic development (*10*). The demonstrated abilities of certain benzoquinolinediones to induce extra body structures in this insect, as well as to disrupt embryonic morphogenesis in the moth *Manduca sexta* (L.), the bug *Pyrrhocoris apterus* (*11*), and the cricket *Gryllus rubens* Scudder (*12*), indicate that these chemicals will be useful tools for exploring the processes of embryonic cellular differentiation, pattern formation, and chemically induced teratogenesis. We propose that the more potent insect teratogen, benz[*g*]isoquinoline-5,10-dione, be called "biquidone," a shortened version of the full chemical name.

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References and Notes

1. Chemical induction of duplicated body structures in crickets occurred after eggs were treated with liquids produced by several coal liquefaction processes, ether-soluble bases isolated from a coal liquid, and soil contaminated by a coal liquid spill [B. T. Walton, M. V. Buchanan, C.-h. Ho, in *Energy and Environmental Chemistry*, L. H. Keith, Ed. (Ann Arbor Science, Ann Arbor, Mich., 1982), vol. 1, p. 249; R. F. Strayer, N. T. Edwards, B. T. Walton, V. Charles-Shannon, *Environ. Toxicol. Chem.*, in press].
2. B. T. Walton, *Science* **212**, 51 (1981).
3. ———, *Fundam. Appl. Toxicol.*, in press.
4. A. Philips, *Chem. Ber.* **27**, 1923 (1894); Zd. H. Skraup and A. Cobenzl, *Monatshefte* **4**, 436 (1883). Each isomer was purified by high-performance liquid chromatography to 99 percent or more. Structures were confirmed with mass spectrometry and with infrared and ^{13}C nuclear magnetic resonance spectra.
5. A Hamilton syringe attached to a power-driven micrometer was used to apply 0.05 μl of various chemicals dissolved in dimethyl sulfoxide to eggs at 16 to 24 hours after oviposition. Eggs were incubated at 31°C on moist filter paper in covered petri plates (20 eggs per plate). The eggs were scored for multiple eyespots on days 8 to 11 of development.
6. The acridine used in these experiments, which contained less than 2 percent impurities, was prepared by vacuum distillation of coal tar (A. Bader, Aldrich Chemical Company, personal communication).
7. C. Y. Ma, C.-h. Ho, B. T. Walton, G. L. Kao, M. Guerin, in preparation. The isolation of the teratogenic impurity from commercial acridine samples and its identification as benz[*g*]isoquinoline-5,10-dione was reported at the March 1983 meeting of the Society of Toxicology [C.-h. Ho, C. Y. Ma, B. T. Walton, M. R. Guerin, *Toxicologist* **3**, 29 (1983)]. The eight isomers of $C_{13}H_9N$ were obtained as follows: acridine, phenanthridine, benzo[*h*]quinoline, and benzo[*f*]quinoline were purchased from Aldrich. Benz[*f*]isoquinoline and benz[*h*]isoquinoline were synthesized via the photochemical cyclization of 3-stilbazole and 4-stilbazole, respectively [C. E. Loader, M. V. Sargent, C. J. Timmons, *Chem. Commun.* **7**, 127 (1965)]. Manuscripts in preparation describe the syntheses of benzo[*g*]quinoline and benz[*g*]-isoquinoline and the embryotoxicity data for all eight isomers.
8. Benzo[*g*]quinoline-5,10-dione was synthesized according to (*4*).
9. The mean weight of the *A. domesticus* egg after water uptake is 606 ± 44 μg. Thus the LD_{50}'s of **1** and **2**, expressed as the ratio of chemical weight to egg weight, are 21.7 and 140 mg/kg, respectively.
10. J. S. Edwards and S.-W. Chen, *Wilhelm Roux' Arch. (Dev. Biol.)* **186**, 151 (1979); L. Lauga, *Ann. Sci. Nat. Zool. Biol. Anim.* **11**, 483 (1969); E. Mahr, *Wilhelm Roux' Arch. Entwicklungsmech. Org.* **15**, 662 (1961).
11. B. T. Walton and L. M. Riddiford, unpublished data.
12. B. T. Walton, J. B. Nardi, E. G. O'Neill, unpublished data.
13. We thank A. Bader, Aldrich Chemical Company; G. J. Sloan, E. I. duPont de Nemours and Company; and G. R. Southworth, Oak Ridge National Laboratory, for discussions. Research sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation. This is publication No. 2222, Environmental Sciences Division, Oak Ridge National Laboratory.

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Monoclonal Antibodies in the Lymphatics: Selective Delivery to Lymph Node Metastases of a Solid Tumor

Abstract. *After subcutaneous injection, monoclonal antibodies directed against a tumor can enter local lymphatic vessels, pass to the draining lymph nodes, and bind to metastases there. Lymphatic delivery of antibody to early metastases is more efficient than intravenous administration, and the lymphatic route can be used to image smaller metastatic deposits. Perhaps more important, the lymphatic route minimizes binding of antibodies to circulating tumor antigens and to cross-reactive antigens present on normal tissues. Antibodies inappropriate for intravenous use because of binding to normal tissues may therefore be useful against lymph node metastases when injected subcutaneously or directly into lymphatic vessels.*

Intravenous injection of monoclonal antibodies is being studied in numerous laboratories and hospitals for diagnosis and therapy of cancers (*1*). Antibodies with gamma emitters attached can be used for diagnostic imaging of tumors (*2*). For therapy, antibodies can mark tumor cells for destruction by host defenses (*3*) or carry an attached drug, toxin, radionuclide, or liposome (*4*). We recently reported an alternative to the intravenous route of administration: after subcutaneous injection, monoclonal antibodies enter local lymphatic capillar-