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# Influence of Route of Entry on Toxicity of Polycyclic Aromatic Hydrocarbons to the Cricket (*Acheta domesticus*)[a]

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Several polycyclic aromatic hydrocarbons (PAHs) have been shown to be toxic to vertebrates by oral as well as dermal routes of administration (POEL 1963, SHUBIK & DELLA PORTA 1957), but few studies have been conducted in invertebrates to establish the effectiveness of these portals of entry. Such studies are particularly needed for predicting ecological consequences of PAH releases during production, transport, and use of coal-derived synthetic fuels. Knowledge of natural barriers to PAH entry at the organismal level can provide a basis for identifying species of invertebrates most vulnerable to PAH exposure through habitat and food sources.

In the present study, acute and chronic toxicities of five PAHs (naphthalene, anthracene, benz[a]anthracene, pyrene, and benzo[a]pyrene) and an oxygenated PAH derivative (anthrone) were determined in a terrestrial insect, Acheta domesticus (L.), with emphasis on the influence of route of entry on toxicity. Hexamethylphosphoric triamide (hempa) was included in the chronic tests since this chemosterilant is a useful reference compound for investigations of reproductive effects of chemicals in insects.

## MATERIALS AND METHODS

Experimental Animals. Insects were purchased as early instar nymphs from a commercial supplier, reared at 32°C, 12L:12D, 54% mean relative humidity, and fed Patton's diet No. 16 (PATTON 1967). Experimental treatments were begun when the animals were last instar nymphs (ca. 4 weeks old).

Chemicals. All chemicals tested for toxicity were obtained from commercial suppliers. Naphthalene and anthrone were purchased from Matheson, Coleman, and Bell; anthracene and benz[a]anthracene from Eastman Kodak Company; and pyrene, benzo[a]pyrene, and hempa from Aldrich Chemical Company. The chemicals were exposed only to yellow fluorescent lights (wavelengths > 500 nm) during experimentation to minimize light

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[a]Orthoptera: Gryllidae

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catalyzed alterations. Reagent grade acetone was used as a solvent for administering chemicals.

Acute Toxicity. For dermal tests, crickets were immobilized by brief exposure to carbon dioxide and acetone solutions of chemicals were applied to the dorsal abdomens. Solutions were dispensed with a 0.25-cc syringe attached to a power-driven microapplicator.

For oral toxicity experiments, PAHs were added to ca. 20 mg of food and presented to food-deprived crickets. Insects requiring more than 15 min to consume the food were excluded from the experiment. Mortality was recorded 24 h after administration in both oral and topical toxicity tests.

Chronic Oral Toxicity. Crickets (equal numbers of males and females) were fed for up to 18 days on diets containing 0.1% by weight of the individual chemicals, and daily records were kept of mortality and adult eclosion. Treated food was prepared by mixing a stock diet containing 1.0% PAH, then diluting the stock with untreated food to give a final chemical concentration of 0.1%. Reagent grade acetone was added to the food to facilitate mixing, then removed by evaporation. The insects were held individually in 250-mL containers with food and water provided ad libitum.

Crickets were fed acetone treated food as a control diet, and food consumption was determined gravimetrically every two days for 18 days. These data were used to calculate chemical consumption by the crickets so that equivalent doses could be applied topically. The addition of the chemicals to the diet did not affect the palatability of the food, which was demonstrated by monitoring food consumption rates for benz[a]anthracene, anthrone, and pyrene diets over the 18 days.

$LT_{50}$'s were calculated by interpolation from the linear portion of curves resulting from plots of cumulative percent mortality vs time. The method of least squares was used to fit lines to the data. In cases where the $LT_{50}$ was greater than 18 days, the chi-square test was used to determine statistical significance of mortality in treatments.

Chronic Topical Toxicity. Naphthalene, pyrene, benz[a] anthracene, and hempa were applied topically every other day for 18 days in a dosage equal to 0.1% of the mean weight of food consumed by female crickets per 2-day interval. The application schedule was adjusted during the course of the experiment to reflect the higher feeding rate in the latter days of the experiment, i.e., 103 ± 34 mg food per 2 days per cricket for the first 6 days and 183 ± 17 mg food per 2 days per cricket for the last 12 days. The total amount of each PAH applied over the 18 day period was 1,224 μg. Control crickets were treated with acetone only.

## RESULTS AND DISCUSSION

Acute Toxicity. The acute toxicities of the PAHs were low when administered orally or topically. Naphthalene and benzo[a]pyrene in the food resulted in $LD_{50}$'s > 15,000 μg/g; anthracene, anthrone, benz[a]anthracene, and pyrene produced $LD_{50}$'s > 580 μg/g. Topical application of naphthalene, anthrone, benz[a]anthracene, and pyrene likewise caused $LD_{50}$'s exceeding 580 μg/g. The low solubility of anthracene and benzo[a]pyrene in suitable organic solvents prevented determination of acute as well as chronic topical toxicity.

Chronic Toxicity. Chronic toxicities of the PAHs, hempa, and acetone are listed in Table 1. Hempa proved to be the most toxic of the chemicals tested, and naphthalene the most toxic of the PAHs. While anthracene, anthrone, benz[a]anthracene, pyrene, and benzo[a]pyrene $LT_{50}$'s were greater than 18 days, total mortality (shown in parentheses in Table 1) exceeded that of control treatments for all chemicals except anthrone.

TABLE 1. Chronic toxicity of equivalent doses of polycyclic aromatic hydrocarbons and a chemosterilant (hempa) to Acheta domesticus after oral and topical administration

| | Toxicity | | | |
|---|---|---|---|---|
| Compound $LT_{50}$(days) | Dietary $LT_{50}$(days) | N | Topical | N |
| Control (acetone) | >18 (6%)[a] | 48 | >18 ( 9%) | 21 |
| Hempa | 8.3 ± 0.29[b] | 48 | >18 (33%)*** | 24 |
| Naphthalene | 12.0 ± 0.41 | 48 | >18 (25%)** | 24 |
| Anthracene | >18 (39%)*** | 46 | ---- | -- |
| Anthrone | >18 ( 6%) | 48 | ---- | -- |
| Benz[a]anthracene | >18 (26%)*** | 66 | 18 (29%)** | 24 |
| Pyrene | >18 (23%)*** | 69 | 18 (26%)** | 23 |
| Benzo[a]pyrene | >18 (32%)*** | 22 | ---- | -- |

[a]Number in parentheses is percent morality after 18 days.

[b]$LT_{50}$ ± S. E.

***Percent mortality significantly different from control mortality, $P < 0.001$.

**Percent mortality significantly different from control mortality, $P < 0.01$.

Cumulative mortality did not increase linearly with time for insects feeding on PAH diets. Few deaths occurred in the first and last few days of these experiments with most deaths (77% of all mortality for PAH diets) taking place between days 5 and 11. Only 6% of the deaths occurred in the last 6 days. Food consumption was not significantly reduced after day 11, so lower PAH intake is not a reasonable explanation for the drop in the death rate. In addition, topically treated insects also had low mortality (11% of all deaths in PAH treatments) after day 11, although dose was constant for days 6-18.

Deaths of crickets did not appear to be directly associated with eclosion; however, 99% of the crickets had molted by day 11, the period during which 94% of the deaths occurred. In addition, most deaths (70%) occurred prior to the molt or within two days after the molt to the adult stage. These data suggest that mortality might have been higher if crickets had undergone more than one molt or lower if the insects had been treated after the last molt.

Hempa and naphthalene proved to be more toxic when ingested than when applied topically (Table 1); however, benz[a]anthracene and pyrene toxicities were not markedly different by these two routes of entry. Anthrone, which proved to be non-toxic in food, was not tested for chronic dermal toxicity nor were anthracene and benzo[a]pyrene because of low solubility in suitable organic solvents.

The fact that mortality did occur after consuming the chemicals suggests that PAHs move across the gastrointestinal tract of crickets. This conclusion is further supported by observations of dissected body tissues under ultraviolet light. Tissues and unlaid eggs of pyrene-fed crickets fluoresced deep blue under long-wave ultraviolet light; whereas, tissues of control crickets did not, indicating that pyrene, which is fluorescent, or pyrene metabolites were present.

Uptake of PAHs from food has been reported for other species of insects, although the data are not conclusive with respect to unaided movement into the animals. CORWIN & GOTTLIEB (1978) found that anthracene in the diet of Drosophila melanogaster larvae increased the frequency of melanotic tumors in $F_1$ and $F_2$ progeny of treated flies. The diets contained 5% of the detergent HaemoSol to solubilize the anthracene, which may have affected movement across the gut wall. RIGDEN & NEAL (1967) found benzpyrene (isomer not specified), pyrene, and anthracene in whole body extracts of Periplaneta americana adults that were fed diets containing these PAHs, although extractions were made without removing the gastrointestinal tracts so that food remaining in the gut may have been the source of recovered PAH. However, in the case of benzpyrene, fluorescence of the intact body was observed, indicating the movement of the parent compound or metabolites from the gut into the haemocoel. In addition, body extracts were fluorescent when treatment diets had been replaced by standard diets 16 days

prior to extraction, by which time neither benzpyrene nor fluorescent metabolites were detectable in fecal extracts.

In the present study, chronic ingestion of naphthalene, anthracene, benz[a]anthracene, pyrene, and benzo[a]pyrene by Acheta domesticus nymphs and adults has been shown to cause mortality significantly higher than that of controls. Results also indicate that PAHs found in food and on substrates contacted by litter-dwelling, omnivorous insects such as crickets can enter these organisms by uptake from the gastrointestinal tract as well as by movement across the integument. It appears that further investigations of sublethal effects of these compounds on parameters such as reproduction and behavior can be made by simply administering the PAHs in concentrations less than 0.1% by weight in the diet. Such studies will contribute to prediction of the consequences of PAH pollutants to omnivorous, terrestrial insects.

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