

# Systemic animal external parasitocidal activity of perfluoroalkylbenzimidazoles and their aminoanilide precursors

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**Summary.** In vitro and in vivo efficacy data indicate that chemical activity is optimized at the 2 carbon chain length and that these compounds have injectable potency and spectrum of activity against major animal external parasite species.

Benzimidazole compounds are known to possess anti-fungal, anthelmintic, herbicidal, insecticidal and nematocidal properties in animals and plants. Their use in animals is primarily by oral administration. However, recently it was reported that certain orally active benzimidazoles have excellent injectable potency and spectrum of anthelmintic activity<sup>2</sup>. We wish to report that 2-perfluoroalkylbenzimidazoles and their 2'-aminoanilide precursors are systemic insecticides. Activity has been demonstrated against experimental infestations of fleas, migrating larvae of *Cuterebra*, screw-worm larvae and ticks in laboratory animals.

**Materials and methods.** Insecticidal activity of 2-perfluoroalkylbenzimidazole compounds and their precursors was discovered and elaborated upon by the use of an in vitro black blow fly (*Phormia regina* Meigen) larval screen<sup>3</sup>. The most active compounds were further evaluated in guinea-pigs by oral and parenteral routes of administration. 24 h following the administration of the compounds, blood was collected and the serum was used in the in vitro blow fly larval test to determine systemic activity. Compounds exhibiting activity were then evaluated in a standard guinea pig assay<sup>4</sup>.

Tick efficacy was determined in male guinea-pigs which had been infested with nymphs of the lone star tick (*Amblyomma americanum* [L]) 48 h before treatment. 24 h before treatment, the guinea-pigs were wounded and the wounds infested with larvae of the flesh fly species, black blow fly and the secondary screw-worm (*Cochliomyia macellaria* Fabricus). Candidate insecticides, formulated in Tween-20®, were administered orally and s.c. to the infested guinea-pigs. 24 and 48 h after treatment, stable flies (*Stomoxys calcitrans* [L]) were fed on the guinea-pigs. Engorged flies were observed for 24 h to determine if they were killed by the blood ingested. 24 h after treatment the wounds were examined to determine if the fly larvae were killed. Ticks were observed for mortality during the engorgement period. Compounds that were 90% lethal to one or more of the parasites were considered to be active. The active compounds were then incorporated in a stable fly toxicant and repellent spot-test<sup>5</sup>, in an in vitro screen for prevention of reproduction of *Boophilus* ticks<sup>6</sup> and in a mouse *Cuterebra* screen<sup>7</sup>. Finally, several benzimidazoles were tested in dogs which were naturally infested with the brown dog tick, (*Rhipicephalus sanguineus*) and the common flea (*Ctenocephalides canis*).

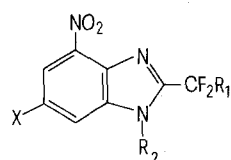
**Results.** Based on overall external parasitocidal activity, 2 of the most potent compounds were 6'-amino-

2,2,3,3,4,4,4-heptafluoro-5'-nitro-m-propionotoluidide (EL-968) and 4-nitro-2-(1,1,2,2-tetrafluoroethyl)-6-trifluoromethylbenzimidazole (EL-919). A single oral dose or s.c. injection of perfluoroalkyl benzimidazole at 10 mg/kg to experimentally infested guinea-pigs produced broad spectrum activity against flesh fly larvae, stable flies and tick nymphs. Excellent activity was obtained with lower s.c. doses of 1.0 and 2.5 mg/kg in guinea-pigs on flesh fly larvae and tick nymphs, respectively; the higher dose level was needed for complete control of the stable fly. The oral and dermal LD<sub>50</sub>s of EL-968 on the migrating rodent bot, *Cuterebra*, in mice were 13.7 mg/kg and 0.39% liquid concentration, respectively. EL-919 had insecticidal LD<sub>50</sub>s of 3.7 mg/kg orally and 0.18% dermally. Certain 2-(1,1-difluoroalkyl)-benzimidazoles and their 1(N)-carboxylic acid esters exhibited oral and dermal levels of 2.6 and 0.005% against *Cuterebra*.

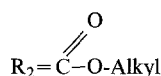
The 2-perfluoroalkylbenzimidazoles and their precursors formulated for the tick dip test as 1% emulsifiable concentrates effectively inhibited the reproductive potential of the tropical horse tick, (*Anocentor nitens* Neumann), the winter tick (*Dermacentor albipictus* Packard) and the *Boophilus annulatus* (Bay) and *B. microplus* species. Candidate insecticides screened as animal protectant sprays by the spot-test method effectively controlled adult stable flies for 4 days or more. However, the perfluoroalkylbenzimidazoles and their carboalkoxy analogues were observed to be more active as toxicants and repellants at 0.5% concentration than their precursors (example, EL-968) showing activity at 5% concentration. EL-919 employed at the lower dose sustained both properties for 8 days or more. 2 compounds LY74281 [2-(chlorodifluoromethyl)-4-nitro-6-(trifluoromethyl)-1-benzimidazolecarboxylic acid, isopropyl ester] and LY103435 [4-nitro-2-(1,1,2,2-tetrafluoroethyl)-6-(trifluoromethyl)-1-benzimidazolecarboxylic acid, isopropyl ester] were evaluated in the United States Department of Agriculture at Gainesville, Florida, oriental flea guinea-pig systemic insecticide test. 5 h following oral treatment at 10 mg/kg, complete kill of fleas was observed. The USDA Gainesville laboratory also tested compound LY69273 [isopropyl 4-nitro-2,6-bis(trifluoromethyl)-1-benzimidazole-carbamate] against the human body louse and at 1% concentration was similar to DDT in activity.

12 dogs heavily infested with fleas and ticks were separated into 2 groups and injected s.c.: 1 group with 25 mg of compound LY69273/kg b. wt and the other with 40 mg/kg of compound LY110972 [2-(chlorodifluoromethyl)-4-nitro-6-(trifluoromethyl)-1-benzimidazolecarboxylic acid, ethyl ester]. No fleas were found on dogs 48 h following treatment with either compound. At 72 h postinjection, efficacy against ticks ranged from 30 to 100%. In general, the effect on male ticks was more pronounced than on the females. This effect may be due to differences in feeding habits of male and female ticks. Dead male ticks were observed at 48 h. Females that engorged soon after treatment died 72 h later.

In vitro and in vivo data on the 2-perfluoroalkylbenzimidazoles and their aminoanilide precursors indicate 1. that their activity is optimized at the 2 carbon chain length



R<sub>1</sub> = F, Cl, CF<sub>2</sub>H, CF<sub>3</sub>, etc.



X = halogen, CF<sub>3</sub>

2-Perfluoroalkylbenzimidazole

and 2. that they are exceptional external parasitocides with a potential as animal systemic insecticides applied by dermal, oral or parenteral routes of administration.

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## Amounts of nuclear DNA in anurans of the USSR

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**Summary.** The amounts of nuclear DNA in blood erythrocytes of 18 species of Anura from the USSR have been determined to be in the range of 4.0–20.6 pg ( $10^{-12}$  g). Brown frogs of genus *Rana* have lower mean genome sizes than green frogs. Palearctic Anura, as a whole, have a greater content of nuclear DNA than the species of the same families from regions further south.

Only 22 species of about 2000 living anurans inhabit the USSR (3 species of *Bombina*, 2 of *Hyla*, 4 of *Bufo*, 2 of *Pelobates*, 1 of *Pelodytes*, and 10 of *Rana*). The fauna of this vast region of the world, occupying a considerable part of the Palearctic area, was formed during the postglacial period. Some of the frogs, e.g., *Rana arvalis*, *R. temporaria*<sup>1</sup> and *Bufo bufo*<sup>2</sup>, were able to move far to the North and cross the Polar Circle.

The present work is concerned with determination of the content of nuclear DNA in 18 species of Anura that constitute more than 80% of the USSR fauna. The data obtained fill the gap in the knowledge of Anura from this region and give additional information about palearctic species, as a whole<sup>3,4</sup>.

**Material and methods.** The animals were collected by us in various regions of the USSR in the period from 1974 to 1978. *Bufo raddei* was kindly given to us by Dr T.O. Alexandrovskaya (the Zoological Museum of Moscow State University), *B. orientalis*, *H. japonica*, *R. dalmatina*, *R. chensinensis* by Dr L.Ya. Borkin (the Zoological Institute, USSR Academy of Sciences, Leningrad). Blood smears were fixed with a mixture of methanol and acetic acid (3:1) or with 4% buffered formalin. In each series of experiments, smears from *Xenopus laevis* were used as a standard. The size of the *X. laevis* genome was assumed to be 6.3 pg<sup>5</sup>. Hydrolysis of the smears was carried out in 5 N HCl at  $37 \pm 0.5^\circ\text{C}$  for 15–35 min, the time interval corresponding to the middle part of the hydrolysis curves of the majority of species. The dye content of the Feulgen stained nuclei was measured at 546 nm by a scanning and integrating microdensitometer, type SIM-I. Not less than 100 nuclei of intact erythrocytes of each species were measured on 4–5 slides hydrolyzed for various periods of time; the results obtained were averaged.

**Results and discussion.** As seen from the table, the content of nuclear DNA in the species studied varies from 4.0 to 20.6 pg and the range of chromosomal sets in them from 22 to 26. The genome of *Bombina bombina* was found to be the largest, i.e.  $20.6 \pm 1.5$  pg in size. *B. variegata* that forms hybrids with *B. bombina* in the sympatric zone<sup>6</sup> has a smaller genome. The Far East species *B. orientalis*, that is allopatric to it, contains a similar amount of DNA. Disco-

glossidae seem to have the highest content of nuclear DNA among diploid Anura<sup>3,4</sup>.

The Caucasian endemic species *Pelodytes caucasicus* has the smallest genome among all the species studied by us, i.e.  $4.0 \pm 0.4$  pg, which is almost half the size of that of *Pelobates fuscus* ( $7.8 \pm 0.9$  pg) belonging to the same family. *P. caucasicus*, together with *P. punctatus*, whose karyotypes are of a similar structure<sup>7,8</sup>, form one of the isolated evolutionary branches of Pelobatidae. The latter occupies an intermediate position between *Scaphiopus* (1.6–3.6 pg)<sup>3</sup> and the true *Pelobates* (5.6–9.0 pg)<sup>3,7</sup>. The Caucasian relict subspecies, *Bufo bufo verucosissima*, ( $13.6 \pm 0.3$  pg) differs by its genome value from the Far East form *B. b. asiaticus* ( $11.2 \pm 0.4$  pg). Another Far East species, *B. raddei*, is close to *B. viridis* which is characterized by the lowest content of DNA among the species of toads studied (table). Despite significant discrepancies in the literature data (e.g., for *Bufo bufo* from 9.2 to 15.5 and for *B. viridis* from 6.7 to 13.3 pg<sup>3,9</sup>), there is an impression that *Bufo* species from the tropics, like those of *Rana*<sup>4,10</sup>, have smaller DNA values than those from the temperate zone.

The 2 allopatric species of tree frogs, *Hyla arborea* and *H. japonica*, which, probably, were a single species in the preglacial epoch, hardly differ in the genome size. The DNA content in them is higher than the average amount of DNA (5.7 pg) in the 30 studied species (most of which are tropical) of this family<sup>3</sup>.

Among brown (grass) frogs, the Far East *R. chensinensis* and the Carpathian *R. dalmatina* have the smallest genomes and the Caucasian *R. macrocnemis* has the largest one. Sympatric *R. ridibunda* and *R. lessonae*, whose hybrid is known as *R. esculenta*, differ significantly in the size of their genomes (table). It is noteworthy that, in this case, as well as in the case of *Bombina*, *Bufo* and a number of other species, significant differences in genome size between parental species cannot prevent natural interspecific hybrids from being formed.

As seen from the table, brown frogs, on the whole, contain a smaller amount of DNA (9.7 pg), compared with green frogs (13.6 pg). Summarizing the data of Bachmann and Nishioka<sup>4</sup> and ours, we can give the mean values for 11 species of brown frogs and for 8 pond ranids, which are