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"Central" and "Peripheral" Benzodiazepines and Kinetics of Lindane-Induced Toxicity[1]

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GRIFFITH, J. A. AND D. E. WOOLLEY. *"Central" and "peripheral" benzodiazepines and kinetics of lindane-induced toxicity.* PHARMACOL BIOCHEM BEHAV 32(2) 367–376, 1989.—Because hypothermia and anorexia were previously found to be more sensitive indices of the effects of lindane than were convulsions, these endpoints were used to quantify the ability of benzodiazepines (BDs) and phenytoin either to ameliorate or exacerbate the toxicity of lindane in the rat. After administration of lindane (40 or 50 mg/kg) in oil per os, toxicity was counteracted by phenytoin and the "central" BD agonists diazepam and clonazepam, but was worsened by Ro 5-4864 a "peripheral" BD agonist. Clonazepam and diazepam were each more effective in counteracting lindane-induced anorexia than in stimulating food intake, presumably because the animals had been fasted and probably even controls ate maximally when food was presented. Diazepam alone (3 injections in 1 day) produced withdrawal-induced decreased food intake the following day. Clonazepam and diazepam alone each transiently decreased colonic temperature, yet effectively blocked the more severe hypothermia produced by lindane. Ro 5-4864 by itself did not produce any measurable effects, yet exacerbated all of the effects, including lethal effects, of lindane. The present findings are compatible with other evidence that lindane and Ro 5-4864 act at the picrotoxinin receptor of the $GABA_A$-activated chloride channel and that systemic administration of agents acting at this site may produce a constellation of effects, including seizures, hypothermia and anorexia.

Clonazepam Convulsions Diazepam Food intake Lindane Phenytoin Ro 5-4864 Temperature regulation

LINDANE, the gamma isomer of hexachlorocyclohexane, is a neurotoxicant used in both human and veterinary medicine to treat ectoparasites. It is considered safe because dermal absorption is usually low. However, dermal use of lindane has occasionally been associated with serious illness and even death (14,55). Death has also resulted from oral intake of lindane, because directions for its use were misunderstood (13). In addition, use of lindane as a general insecticide leads to the possibility of ingesting it via food. Although its use is restricted in some countries (62), lindane continues to be used as a general insecticide in many parts of the world and is registered for limited use on some food crops in the United States (18).

Lindane is of interest to the neurobiologist, not only because of continuing public health problems associated with its use, but also because the mechanism of action of this potent convulsant continues to be actively investigated. The first mechanism of action proposed was that lindane increases spontaneous and/or evoked neurotransmitter release, perhaps because of increased calcium entry into nerve terminals or release of calcium from internal stores [reviewed in (28,68)]. It may also increase postsynaptic response to released neurotransmitter (28, 67, 68). Recently, lindane was found to bind to the picrotoxinin (PTX)/t-butylbicyclophosphorothionate (TBPS) receptor of the $GABA_A$-activated chloride channel (1, 35, 41). An action of lindane at this site has been found to inhibit GABA-stimulated chloride flux in vitro (2, 7, 20) and could account for the convulsant effects of lindane. In synaptosomes from rat brain, the IC_{50} for lindane in inhibiting TBPS binding, i.e., 1.7 μM (35) or 0.15 μM (1) is similar to that for inhibiting GABA-stimulated chloride uptake, i.e., about 1.0 μM (2). An antagonistic action of lindane at the $GABA_A$ ionophore could explain the effectiveness of some benzodiazepines (BDs) in treating lindane poisoning (67,68) since these BDs potentiate GABAergic transmission by an action at the "central" BD receptor site on the $GABA_A$-activated chloride channel, separate from, but near to, the GABA receptor site [reviewed by (17)].

This laboratory has previously demonstrated that a single dose (40 mg/kg) of lindane per os (PO) produces profound hypothermia and anorexia which last longer and have a lower threshold than do overt seizures in the female rat. Repeated administration of either diazepam or phenobarbital prevented the lindane-induced convulsions, anorexia and hypothermia (67,68). In the present studies, hypothermia and anorexia were used as quantifiable endpoints to investi-

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gate further the effects and mechanisms of action of lindane in the rat. To do this, we compared the effectiveness of ligands for "central" versus "peripheral" BD receptors in modulating lindane toxicity. It has not been clear which of these receptors plays the major role in mediating the antidotal effect of diazepam in lindane poisoning, since diazepam binds to both, as well as to a "micromolar" BD site. To help determine this, the effectiveness of diazepam in modulating the effects of lindane was compared with that of clonazepam, an agonist for only the "central" sites, and with that of Ro 5-4864, a ligand for the "peripheral" sites. Although it was originally thought that "peripheral" BD receptors were located only in peripheral tissues, sites which bind nanomolar concentrations of Ro 5-4864 were later also found in the CNS (53). In brain, "central" BD sites bind nanomolar concentrations of diazepam and clonazepam and micromolar concentrations of Ro 5-4864, "peripheral" BD sites bind nanomolar concentrations of diazepam and Ro 5-4864 and micromolar concentrations of clonazepam (40), and "micromolar" BD sites bind micromolar concentrations of these same three drugs, as well as micromolar concentrations of phenytoin (9). In addition, Ro 5-4864 was recently shown to bind to the PTX/TBPS site on the GABA-activated chloride channel (59,64) and to inhibit GABA-stimulated chloride influx (45). IC_{50}s for these effects were about 5–20 μM, suggesting that Ro 5-4864 is less effective than lindane (see above) in these actions.

For comparison with the effects of BDs, the effects of phenytoin (5,5'-diphenylhydantoin), which has been used clinically to treat symptoms of lindane poisoning (32), were determined first. Although phenytoin has several effects, including potentiation of the postsynaptic effect of GABA, its ability to slow recovery of sodium channels from inactivation is believed to be the primary basis for its anticonvulsant effect in the therapeutic dosage range (39). Interestingly, a relation between BDs and phenytoin is suggested by observations that diazepam and Ro 5-4864, as well as other BDs, enhance phenytoin binding (65).

METHOD

General Procedures

Adult male or female Sprague-Dawley rats (bred from rats obtained from Simonsen's Laboratories, Gilroy, CA) were housed individually in stainless steel hanging cages in a temperature-controlled room (23±0.5°C) under controlled lighting (14 L:10 D). Rats were allowed free access to water and to ground rodent chow (Purina 5001; St. Louis, MO) except when the chow was removed the night before dosing. Food intake, body weight, and colonic temperatures were measured daily for several days prior to administration of the drugs to assure that they were stable. For food intake measurements, unaccounted spillage of food was minimized by using food cups centered and glued to the inside of larger metal containers. Colonic temperatures were measured with a rectal thermistor probe connected to a telethermometer (Yellow Springs Instrument, model 42SC; Yellow Springs, OH).

On the morning after fasting, "zero hour" colonic temperatures and body weights were measured, then lindane, drugs, or both were administered one hr later, except in Experiment 1, when either phenytoin or vehicle was administered 1 hr prior to zero hour measurements.

Rats were observed for the appearance of convulsions for at least 2 hr following administration of lindane, drugs, and/or vehicle(s). After dosing, temperatures, food intake and body weights were measured at the times indicated in the Results section and in the appropriate figures. Food cups were returned following temperature measurements 2 hr after dosing.

Lindane (Sigma Chemical Co., St. Louis, MO) was dissolved in oil and administered PO by gastric intubation in a volume of 1 ml/kg body weight. Diazepam, clonazepam and Ro 5-4864 (provided by Dr. Peter Sorter, Hoffmann-La Roche Inc., Nutley, NJ) were dissolved in DMSO and injected SC in a volume of 0.5 ml/kg body weight. Phenytoin sodium salt (Sigma) was dissolved in distilled water:ethanol (2:1, v/v) and administered by intubation in a volume of 1.5 ml/kg body weight. All animals not receiving either drugs or lindane received the appropriate vehicle(s) at the same times. Dosing and measurements were made on one rat from each group before proceeding to the second rat from each group, etc.

Experimental Protocols

In Experiment 1, 23 female rats were divided into 3 groups of 7 or 8 rats each. The two experimental groups each received lindane (40 mg/kg) in oil PO. One of these was pretreated (1 hr) with 100 mg/kg phenytoin in saline:ethanol PO.

In Experiment 2, 15 male rats were divided into 3 equal groups. The two experimental groups received 40 mg/kg lindane PO; one of these also received 5 mg/kg diazepam SC just prior to administration of lindane.

In Experiment 3, 30 male rats were divided into a control and 3 experimental groups of 7 or 8 rats per group. Two groups received 50 mg/kg lindane (PO); one of these also received 3 administrations of diazepam (5 mg/kg SC) at 8-hr intervals during the first 24 hr, starting just before lindane was administered. A third group received only diazepam at the same dose and times.

In Experiment 4, 24 female rats were divided into 4 equal groups and injected SC with 0.2, 1, or 5 mg/kg clonazepam or the vehicle (controls). In this and the succeeding experiments, food intake was measured at 2-hr intervals for 10 hr after return of the food cups, instead of only daily.

In Experiment 5, 35 female rats were divided into 5 equal groups. Animals in the 4 experimental groups received lindane (40 mg/kg) by intubation, as well as 0.2, 1, or 5 mg/kg clonazepam or the vehicle SC.

In Experiment 6, 23 female rats were divided into groups as follows: 7 rats receiving only the vehicles (controls), 7 lindane-treated rats, 5 Ro 5-4864-treated rats and 4 rats treated with Ro 5-4864 plus lindane. Forty mg/kg lindane was administered in oil PO and 10 mg/kg Ro 5-4864 was administered in DMSO SC. Not enough Ro 5-4864 was available to dose more animals.

Statistical Analysis

The significance of differences between the means of treated and control groups was determined after analysis of variance (ANOVA) when the overall F ratio was significant at the $p<0.05$ level. The post hoc test was Fisher's Least Significant Difference (LSD) test, with the level of significance corrected for the number of comparisons by the Bonferroni method (44).

RESULTS

Experiment 1. Effects of 100 mg/kg Phenytoin PO on Lindane-Induced Toxicity in Female Rats (Fig. 1)

In an initial study, 75 mg/kg phenytoin, administered 30

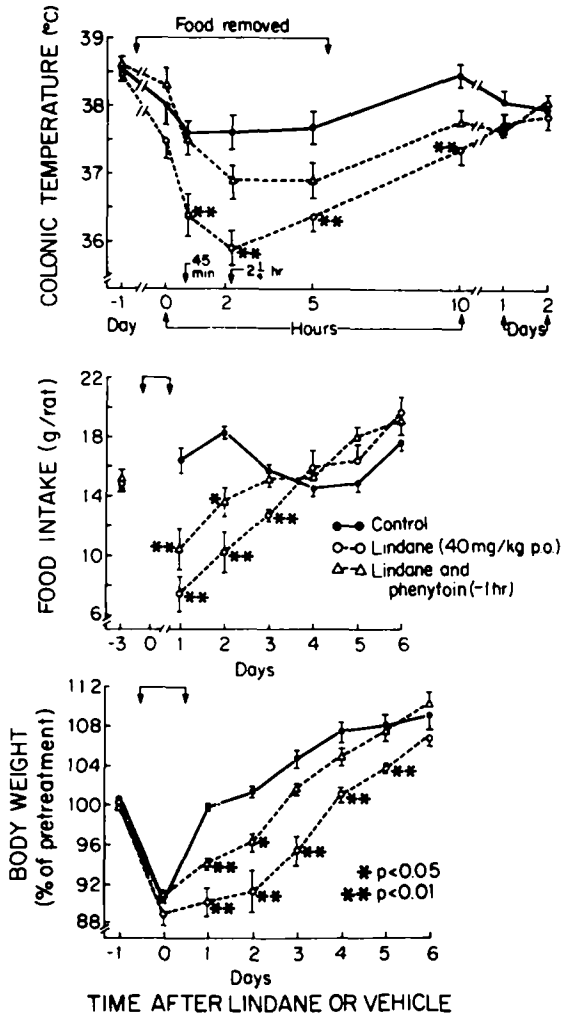

FIG. 1. Effects of pretreatment (−1 hr) with phenytoin (100 mg/kg) in distilled water: ethanol (2:1 v/v) PO on lindane-induced toxicity in female rats. In this and the following figures bracketed vertical lines represent SEMs and asterisks indicate the significance of the differences between the means of treated and control groups. The horizontal bars over the figures indicate the time food was removed in this and the following figures.

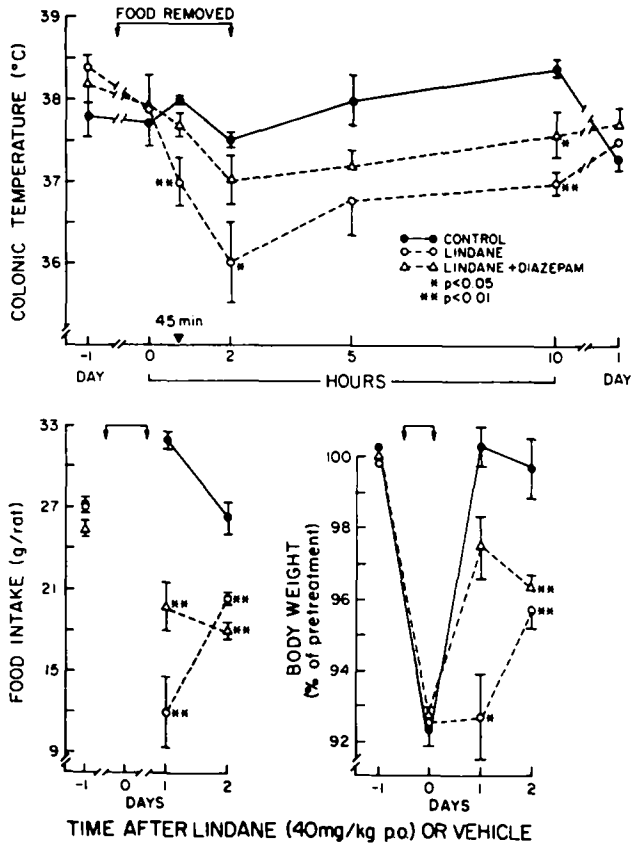

FIG. 2. Effect of a single administration of diazepam (5 mg/kg) in DMSO administered SC on the effects of lindane in oil administered PO in male rats.

min before lindane, hastened the recovery from, but only partially blocked, lindane-induced toxicity. Therefore, in the present study, the dose of phenytoin was increased from 75 to 100 mg/kg and the time of pretreatment was extended from 30 min to 1 hr.

As usual, the first behavioral effect of lindane (40 mg/kg) was to reduce motor activity, e.g., the rats would stand quietly facing the back of the cage. Seizures first appeared 30–60 min after dosing and consisted, initially, of myoclonic (whole body) jerks. As severity of the effects increased, masticatory "jawing," forelimb and head clonus, as well as rearing, occasionally with loss of the righting reflex, also occurred. The most severe seizure activity in this experiment was a stereotyped sequence which began with brief tonic neck flexion, followed by tonic neck extension accompanied by tight closure of the eyes, wide opening of the mouth and a tonic spasm of the trunk muscles including a partial rotation of the body at midthorax severe enough to cause loss of righting so that the animal fell briefly on its side and then recovered, usually to exhibit forelimb clonus. Although this or a similar sequence has been observed hundreds of times in this laboratory, following either electroshock seizure stimulation or administration of lindane, dieldrin, chlordane or picrotoxin, we have not yet found an adequate description of it in the literature, and so we are naming it "minimal tonic seizure plus clonus." This is to contrast with maximal tonic seizures, characterized by tonic limb flexion or extension.

Pretreatment with phenytoin reduced the number of rats exhibiting seizures from 4 out of 8 in the group receiving only lindane to 1 out of 8 in the group of rats receiving lindane plus phenytoin. Also, time to onset of seizures (158 min) in the one phenytoin-treated rat which showed seizures was longer than in any of the 4 rats exhibiting seizures in the group receiving only lindane (mean±S.E. for latency=47±14 min). Phenytoin also counteracted lindane-induced hypothermia through 10 hr after dosing. Although both experimental groups exhibited anorexia and reduced body weight gain on day 1, food intake and body weight recovered earlier in the group receiving lindane and phenytoin than in the group receiving only lindane. Body weights of lindane-treated rats required 6 days to equal those of the control group.

Experiment 2. Effects of a Single Dose of Diazepam in DMSO on Lindane-Induced Toxicity in Male Rats (Fig. 2)

Convulsions were not observed in the 5 rats that had re-

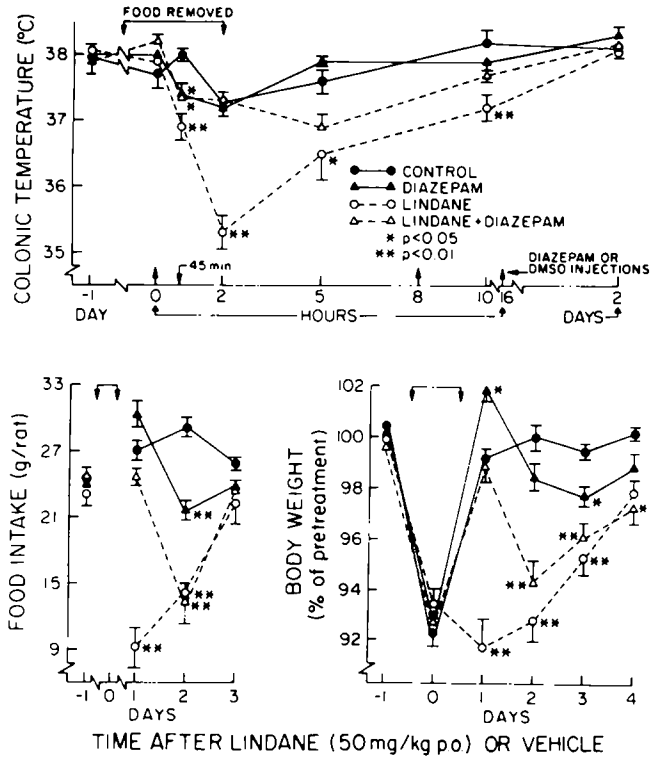

FIG. 3. Effect of diazepam (5 mg/kg) in DMSO, administered 3 times SC at the times indicated by arrows, on the effects of lindane in oil administered PO in male rats.

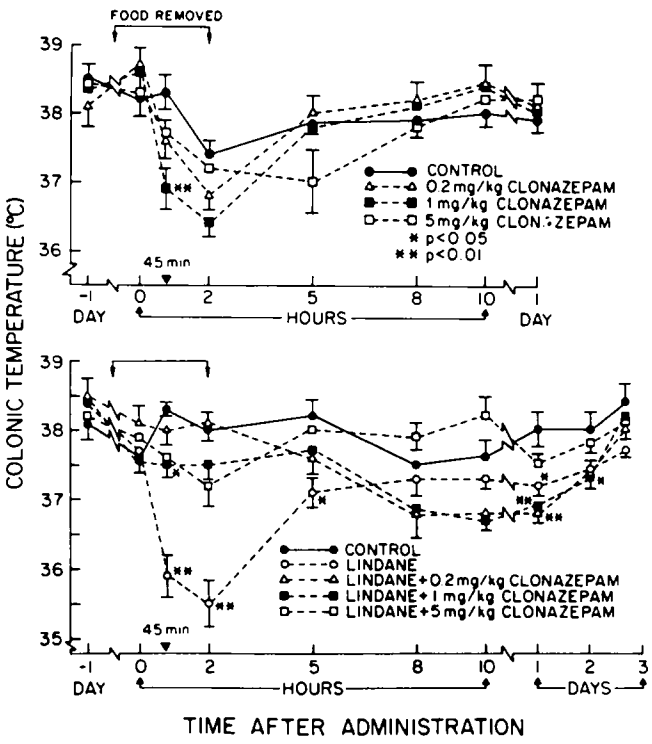

FIG. 4. Effects on colonic temperature of 3 different single doses of clonazepam in DMSO administered SC (top) in Experiment 3 compared with the effects of the same doses of clonazepam in blocking lindane-induced hypothermia (bottom) in Experiment 4. Clonazepam was administered immediately before lindane was given. Female rats were used. Compare with Figs. 5 and 6.

ceived diazepam (5 mg/kg SC) as well as lindane (40 mg/kg, PO), whereas seizures occurred 30–45 min after dosing in all but 1 of the 5 rats receiving lindane only. Diazepam blocked lindane-induced hypothermia at 45 min and 2 hr after dosing and still partially blocked at 10 hr. By day 1 after dosing, temperatures did not differ between groups. Diazepam partially blocked lindane-induced anorexia and antagonized body weight gain on day 1 only. Between days 1 and 2, the effects of diazepam had clearly worn off, and the effects of lindane had returned.

Experiment 3. Effects of Diazepam Administered 3 Times SC on the Effects of Lindane in Male Rats (Fig. 3)

Since a single injection of diazepam (5 mg/kg in DMSO) at the time of dosing with lindane (50 mg/kg in oil PO) was no longer effective in blocking lindane-induced toxicity 10–24 hr later in Experiment 2, additional injections of diazepam were made 8 and 16 hr after administration of lindane. Colonic temperatures of all experimental groups, including those of the group that had received diazepam only, were reduced by 45 min. Even though diazepam alone produced a slight hypothermia at 45 min, it effectively antagonized the more marked hypothermic effects of lindane at this time and at 2 hr after dosing.

The group receiving only diazepam tended to eat more than did the control group on day 1 (though this was not statistically significant) and ate significantly less than did the control group on day 2, perhaps because of diazepam withdrawal-induced reduction in food intake. Diazepam blocked the lindane-induced effects on food intake and body weight on day 1, but, by day 2, the effects of diazepam had worn off with the result that body weights and food intake of both lindane-treated groups, with or without diazepam, were indistinguishable. As in the previous experiments, the effects of lindane on food intake lasted longer than the effects on colonic temperature. Despite the marked effects of lindane on food intake and body temperature, convulsions were not observed.

Experiment 4. Dose-Response Effects of Clonazepam (0.2, 1, or 5 mg/kg) in DMSO SC on Colonic Temperature (Fig. 4), Food Intake and Body Weight (Fig. 5) in Female Rats

Before determining the effects of clonazepam on lindane-induced toxicity, the dose-response effects of clonazepam alone were determined. Nearly all of the 6 rats in the highest dosage group showed some type of abnormal behavior, e.g., wet-dog shakes, head jerks, fast jaw movements, ataxia or marked inactivity. "Catatonic" behavior, i.e., remaining immobile and holding up a paw for several min, jerky movements, irregular breathing, inactivity, ataxia, and hyperactivity were observed in rats of the two lower dosage groups. The following sequence of abnormal behavior was found to be typical of the highest dosage group: strong wet-dog shakes, followed by loss of balance, which was followed, in turn, by ravenous feeding on a food pellet, or sawdust or feces, even though food was available. Stereotypic, ingestive behaviors, such as chewing in the absence of a substrate,

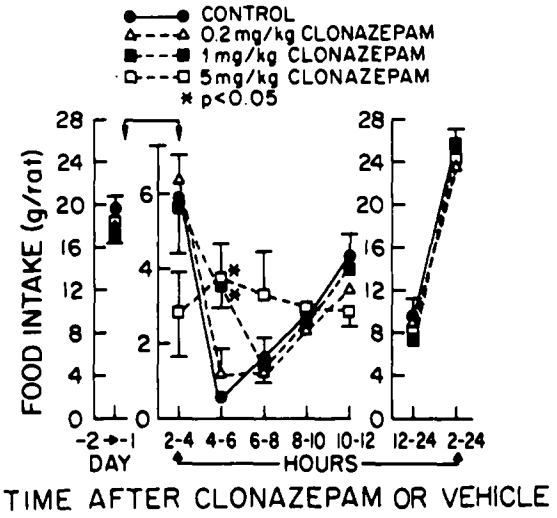

FIG. 5. Effects of 3 different single doses of clonazepam on food intake and changes in body weight in female rats in Experiment 3.

rhythmic tongue protrusions, and pawing at food, were similar to behaviors following administration of chlordiazepoxide, described by others (6). Hyperactivity and stereotypic behavior were followed by inactivity, during which the only movement observed was deep, labored breathing.

A trend toward clonazepam-induced hypothermia at 45 min and 2 hr after dosing reached statistical significance only in the middle dosage group at 45 min (Fig. 4).

In order to obtain more detailed information on food intake than in the previous experiments, food intake was measured at 2 hr intervals until 12 hr after drug administration, rather than only daily. All but the group receiving the highest dose of clonazepam ate more during the first 2 hr interval that food was available than at later intervals (Fig. 5). As the normal "lights off" time (10 hr after dosing) approached, the rats increased their food intake. Food intake of the highest dosage group was relatively low when food was first returned. Thus, food intake to compensate for the overnight fast for this group was delayed until the 4–6 hr period when it was higher than for the controls. Food intake for the middle dosage group was also significantly higher than that for the controls during the 4–6 hr interval, even though food intake during the 2–4 hr period for this group was equal to that of the controls, i.e., food intake to compensate for the overnight fast was not delayed, making the increased food intake during the 4–6 hr period even more significant. Cumulative food intake over the 2–12 hr (not shown), 12–24 hr, and 2–24 hr periods did not differ between groups. Body weights (not shown) were not affected by clonazepam.

Experiment 5. Dose-Response Effects of Clonazepam (0.2, 1, or 5 mg/kg SC DMSO) on Lindane-Induced Toxicity in Female Rats (Figs. 4 and 6)

To determine the effectiveness of clonazepam in antagonizing lindane-induced toxicity, a single dose of 0.2, 1, or 5 mg/kg was given just prior to administration of lindane (40 mg/kg in oil) PO. Lindane alone elicited myoclonic whole

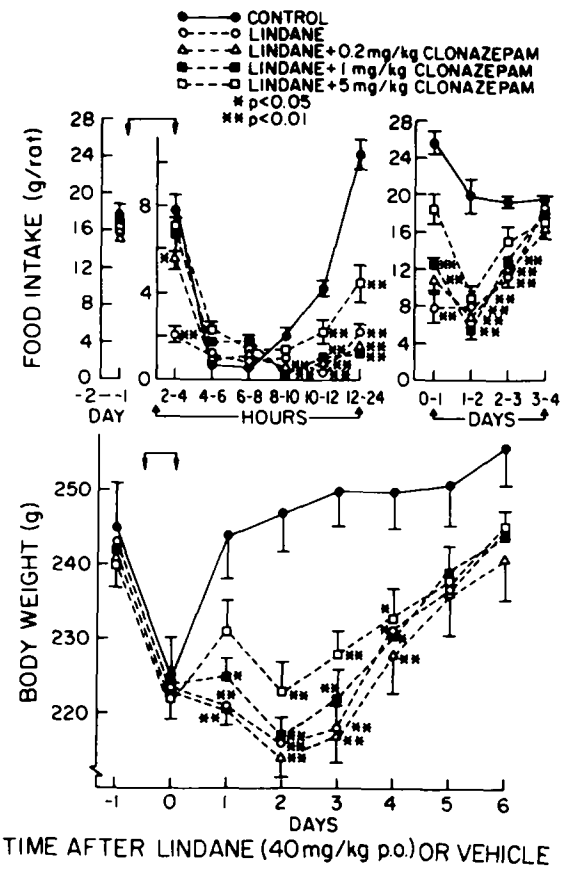

FIG. 6. Effects of 3 different single doses of clonazepam on food intake and changes in body weight in lindane-treated female rats in Experiment 4. Clonazepam was given a few minutes before lindane.

body jerks and mild clonic convulsions in 3 out of 7 animals, beginning about 50 min after dosing and ending about 75 min after dosing. Only a single incidence of myoclonus was observed in 1 of 7 animals in the group that had received lindane plus the lowest dose of clonazepam. The remaining animals were limp and inactive. Although lindane-induced convulsions were not observed in the groups that had received either the middle or high dose of clonazepam, 4 out of 7 animals from the highest clonazepam dosage group exhibited intermittent wet-dog shakes, a sign of clonazepam-induced toxicity. Wet-dog shakes began about 30 min and ended about 100 min after dosing.

All doses of clonazepam antagonized the marked hypothermic effects of lindane from 45 min through 5 hr (Fig. 4). Thus, even though the lindane-treated rats given clonazepam were limp and inactive, their temperatures were normal. Also, even though clonazepam alone tended to produce hypothermia, it effectively blocked lindane-induced hypothermia. Only the highest dose of clonazepam completely protected the rats throughout the first day against lindane-induced hypothermia.

As expected, the controls ate when food was first returned, then reduced their intake until near the "lights off" period, 10 hr after dosing (Fig. 6). Clonazepam antagonized lindane-induced anorexia during the 2–4 hr interval after dosing. The protective effect of even the highest dose of

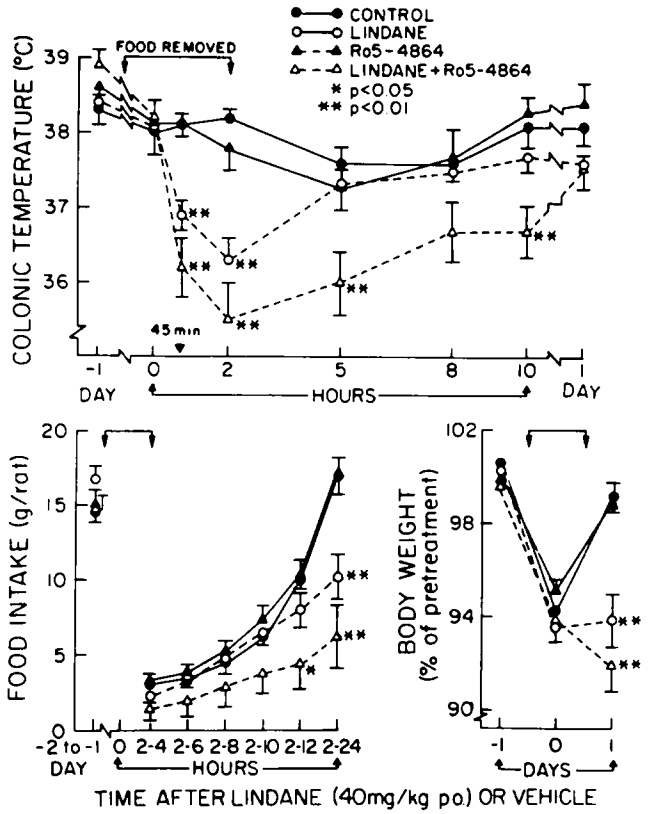

FIG. 7. The exacerbating effects of Ro 5-4864 (10 mg/kg in DMSO SC) on lindane-induced hypothermia, anorexia, and loss of body weight in female rats.

clonazepam had started to wear off 10–12 hr after administration, though food intake of this group was still significantly greater than that of the group dosed with lindane only. Only the highest dose of clonazepam provided significant protection against lindane-induced anorexia when cumulative food intake was measured over the first 24 hr (Fig. 6). All groups receiving lindane, with or without clonazepam, showed reduced food intake from 10 hr through 2–3 days after dosing.

The body weights of all experimental groups were significantly lower than were weights for the controls from days 1 through 4 after dosing, except on day 1 for the lindane-treated group which received the highest dose of clonazepam.

Experiment 6. Exacerbating Effects of Ro 5-4864 on Lindane-Induced Toxicity in Female Rats (Fig. 7)

Although neither lindane (40 mg/kg in oil PO) nor Ro 5-4864 (10 mg/kg in DMSO SC) elicited convulsions when administered alone, together they produced convulsions in 3 out of 5 animals. Convulsions, typical of lindane poisoning, began 1 hr after dosing, peaked at 2 hr, and had subsided by 2.5 hr in 2 of the 3 animals. However, the third animal died after numerous convulsive episodes, including hopping seizures (i.e., bilateral, synchronous clonic movements of the limbs, strong enough to propel the animal upward into a "hop") and tonic forelimb and hindlimb extension. This was the only animal to die in any of the 6 experiments.

Ro 5-4864 alone did not affect food intake, colonic temperature or body weight, However, Ro 5-4864 exacerbated all of the effects of lindane on these endpoints during the first 24 hr after dosing. Significant colonic hypothermia was observed 45 min after dosing in the groups receiving either lindane plus Ro 5-4864 or lindane only. Although hypothermia continued through 10 hr after dosing for the group receiving both the toxicant and the drug, hypothermia in the group receiving only lindane had subsided by 5 hr after dosing.

Reduction in food intake became statistically significant first in the group receiving both lindane and Ro 5-4864 during the 2–12 hr interval, but not until the 2–24 hr interval for the group receiving lindane only. By day 1, both groups receiving lindane weighed significantly less than did the control group.

DISCUSSION

The present findings confirm and extend previous observations from this laboratory (67,68) that lindane, administered PO, produces anorexia which lasts for days, in contrast to the relatively shorter effect on colonic temperature (e.g., about 1 day) and even briefer effect in producing observable seizures (e.g., 2–3 hr).

In the present study, agents which either ameliorated or exacerbated one of the lindane-induced effects generally affected all of them in the same way. Thus, phenytoin, diazepam, and clonazepam antagonized lindane-induced seizures, hypothermia, and anorexia, whereas Ro 5-4864 exacerbated all of these effects.

Effects of the BDs: Diazepam, Clonazepam, and Ro 5-4864

Temperature regulation. The present results are consistent with the hypothesis that systemic administration of agents that promote GABAergic transmission produce hyperthermia, or have the ability to antagonize hypothermia, whereas agents that inhibit GABAergic transmission produce hypothermia. Thus, lindane, believed to be an antiGABAergic agent (see Introduction), induced hypothermia, and Ro 5-4864, which may also be antiGABAergic (see Introduction), worsened the lindane-induced hypothermia. Other work in this laboratory (66) has shown that a subconvulsant dose of Ro 5-4864 itself produced hypothermia. Clonazepam and diazepam, agonists for "central" BD receptors that promote GABAergic transmission, counteracted lindane-induced hypothermia. Also, GABA injected into the hypothalamus increased body temperature (8), whereas inhibition of hypothalamic GABA synthesis decreased body temperature in the rat (37). However, the role of GABA in thermoregulation is complex and varies with species, the target brain sites involved, and ambient temperature [reviewed in (15,37)].

It seems contradictory that clonazepam and diazepam each transiently decreased colonic temperature, yet were so effective in blocking the more severe hypothermia induced by lindane. However, others also have found that diazepam reduces body temperature in rats at ambient temperatures of 22–23°C, though not at 33°C (4). (The thermoneutral zone for adult rats is 27–28°C [(47), p. 681].) The hypothermic effects of 1 mg/kg of clonazepam in the present study agree with the marked decrease in body temperature produced by clonazepam (4 mg/kg IP) in mice at normal room temperature though not at 33°C (27). It is also possible that the reduced motor activity observed in our animals after administration of either clonazepam or diazepam contributed to the slight decrease in temperature.

Withdrawal from repeated administration of clonazepam

for 2 weeks in dogs led to hyperthermia (52). Although all groups receiving a single dose of clonazepam in the present study had higher colonic temperatures after the initial hypothermia than did the controls at 10 hr and 1 day after dosing, this did not reach statistical significance.

Food intake. The present results support the hypothesis that systemic administration of agents that promote $GABA_A$ergic transmission, such as agonists for the "central" BD receptor, increase food intake, whereas administration of agents believed to inhibit $GABA_A$ergic transmission, such as lindane or Ro 5-4864 (see Introduction), reduce food intake through actions on the brain or gut or both. BD-induced feeding behavior was recognized early (49) and is believed to be secondary to enhancement of GABAergic activity in some brain systems (10,43). Not surprisingly, when agents which modulate GABAergic activity were injected into specific brain regions in the rat, the effects varied depending on the brain area involved [reviewed in (10, 30, 31, 33, 43)]. Injection of GABA, muscimol (a GABA agonist) or BDs into the ventromedial hypothalamus (the "satiety center"), the paraventricular nucleus, or the dorsal raphe nucleus increased food intake, presumably because of inhibition of areas inhibitory to food intake (10, 30, 31, 43). Similarly, injections of agents to counteract the effects of GABA (PTX, bicuculline) into the ventromedial "satiety center" suppressed feeding (10, 30, 31). Injection of muscimol into the lateral hypothalamic area (the "feeding center") reduced food intake presumably because of inhibition of areas stimulatory to food intake (31). The effectiveness of muscimol injected into the lateral hypothalamic area in increasing food intake was reversed by GABAergic blockade with bicuculline (10). Physiologically, increased food intake has been associated with increased levels of GABA in the ventromedial hypothalamus and with decreased levels in the lateral hypothalamus (33).

Cooper proposed that control of food intake at the "central" BD receptor was bidirectional, i.e., agonists for "central" BD receptors produced hyperphagia, inverse agonists (ligands that bind to a receptor but produce an effect opposite to that of the agonist) produce anorexia, and antagonists block both actions (11). Cooper and Gilbert (12) showed that clonazepam produced hyperphagia in rats and that this was antagonized by the "central" BD receptor antagonist Ro 15-1788. Ro 5-4864 reduced food intake (12), though the possibility that Ro 5-4864 was acting as an inverse agonist was not considered, even though Ro 5-4864 does bind to the "central" site in micromolar levels (see Introduction).

In the present studies, clonazepam and diazepam were each more effective in counteracting lindane-induced anorexia than in stimulating food intake in animals not given lindane, presumably because the animals had been fasted and so the controls ate maximal amounts when food was returned. Diazepam and clonazepam have been shown to promote feeding behavior after acute administration [reviewed in (11,12)]. Withdrawal from chronic administration of diazepam reduced food intake in both animals and man [reviewed in (11, 51, 52)]. In the present work, withdrawal-induced decreased food intake occurred in the rat after only 3 equally spaced administrations of diazepam in a 24 hr interval (Fig. 3). This extends previous work in this laboratory in which withdrawal-induced reduction in food intake occurred on the fourth day following three days of twice daily injections of diazepam [Fig. 8 in (68)].

Because GABA is a neurotransmitter in the myenteric plexus [reviewed in (58)], the possibility arises that, after PO administration, lindane-induced anorexia was due to interference with $GABA_A$ergic transmission in the gut. Activation of $GABA_A$ receptors caused contractions of the guinea pig ileum and altered gastric acid and hormonal secretion (21,58). Perhaps lindane affected secretion and motility of the gut. If so, this could help explain the abdominal pain and nausea reported by patients who received very low oral doses of lindane (0.5 mg/kg daily for 3 days) (22).

BDs also may affect ileal contractions. In vitro, diazepam dose-dependently (10^{-9}–10^{-6} M) potentiated guinea pig ileal contractions caused by activation of $GABA_A$ receptors. The "central" BD antagonist Ro 15-1788 (10^{-5} M) blocked diazepam-induced potentiation of GABA-mediated contractions, while the "peripheral" BD antagonist PK 11195 (10^{-5} M) was ineffective (38). By contrast, micromolar levels of either diazepam or Ro 5-4864, or even higher levels of clonazepam, inhibited contractions of the guinea pig ileum elicited by electrical stimulation (26). Thus, a micromolar BD receptor mediates inhibition of ileal contractions and the "central" nanomolar BD receptor mediates potentiation of contractions. This could help explain our observation that the higher dose (5 mg/kg) of clonazepam tended to inhibit food intake during the first 2 hr interval in which the rats had access to food, though this reduction did not reach statistical significance. This high dose could have inhibited food intake by activation of the micromolar BD receptor. Later, the middle and even highest doses of clonazepam increased food intake, presumably when clonazepam dropped from micromolar to nanomolar levels, and thus activated only the "central" BD receptor.

Perhaps, then, the anorexic effect of lindane could have been due to antiGABAergic actions on gut or brain or both. Likewise, the potentiation of GABAergic transmission by diazepam or clonazepam at the "central" nanomolar BD site in either gut or brain or both could explain how each blocked the anorexic effect of lindane.

Proconvulsant Effects of Ro 5-4864

Ro 5-4864 was initially considered to be anticonvulsant in electroshock seizure tests and was even selected for clinical trials on this basis. Upon retesting, the anticonvulsant effect could not be confirmed and higher doses were found to have marked convulsant properties (70). Similarly, the reductions in locomotor activity produced by Ro 5-4864, which were interpreted as evidence for a sedative effect (46), may be better interpreted as an indication of prodromal effects produced by convulsants. The first behavioral effect produced by lindane in the rat which we observed was decreased locomotor activity. A GABAergic mechanism in the convulsant action of Ro 5-4864 in the rat has been proposed (50). The importance in vivo of an action by Ro 5-4864 at the PTX/TBPS site, demonstrated in vitro (45, 59, 64), is suggested by the fact that Ro 5-4864 lowered the threshold for seizures produced by PTX (19). Another convulsant BD, Ro 5-3663, also bound to the PTX/TBPS site (36).

Although Ro 5-4864 also acts on voltage-dependent calcium channels, it is hard to see how an action here can explain the present findings. In contrast to their different effects on lindane-induced toxicity, diazepam, clonazepam and Ro 5-4864 in micromolar levels each inhibited depolarization-induced entry of calcium into synaptosomes from rodent brain. This effect was additive with that of the known calcium channel antagonists (48,57).

Thus, of the several possible sites of action, an action at

the PTX/TBPS site seems most likely to explain the proconvulsant action of Ro 5-4864, though more work is needed.

Blood-brain barrier. One must consider that Ro 5-4864 could have exacerbated the effects of lindane by facilitating the entry of lindane into the CNS. The highest density of sites that bind Ro 5-4864, besides those in the olfactory nerve and glomerular layers of the olfactory bulb, is in the ventricular ependymal cells and choroid plexus (3). If Ro 5-4864 could affect metabolism or have some other effect in cells of the ependyma or choroid plexus, this, in turn, might affect transport between blood and cerebral spinal fluid. However, such an effect in these cells has not yet been investigated. Since 1–15 mg/kg Ro 5-4864 in mice exacerbated audiogenic seizures (5), a seizure model in which movement of a drug into the brain is not involved, it is likely that the proconvulsant/convulsant effects of Ro 5-4864 do not depend on transport across the blood-brain barrier.

Effects on metabolism. Since Ro 5-4864 prolonged and intensified lindane-induced toxicity, perhaps Ro 5-4864 inhibited metabolic degradation of lindane. In support of the possibility that Ro 5-4864 may influence metabolism is the finding that "peripheral" BD receptors are found on the outer mitochondrial membrane in a number of tissues, e.g., adrenal cortex, liver and kidney. The endogenous ligands for these sites appear to be physiologically relevant porphyrins (54). Again, since Ro 5-4864 has been shown to enhance audiogenic seizures in mice (5), an effect which would not seem to depend on altered metabolism, at least some of the proconvulsant/convulsant effects of Ro 5-4864 would not appear to depend on changes in metabolism.

Phenytoin

Tilson *et al.* (61) showed that pretreatment with 75 mg/kg phenytoin did not block the seizure activity induced by 60 mg/kg lindane PO, nor did it counteract the disruptive effect of lindane (30 mg/kg) in a learning test in the rat. Furthermore, phenytoin increased the sensitivity to a startle stimulus of lindane-treated rats (60). Also, in the rat, phenytoin (30 mg/kg) was ineffective in counteracting the proconvulsant effects of Type II pyrethroids (16), which are believed to have an effect similar to that of lindane. In agreement with these studies, the present work shows that phenytoin was not as effective as either clonazepam or diazepam in preventing all of the effects of lindane. In particular, it was less effective in preventing the initial lindane-induced anorexia. However, in the present study, pretreatment with 100 mg/kg phenytoin reduced the incidence of seizures produced by 40 mg/kg lindane PO from 50% to 12½% and also antagonized lindane-induced hypothermia. Although phenytoin was not as effective as either diazepam or clonazepam in counteracting lindane-induced anorexia during the first day after administration of lindane, the antidotal effect of phenytoin against this endpoint lasted longer, perhaps because of the relatively slow excretion of phenytoin (42).

Although BDs and phenytoin interact at specific neuronal membrane sites (65), the greater effectiveness of clonazepam and diazepam than of phenytoin in counteracting all of the effects of lindane, suggests that the mechanisms shared by the BDs and phenytoin are the less effective ones for treatment of lindane toxicity. Also, both Ro 5-4864 and diazepam enhance phenytoin binding (65), whereas they have opposite effects on lindane-induced toxicity.

Summary of Mechanisms of Action of Lindane and Signs of Toxicity

The present and previous findings suggest that a number of agents that bind to the PTX/TBPS site produce a similar constellation of effects, i.e., convulsions, anorexia, and hypothermia. Insecticides that bind to the PTX/TBPS site (1, 17, 29, 34, 35, 41) and inhibit GABA-stimulated chloride flux (2, 7, 17, 20) include lindane, Type II pyrethroids, dieldrin, alpha (cis)-chlordane, endrin, heptachlor and heptachlor epoxide. These pesticides produce convulsions (23, 28, 56, 68, 69), hypothermia (23–25, 56, 63, 67, 68) and anorexia [reviewed in (67,68)], in those cases in which food intake was tested.

The similar signs of poisoning produced by these insecticides contrast with the effects produced by their isomers with lower affinity for this site. Type II pyrethroids are convulsant, produce hypothermia, and bind with high affinity to the PTX/TBPS site, whereas Type I pyrethroids, with very similar structures, produce tremors and hyperthermia and only bind with low affinity to this site (23,34). Similarly, picrotoxin reduces food intake (30) and produces hypothermia (66), as well as seizures, in the rat. Ro 5-4864 is believed to act at this site (45, 59, 64) and is both convulsant (19, 50, 64, 70) and anorexic (12). In addition, work in this laboratory (66) has shown that Ro 5-4864 produces hypothermia. Furthermore, Ro 5-3663 is also a convulsant BD which binds competitively to the PTX site (36) and produces anorexia in a dose-dependent manner (12). Clonazepam completely reversed the anorexic effect of Ro 5-3663 (12), just as it reversed the anorexic and other effects of lindane in the present study.

REFERENCES

1. Abalis, I. M.; Eldefrawi, M. E.; Eldefrawi, A. T. High-affinity stereospecific binding of cyclodiene insecticides and gamma-hexachlorocyclohexane to gamma-aminobutyric acid receptors of rat brain. Pest. Biochem. Physiol. 24:95–102; 1985.
2. Abalis, I. M.; Eldefrawi, M. E.; Eldefrawi, A. T. Effects of insecticides on GABA-induced chloride influx into rat brain microsacs. J. Toxicol. Environ. Health 18:13–23; 1986.
3. Anholt, R. R. H.; Murphy, K. M. M.; Mack, G. E.; Snyder, S. H. Peripheral-type benzodiazepine receptors in the central nervous system: localization to olfactory nerves. J. Neurosci. 4:593–603; 1984.
4. Bartholini, G.; Keller, H.; Pieri, L.; Pletscher, A. The effect of diazepam on the turnover of cerebral dopamine. In: Garattini, S.; Mussini, E.; Randall, L. O., eds. The benzodiazepines. New York: Raven Press; 1973:235–240.
5. Benavides, J.; Guilloux, F.; Allam, D. E.; Uzan, A.; Mizoule, J.; Renault, C.; Dubroeucq, M. C.; Gueremy, C.; Le Fur, G. Opposite effects of an agonist, Ro5-4864, and an antagonist, PK 11195, of the peripheral type benzodiazepine binding sites on audiogenic seizures in DBA/2J mice. Life Sci. 34:2613–2620; 1984.

6. Berridge, K. C.; Treit, D. Chlordiazepoxide directly enhances positive ingestive reactions in rats. Pharmacol. Biochem. Behav. 24:217–221; 1986.
7. Bloomquist, J. R.; Adams, P. M.; Soderlund, D. M. Inhibition of gamma-aminobutyric acid-stimulated chloride flux in mouse brain vesicles by polychlorocycloalkane and pyrethroid insecticides. Neurotoxicology 7(3):11–20; 1986.
8. Boros-Farkas, M.; Illei-Donhoffer, A. The effect of hypothalamic injections of noradrenaline, serotonin, pyrogen, gamma-amino-n-butyric acid, triiodothyronine, triiodothyroacetic acid, DL-phenylalanine, DL-alanine and DL-gamma-amino-beta-hydroxy butyric acid on body temperature and oxygen consumption in the rat. Acta Physiol. Acad. Sci. Hung. 36:105–116; 1969.
9. Bowling, A. C.; DeLorenzo, R. J. Micromolar affinity benzodiazepine receptors: identification and characterization in central nervous sytem. Science 216:1247–1250; 1982.
10. Cooper, S. J. GABA and endorphin mechanisms in relation to the effects of benzodiazepines on feeding and drinking. Prog. Neuropsychopharmacol. Biol. Psychiatry 7:495–503; 1983.
11. Cooper, S. J. Bidirectional control of palatable food consumption through a common benzodiazepine receptor: Theory and evidence. Brain Res. Bull. 15:397–410; 1985.
12. Cooper, S. J.; Gilbert, D. B. Clonazepam-induced hyperphagia in nondeprived rats: Tests of pharmacological specificity with Ro 5-4864, Ro5-3663, Ro15-1788 and CGS 9896. Pharmacol. Biochem. Behav. 22:753–760; 1985.
13. Crosby, A. D.; D'Andrea, G. H.; Geller, R. J. Human effects of veterinary biological products. Vet. Hum. Toxicol. 28:569–571; 1986.
14. Davies, J. E.; Dedhia, H. V.; Morgade, C.; Barquet, A.; Maibach, H. I. Lindane poisonings. Arch. Dermatol. 119:142–144; 1983.
15. DeFeudis, F. V. Involvement of GABA and other inhibitory amino acids in thermoregulation. Gen. Pharmacol. 15:445–447; 1984.
16. Devaud, L. L.; Szot, P.; Murray, T. F. PK 11195 antagonism of pyrethroid-induced proconvulsant activity. Eur. J. Pharmacol. 120:269–273; 1986.
17. Eldefrawi, A. T.; Eldefrawi, M. E. Receptors for gamma-aminobutyric acid and voltage-dependent chloride channels as targets for drugs and toxicants. FASEB J. 1:262–271; 1987.
18. Environmental Protection Agency. Lindane; amendment of notice of intent to cancel pesticide products containing lindane. Fed. Reg. 50(27):5424–5425; 1985.
19. File, S. E.; Green, A. R.; Nutt, D. J.; Vincent, N. D. On the convulsant action of Ro 5-4864 and the existence of micromolar benzodiazepine binding site in rat brain. Psychopharmacology (Berlin) 82:199–202; 1984.
20. Ghiasuddin, S. M.; Matsumura, F. Inhibition of gamma-aminobutyric acid (GABA)-induced chloride uptake by gamma-BHC and heptochlor epoxide. Comp. Biochem. Physiol. 73C:141–144; 1982.
21. Giotti, A.; Luzzi, S.; Spagnesi, S.; Zilletti, L. $GABA_A$ and $GABA_B$ receptor-mediated effects in guinea-pig ileum. Br. J. Pharmacol. 78:469–478; 1983.
22. Graeve, K.; Herrnring, G. Uber die toxizitat des gamma-hexachlorcyclohexan. Arch. Int. Pharmacodyn. 85:64–72; 1951.
23. Gray, A. J.; Pyrethroid structure-toxicity relationships in mammals. NeuroToxicology 6(2):127–138: 1985.
24. Hrdina, P. D.; Peters, D. A. V.; Singhal, R. L. Role of noradrenaline, 5-hydroxytryptamine and acetylcholine in the hypothermic and convulsive effects of alpha-chlordane in rats. Eur. J. Pharmacol. 26:305–312; 1974.
25. Hrdina, P. D.; Singhal, R. L.; Peters, D. A. V. Changes in brain biogenic amines and body temperature after cyclodiene insecticides. Toxicol. Appl. Pharmacol. 29:119; 1974 (abstract).
26. Hullihan, J. P.; Spector, S.; Taniguchi, T.; Wang, J. K. T. The binding of [^{3}H]-diazepam to guinea-pig ileal longitudinal muscle and the *in vitro* inhibition of contraction by benzodiazepines. Br. J. Pharmacol. 78:321–327; 1983.
27. Jenner, P.; Pratt, J. A.; Marsden, C. D. Mechanism of action of clonazepam in myoclonus in relation to effects on GABA and 5-HT. In: Fahn, S.; Marsden, C. D.; Van Woert, M. H., eds. Advances in neurology, vol. 43: Myoclonus. New York: Raven Press; 1986:629–643.
28. Joy, R. M. Mode of action of lindane, dieldrin and related insecticides in the central nervous system. Neurobehav. Toxicol. Teratol. 4:813–823; 1982.
29. Kadous, A. A.; Ghiasuddin, S. M.; Matsumura, F.; Scott, J. G.; Tanaka, K. Difference in the picrotoxinin receptor between the cyclodiene-resistant and susceptible strains of the German cockroach. Pest. Biochem. Physiol. 19:157–166; 1983.
30. Kelly, J.; Grossman, S. P. GABA and hypothalamic feeding systems. II. A comparison of GABA, glycine and acetylcholine agonists and their antagonists. Pharmacol. Biochem. Behav. 11:647–652; 1979.
31. Kelly, J.; Rothstein, J.; Grossman, S. P. GABA and hypothalamic feeding systems. I. Topographic analysis of the effects of microinjections of muscimol. Physiol. Behav. 23:1123–1134; 1979.
32. Khare, S. B.; Rizvi, A. G.; Shukla, O. P.; Singh, R. R. P.; Perkash, O.; Misra, V. D.; Gupta, J. P.; Sethi, P. K. Epidemic outbreak of neuro-ocular manifestations due to chronic BHC poisoning. J. Assoc. Physicians India 25:215–222; 1977.
33. Kuriyama, K.; Kimura, H. Distribution and possible functional roles of GABA in the retina, lower auditory pathway, and hypothalamus. In: Roberts, E.; Chase, T. N.; Tower, D. B., eds. GABA in nervous system function. New York: Raven Press; 1976:203–216.
34. Lawrence, L. J.; Casida, J. E. Stereospecific action of pyrethroid insecticides on the gamma-aminobutyric acid receptor-ionophore complex. Science 221:1399–1401; 1983.
35. Lawrence, L. J.; Casida, J. E. Interactions of lindane, toxaphene and cyclodienes with brain-specific *t*-butylbicyclophosphorothionate receptor. Life Sci. 35:171–178; 1984.
36. Leeb-Lundberg, F.; Napias, C.; Olsen, R. W. Dihydropicrotoxinin binding sites in mammalian brain: interaction with convulsant and depressant benzodiazepines. Brain Res. 216:399–408; 1981.
37. Liliedahl, R. L.; Ryan, C. F.; Peipho, R. W. Hypothermic response following administration of 2-amino-4-pentenoic acid (allylglycine). J. Pharm. Sci. 64:835–838; 1975.
38. Luzzi, S.; Spagnesi, S.; Franchi-Micheli, S.; Rosi, E.; Ciuffi, M.; Zilletti, L. Diazepam potentiates GABA-contraction in guinea-pig ileum. Arch. Int. Pharmacodyn. Ther. 279:29–39; 1986.
39. McLean, M. J.; Macdonald, R. L. Multiple actions of phenytoin on mouse spinal cord neurons in cell culture. J. Pharmacol. Exp. Ther. 227:779–789; 1983.
40. Martini, C.; Lucacchini, A.; Hrelia, S.; Rossi, C. A. Central- and peripheral-type benzodiazepine receptors. In: Biggio, G.; Costa, E., eds. GABAergic transmission and anxiety. New York: Raven Press; 1986:1–10.
41. Matsumura, F.; Ghiasuddin, S. M. Evidence for similarities between cyclodiene type insecticides and picrotoxinin in their action mechanisms. J. Environ. Sci. Health B18:1–14; 1983.
42. Maynert, E. W.; The metabolic fate of diphenylhydantoin in the dog, rat and man. J. Pharmacol. Exp. Ther. 129:275–284; 1960.
43. Morley, J. E.; Levine, A. S. The central control of appetite. Lancet Feb. 19:398–401; 1983.
44. Neter, J.; Wasserman, W.; Kutner, M. H. Applied linear statistical models. Regression, analysis of variance, and experimental designs. 2nd ed. Homewood, IL: Richard D. Irwin, Inc.; 1985.
45. Obata, T.; Yamamura, H. I. Inhibition of GABA-stimulated chloride influx by the convulsant benzodiazepines Ro 5-3663 and Ro 5-4864 into membrane vesicles from rat cerebral cortex. Eur. J. Pharmacol. 136:447–448; 1987.
46. Pellow, S.; File, S. E. Behavioural actions of Ro 5-4864: a peripheral-type benzodiazepine? Life Sci. 35:229–240; 1984.
47. Precht, H.; Christophersen, J.; Hensel, H.; Larcher, W. Temperature and life. New York: Springer-Verlag; 1973.

48. Rampe, D.; Triggle, D. J. Benzodiazepine interactions at neuronal and smooth muscle Ca^{2+} channels. Eur. J. Pharmacol. 134:189–197; 1987.
49. Randall, L. O.; Schallek, W.; Heise, G. A.; Keith, E. F.; Bagdon, R. E. The psychosedative properties of methaminodiazepoxide. J. Pharmacol. Exp. Ther. 129:163–171; 1960.
50. Rastogi, S. K.; Ticku, M. K. A possible role of a GABAergic mechanism in the convulsant action of RO5-4864. Pharmacol. Biochem. Behav. 23:285–288; 1985.
51. Rosenberg, H. C.; Chiu, T. H. Time course for development of benzodiazepine tolerance and physical dependence. Neurosci. Biobehav. Rev. 9:123–131; 1985.
52. Scherkl, R.; Scheuler, W.; Frey, H.-H. Anticonvulsant effect of clonazepam in the dog: development of tolerance and physical dependence. Arch. Int. Pharmacodyn. 278:249–260; 1985.
53. Schoemaker, H.; Bliss, M.; Yamamura, H. I. Specific high-affinity saturable binding of [^{3}H]Ro5-4864 to benzodiazepine binding sites in the rat cerebral cortex. Eur. J. Pharmacol. 71:173–175; 1981.
54. Snyder, S. H.; Verma, A.; Trifiletti, R. R. The peripheral-type benzodiazepine receptor: a protein of mitochondrial outer membranes utilizing porphyrins as endogenous ligands. FASEB J. 1:282–288; 1987.
55. Solomon, L. M.; Fahrner, L.; West, D. P. Gamma benzene hexachloride toxicity. A review. Arch. Dermatol. 113:353–357; 1977.
56. Swanson, K. L.; Woolley, D. E. Comparison of the neurotoxic effects of chlordecone and dieldrin in the rat. NeuroToxicology 3(2):81–102; 1982.
57. Taft, W. C.; DeLorenzo, R. J. Micromolar-affinity benzodiazepine receptors regulate voltage-sensitive calcium channels in nerve terminal preparations. Proc. Natl. Acad. Sci. USA 81:3118–3122; 1984.
58. Tanaka, C. Gamma-aminobutyric acid in peripheral tissues. Life Sci. 37:2221–2235; 1985.
59. Ticku, M. K.; Ramanjaneyulu, R. Ro5-4864 inhibits the binding of [^{35}S]t-butylbicyclophosphorothionate to rat brain membranes. Life Sci. 34:631–638; 1984.
60. Tilson, H. A.; Hong, J. S.; Mactutus, C. F. Effects of 5,5-diphenylhydantoin (phenytoin) on neurobehavioral toxicity of organochlorine insecticides and permethrin. J. Pharmacol. Exp. Ther. 233:285–289; 1985.
61. Tilson, H. A.; Shaw, S.; McLamb, R. L. The effects of lindane, DDT, and chlordecone on avoidance responding and seizure activity. Toxicol. Appl. Pharmacol. 88:57–65; 1987.
62. United Nations Publication, Sales Number E.87.IV.1 06000 ISBN 92-1-130120-3. Consolidated list of products whose consumption and/or sale have been banned, withdrawn, severely restricted or not approved by governments. second issue. 1987:126–127.
63. Wagner, S. R.; Greene, F. E. Dieldrin-induced alterations in biogenic amine content of rat brain. Toxicol. Appl. Pharmacol. 43:45–55; 1978.
64. Weissman, B. A.; Cott, J.; Jackson, J. A.; Bolger, G. T.; Weber, K. H.; Horst, W. D.; Paul, S. M.; Skolnick, P. "Peripheral-type" binding sites for benzodiazepines in brain: relationship to the convulsant actions of Ro 5-4864. J. Neurochem. 44:1494–1499; 1985.
65. Wong, P. T.-H.; Teo, W. L. Diazepam-sensitive specific binding of phenytoin in rat brain. J. Recept. Res. 6:297–309; 1986.
66. Woolley, D. E.; Drummer, H. L. Toxicokinetics of lindane, picrotoxin and Ro5-4864 compared. Soc. Neurosci. Abstr. 14(Part 2):1219; 1988.
67. Woolley, D. E.; Zimmer, L. Effects and proposed mechanisms of action of lindane in mammals: unsolved problems. In: Clark, J. M.; Matsumura, F., eds. Membrane receptors and enzymes as targets of insecticidal action. New York: Plenum Press; 1986:1–31.
68. Woolley, D.; Zimmer, L.; Dodge, D.; Swanson, K. Effects of lindane-type insecticides in mammals: unsolved problems. NeuroToxicology 6(2):165–192; 1985.
69. Woolley, D.; Zimmer, L.; Hasan, Z.; Swanson, K. Do some insecticides and heavy metals produce long-term potentiation in the limbic system? In: Narahashi, T., ed. Cellular and molecular neurotoxicology. New York: Raven Press; 1984:45–69.
70. Zbinden, G.; Randall, L. O. Pharmacology of benzodiazepines: laboratory and clinical correlations. Adv. Pharmacol. 5:213–291; 1967.