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Conditioned Taste Aversion Induced by Organophosphate Compounds in Rats[1]

PAUL L RONEY, JR ,* LUCIO G COSTA†[2]
AND SHELDON D MURPHY†

**Department of Environmental Science, University of Texas School of Public Health P.O Box 20186, Houston, TX 77025*
†Department of Environmental Health, SC-34, University of Washington, Seattle, WA 98195

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RONEY, P L , JR , L G COSTA AND S D MURPHY *Conditioned taste aversion induced by organophosphate compounds in rats* PHARMACOL BIOCHEM BEHAV 24(3) 737–742, 1986 —Three organophosphate compounds, dichlorvos, parathion and diisopropylfluorophosphate were tested as an unconditioned stimulus in the conditioned taste aversion (CTA) test All organophosphates caused a dose-dependent CTA in rats at doses which did not induce any other signs of toxicity Experiments with dichlorvos showed that the minimum dose which caused CTA did not alter the rats' sensitivity to pain or their behavior in either an open field or on an inclined plane Cholinesterase activity was inhibited in a dose-dependent manner in brain and plasma after administration of the organophosphates and CTA was correlated with the degree of plasma cholinesterase inhibition CTA appears to be a sensitive indicator of neurobehavioral effects of mild exposure to organophosphates which causes only 30–40% inhibition of plasma cholinesterase

Conditioned taste aversion Organophosphates Dichlorvos Parathion Diisopropylfluorophosphate Plasma cholinesterase Neurobehavioral testing

IT has been observed that under many conditions when intake of a novel food or liquid is paired with administration of a chemical, this often results in subsequent avoidance of that food or fluid Such conditioned aversions have been observed under natural conditions and can be produced experimentally by pairing a novel flavored substance with exposure to a toxic substance The conditioned stimulus is the novel taste, the unconditioned stimulus is the toxicant and the subsequent avoidance is known as conditioned taste aversion (CTA) [17] A wide variety of chemical and physical agents have been found to serve as unconditioned stimuli and cause CTA [16, 17, 37, 40] This behavioral paradigm has been also successfully used in the control of predation of eggs by crows and predation of sheep and turkeys by coyotes [14,32] Furthermore, CTA appears to play a role in the anorexia present in cancer patients [6,7] In recent years neurotoxicologists, concerned with the development of new methods for detecting and characterizing the behavioral and neurological consequences of exposure to toxic substances, have tested various chemicals for their ability to induce CTA

Compounds which cause CTA include metals such as lead [12], methylmercury [8], copper [9] and organotins [26], pesticides such as chlordimeform [27], methylbromide [28] or 2,4,5-trichlorophenoxyacetic acid [43], and various other toxic chemicals like acrylamide [1], toluene [29] and halogenated hydrocarbons [20] The results of these studies suggest that the CTA method may be a sensitive method for assessing the possible effects of chemicals Moreover, a few studies have shown that CTA is a more sensitive indicator than other behavioral paradigms [1,34]

A class of environmentally relevant compounds which have not been evaluated for their ability to induce CTA is the organophosphorus insecticides Their main mechanism of action is inhibition of acetylcholinesterase with a consequent stimulation of cholinergic receptors by acetylcholine which accumulates in the synaptic cleft Thus, differently from most of the other chemicals tested, whose precise mechanism of action has not been elucidated, measurement of cholinesterase activity represents a reliable marker for assessing exposure to organophosphates and for mechanistic studies

The aim of this study was therefore threefold first, to establish whether organophosphorus insecticides would cause CTA, second, to determine whether CTA is a more sensitive index of neurotoxicity than other behavioral tests, third, to correlate the behavioral effects observed in the CTA test, with a biochemical marker of organophosphate exposure Three organophosphates were used in this study dichlorvos and parathion, which are among the most widely used insecticides in the home and in agriculture, respectively, and diisopropylfluorophosphate, a prototype of irreversible cholinesterase inhibitors

METHOD

Male Sprague-Dawley rats (Timco, Houston, TX) weigh-

[1]Preliminary communications of this study have been presented and were published in abstract form [10,41]

[2]Requests for reprints should be addressed to Lucio G Costa, Ph D , Department of Environmental Health, SC-34, University of Washington, Seattle, WA 98195

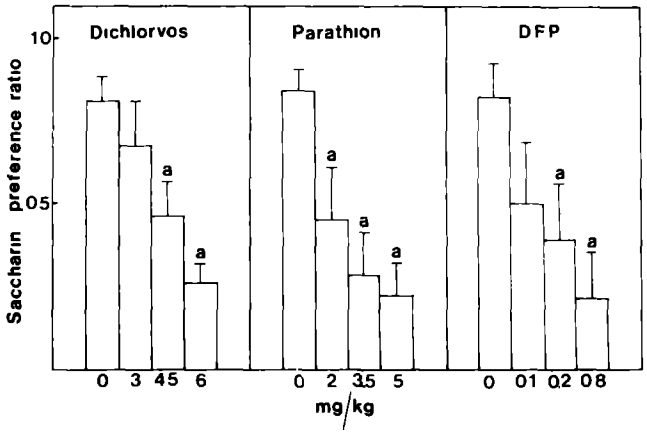

FIG 1 Saccharin preference ratios for rats treated with dichlorvos, parathion and DFP Control animals were injected with the respective vehicles Each bar represents the mean (±SEM) of 7–8 rats [a]Significantly different from control, $p<0.05$

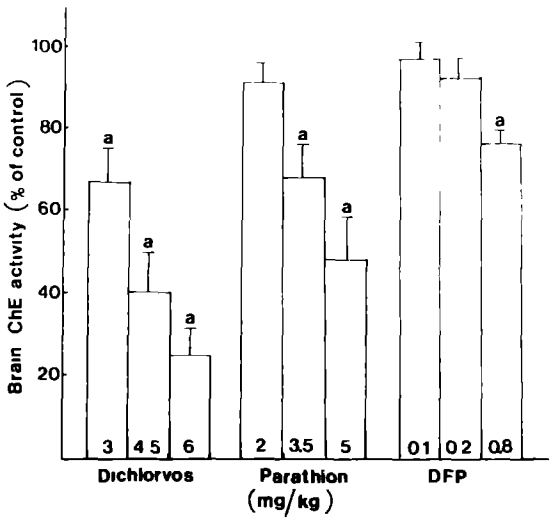

FIG 2 Inhibition of brain cholinesterase activity by organophosphates in rats Each bar represents the mean (±SEM) of five rats Control cholinesterase activity was 9 28±0 47 μmol ATC/min/g of tissue [a]Significantly different from control, $p<0.05$

ing 150–200 g were individually housed in wire bottom cages in air conditioned rooms at a constant temperature (25°C) and light schedule (12 hr dark) and had food (Purina Formulab Chow 5008) and water available ad lib At least seven days after shipment rats were placed on a water restricted regimen, tap water was available 30 min/day between 08 30 and 09 00 Water was presented in modified 60 ml plastic syringes attached to the front of the cage by a spring clip The volume of water consumed in each session was read directly off the syringe which was marked in one ml divisions When water intake had stabilized (usually after 5–7 days) rats were presented with a 0 15% (w/v) solution of sodium saccharin (Aldrich Chemical Co , Milwaukee, WI) Fifteen minutes after this drinking session, rats were injected IP with an organophosphate or its vehicle Parathion (0,0-diethyl-0-(4 nitrophenyl) phosphorothioate, analytical grade, supplied by American Cyanamid Co , Princeton, NJ) and diisopropylfluorophosphate (DFP, Sigma Chemical Co , St Louis, MO) were dissolved in corn oil Dichlorvos (0-(2,2-dichloroethenyl)-0-0-dimethylphosphate, analytical grade, 97%, donated by Shell Chemical Co , Houston TX) was dissolved in physiological saline solution. Lithium chloride (Aldrich), dissolved in distilled water and administered IP at the dose of 3 mEq/kg, served as a positive control All compounds were administered in the volume of 1 ml/kg On the next day rats were given access for 30 minutes to tap water Two days after the conditioning session, rats were presented with the choice between tap water and the saccharin solution This two-bottle procedure has been shown to be more sensitive than the single-bottle test [19] The total fluid consumed was defined as the volume of tap water consumed plus the volume of saccharin solution consumed The saccharin preference ratio (SPR) was calculated as

$$\frac{\text{Volume of saccharin solution consumed}}{\text{Total fluid consumption}}$$

In control animals SPR ranged between 0 80 and 0 85, while a substance causing aversion gave lower SPR values

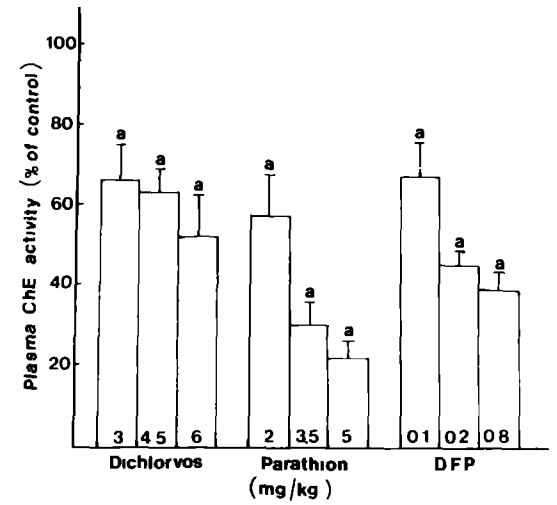

FIG 3 Inhibition of plasma cholinesterase activity by organophosphates in rats Each bar represents the mean (±SEM) of five rats Control cholinesterase activity was 0 51±0 07 μmol ATC/min/ml [a]Significantly different from control, $p<0.05$

Cholinesterase (ChE) activity was determined in whole brain and plasma according to the method of Ellman *et al* [15], as modified by Benke *et al* [4] Rats were anesthetized with ether and blood was collected by cardiac puncture in a heparinized syringe, transferred to heparinized tubes, centrifuged and kept on ice until the assay ChE activities were measured 30 min after dichlorvos, 60 min after DFP, and 120 min after parathion injection These time points were chosen as the time of maximal ChE inhibition, as determined in preliminary experiments Brains were homogenized with a Brinkman polytron in 10 vol of 0 1 M sodium phosphate buffer (pH 8 0) and further diluted (1 13) with the same buf-

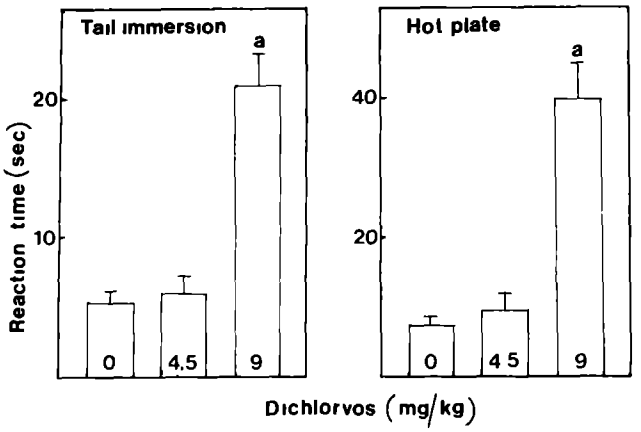

FIG 4 Antinociceptive effect of dichlorvos in rats Reaction times were measured in the tail immersion and in the hot-plate test, 30 min after injection, as described in the method section Each bar represents the mean (±SEM) of five rats *Significantly different from control, $p<0$ 05

TABLE 1

EFFECT OF DICHLORVOS ON OPEN FIELD AND INCLINED PLANE BEHAVIORS

Dichlorvos (mg/kg)	Open Field		Inclined Plane (°)
	Square Crossing	Rearings	
0	18 ± 5	4 ± 2	46 ± 4
4 5	11 ± 5	4 ± 1	47 ± 3
9 0	0*	0*	36 ± 2*

Tests were conducted as described in the Method section, 30 min after administration of dichlorvos or saline Results are the mean (±SEM) of five rats

*Significantly different from control, $p<0$ 05

fer One hundred μl of diluted brain homogenate or 20 μl of plasma (previously diluted 1 2 with buffer) were added to phosphate buffer containing 50 μl of 1 0 mM 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB) in a final volume of 5 ml The reaction was started by the addition of 5 μl of acetylthiocholine (ATC) and the initial absorbance at 412 nm as well as the absorbance after 30 min incubation at 27°C were recorded The change in absorbance is due to the formation of 5-thio-2-nitrobenzoic acid from DTNB and thiocholine, the hydrolytic product of ATC To be consistent with the CTA experiments, rats used for ChE measurement were also on a water restricted regimen

Antinociception was measured by the tail immersion technique and the hot plate test, as previously described [11] In the tail immersion test rats were placed in a plastic restrainer and their tail immersed, to a constant depth, in a water bath maintained at 51 0±0 25°C by a thermostatically controlled heating block The time between immersion and the animal withdrawing its tail was recorded, the nociceptive endpoint being a violent jerk of the tail An arbitrary 30 sec cut-off time was imposed as maximal antinociceptive response In the hot plate test, the plate (IITC Inc , Landing, NJ) was maintained at 55 0±0 1°C and was surrounded by a Plexiglas wall to prevent escape The endpoint was the first lick of a hindpaw and a 60 sec cut-off time was arbitrarily selected as a maximal antinociceptive response

For the open field test a 120 cm square box with 41 cm high black walls was used The floor of the box consisted of alternated black and white 24 cm squares The room was kept dark and the field was illuminated by a red light A trial consisted in placing a rat in the center square and covering it under a confining box for 15 sec The box was then removed allowing the rat free movement for two minutes Square crossing was defined as the rat entering another square with all four paws, rearing was defined as the rat raising its forepaws simultaneously off the floor [45] For each animal the number of squares crossed and the number of rearings made was recorded

For the inclined plane test, a measure of the rat's motor coordination [2], a wooden board covered by a washable plastic sheet was used The initial angle was zero degrees The angle of the board was raised by a torque motor attached through a pulley system to the front of the plane The rat was placed on the plane facing uphill and the plane was raised at a constant rate (15 degrees/sec) The angle of the plane when the rat began to slip was recorded

In all behavioral experiments animals were water deprived for at least seven days as in the CTA studies All tests were done 30 min after the administration of dichlorvos on a blind basis, i e , the investigator was not aware of the treatment the animals had received Data were analyzed for statistical significance by analysis of variance When a significant F value was found, data were further analyzed by the Newman-Keuls test [42]

RESULTS

Five to seven days after the beginning of the restricted drinking regimen, water intake had usually stabilized at 16–18 ml in the 30 min period The only rat that did not reach a constant baseline of water consumption was not used in the studies On the conditioning day rats had free access for 30 min to a 0 15% saccharin solution instead of water Fluid consumption in this testing session did not differ among groups and ranged from 15 5±1 0 ml to 18 5±1 5 ml (±SEM) Two days later animals were presented with the choice of the saccharin solution or water Total fluid consumption (saccharin plus water) did not differ among groups (not shown) Saccharin preference ratios for control (vehicle treated) rats were 0 80 to 0 85, however, animals injected with the organophosphates 15 min after the first conditioning session, drank significantly less saccharin than control rats Figure 1 shows that dichlorvos (4 5 and 6 0 mg/kg), parathion (2 0, 3 5 and 5 0 mg/kg) and DFP (0 2 and 0 8 mg/kg) significantly lowered the rats' saccharin preference ratio as compared to vehicle treated controls Lithium chloride was chosen as a positive control and at a dose of 3 mEq/kg induced an SPR of 0 19±0 09 ($n=5$, $p<0$ 01) At the dosages employed, no signs of organophosphate poisoning were observed, with the exception of highest dose of parathion (5 0 mg/kg) which caused slight diarrhea and salivation

Cholinesterase activity was measured in brain and plasma of rats after administration of the organophosphates (Fig 2) Brain cholinesterase activity was significantly inhibited by all doses of dichlorvos and by the two highest doses of

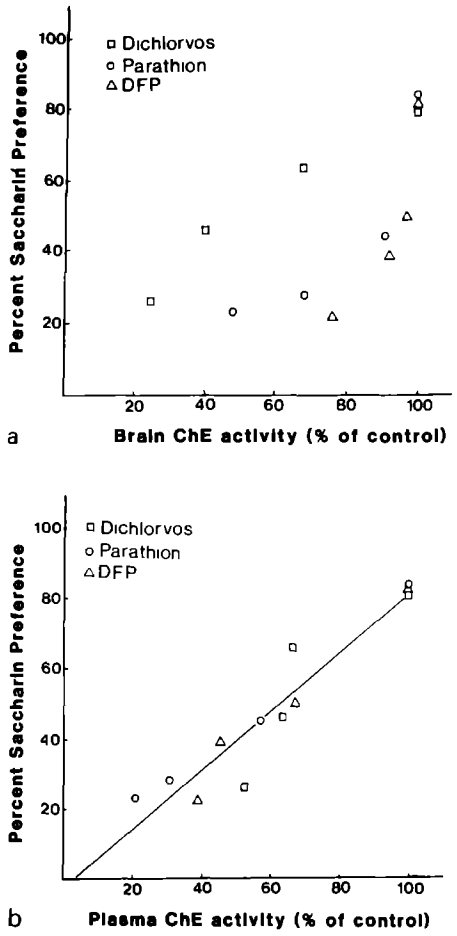

FIG 5 Correlation between percent saccharin preference (SPR × 100) and inhibition of cholinesterase activity in brain (5a) or plasma (5b) The correlation coefficients are 0 617 and 0 947, respectively

parathion and DFP Plasma cholinesterase activity was significantly lowered by all organophosphates at all doses (Fig 3)

In order to determine the sensitivity of CTA, further studies were conducted with dichlorvos The lowest dose of dichlorvos capable of causing CTA (4 5 mg/kg) was administered to rats and tested for its ability to induce antinociception and to impair both motor activity in an open field and motor coordination on an inclined plane A higher dose of dichlorvos (9 0 mg/kg) was used as a positive control Figure 4 shows that at the dose of 4 5 mg/kg, dichlorvos did not alter pain sensitivity in both a tail-immersion and a hot-plate test Moreover, this dose of dichlorvos had no effect on rats' behavior in the open field or on the inclined plane (Table 1) At the higher dose tested (9 0 mg/kg) dichlorvos exerted a significant effect in all three tests (Fig 4 and Table 1)

DISCUSSION

The major aims of this study were (1) to determine whether three organophosphates with different characteristics would cause CTA in rats, (2) to determine whether CTA is a more sensitive paradigm for assessing neurobehavioral effects than other behavioral tests, (3) to determine the extent of central and peripheral ChE inhibition caused by the organophosphates The dimethyl organophosphate dichlorvos is a direct inhibitor of ChE which causes a rapid onset of toxicity but has a relatively short duration of action DFP is also a direct inhibitor of ChE, however it can be considered an almost irreversible inhibitor Parathion, on the other hand, requires metabolic transformation to an active ChE inhibitor and has, therefore, a slower onset of toxicity Despite these differences in the onset and duration of ChE inhibition and of toxicity, all three organophosphates caused CTA at doses which produced no other obvious sign of toxicity These results are consistant with previous studies with the carbamate physostigmine [34,39] and the organophosphates soman [39], sarin [24] and tabun [23] In one study it was found that the carbamate carbaryl caused CTA at high doses and the carbamate propoxur was unable to cause CTA [25] The reasons for the lack of effect of propoxur are not evident but might be related to the time separating dosing and choice testing However, with this exception, ChE inhibitors, of both the carbamate and organophosphate classes, are all able to cause CTA

An issue to be considered in CTA studies with neurotoxic compounds is the sensitivity of this procedure In our study the minimum effective doses of organophosphates able to induce CTA were 25 0, 35 1 and 5 4% of their reported LD_{50} values [3, 21, 44], for dichlorvos, parathion and DFP, respectively Thus, CTA was elicited at doses well below the acute lethal doses We investigated whether one of these organophosphates, dichlorvos, would cause any effect in other behavioral paradigms when administered at the minimal dose causing CTA Dichlorvos, however, at the dose of 4 5 mg/kg had no effect in the tail-immersion and the hot-plate tests, nor altered the rats' behavior and performance in the open-field or inclined-plane tests A lack of any effect in various behavioral tests at doses which caused CTA, was also shown for physostigime [34,39], acrylamide [1] and more recently, for soman, sarin and tabun [23, 24, 39] Thus, CTA appears to be a rather sensitive method to detect effects of toxic agents

Organophosphates are potent ChE inhibitors and measurement of this enzyme's activity represents a good marker of the degree of exposure We attempted to correlate the effects of organophosphates on saccharin preference ratio with their degree of inhibition of brain and plasma cholinesterase (Fig 5) With the possible exception of dichlorvos, there was no clear correlation between the whole brain ChE activity and SPR For example, CTA was induced by a dose of parathion (2 0 mg/kg) or DFP (0 2 mg/kg) which did not significantly inhibit brain ChE, even in the case of dichlorvos, a 3 0 mg/kg dose inhibited brain ChE by about 30% ($p<0$ 05) but did not cause any CTA This does not imply that inhibition of brain ChE is not important in organophosphate induced CTA, it is possible that only a specific area of the brain is involved in CTA and a certain degree of ChE inhibition in this area might be sufficient for CTA to develop

On the other hand, a good correlation ($r=0$ 947, $p<0$ 05) was observed between SPR and inhibition of plasma ChE (Fig 5) This observation is particularly interesting because it suggests that a peripheral site might mediate organophosphate-induced CTA This site could be outside the central nervous system or in an area of the brain which is not protected by the blood-brain barrier Several studies suggest a role of the area postrema in the induction of CTA by different agents Surgical lesion of the area postrema has been found to attenuate or abolish CTA induced by gamma radiation [33], lithium chloride [38] copper sulfate [9] and

scopolamine methylnitrate [5] Cholinergic muscarinic receptors have been recently identified in the area postrema [35] and could represent the target for organophosphate-induced CTA. However, CTA induced by certain drugs such as amphetamine [5] does not seem to require an intact area postrema Therefore, a possible role for the area postrema in organophosphate-induced CTA will be only determined from experiments in lesioned animals

Another issue to be addressed is whether a specific neurotransmitter receptor system is involved in organophosphate-induced CTA Acetylcholine, which accumulates at cholinergic synapses following ChE inhibition, will stimulate cholinergic receptors of the muscarinic and nicotinic type Both carbachol and nicotine have been previously shown to cause CTA [22,30] In preliminary experiments we attempted to selectively block either receptor with the nicotinic antagonist mecamylamine and the muscarinic antagonist atropine However, despite the low doses of antagonists utilized (2 mg/kg), in certain experiments they caused CTA when administered alone Indeed, CTA induced by atropine, scopolamine and mecamylamine had been previously reported [5, 13, 36] Further studies are needed to investigate this mechanistic aspect of organophosphate-induced CTA

Finally, it is of interest to consider the relationship between the degree of inhibition of plasma ChE by organophosphates and evidence for other adverse effects It is known that a lowered plasma ChE activity is often not associated with clinical poisoning Namba *et al* [31] indicated that inhibition of up to 50% of plasma ChE in humans does not result in any sign of toxicity, whereas only mild poisoning is present when plasma ChE is depressed as much as 80% A 30–40% decrease in plasma ChE activity is usually considered sufficient for removal of workers from possible exposure, even in the absence of any clinical sign of poisoning Our study, which showed that CTA could be induced by doses of organophosphates causing 37–45% inhibition of plasma ChE (Fig 3), may represent an example of a subtle behavioral effect associated with such low ChE inhibition This indirectly supports the use of plasma ChE measurements for assessing potential health hazards related to exposure to organophosphates

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REFERENCES

1 Anderson, C E , H A Tilson and C L Mitchell Conditioned taste aversion following acutely administered acrylamide *Neurobehav Toxicol Teratol* **4:** 497–499, 1982
2 Arvola, A , L Sammalisto and M Wallgreen A test for level of intoxication in the rat *Q J Stud Alcohol* **19:** 563–572, 1978
3 Benke, G M and S D Murphy The influence of age on the toxicity and metabolism of methylparathion and parathion in male and female rats *Toxicol Appl Pharmacol* **31.** 254–269, 1975
4 Benke, G M , K L Cheever, R E Mirer and S D Murphy Comparative toxicity, anticholinesterase action and metabolism of methylparathion and parathion in sunfish and mice *Toxicol Appl Pharmacol* **28:** 97–109, 1974
5 Berger, B D , C D Wise and L Stein Area postrema damage and bait shyness *J Comp Physiol Psychol* **82.** 475–479, 1973
6 Bernstein, I L Learned taste aversions in children receiving chemotherapy *Science* **206.** 1302–1303, 1978
7 Bernstein, I L and R A Sigmundi Tumor anorexia a learned food aversion? *Science* **209·** 416–418, 1980
8 Braun, J J and D R Snyder Taste aversion and acute methylmercury poisoning in rats *Bull Psychon Soc* **1·** 419–420, 1973
9 Coil, J D and R Norgren Taste aversions conditioned with intravenous copper sulfate attenuation by ablation of the area postrema *Brain Res* **212·** 425–433, 1981
10 Costa, L G , P Roney and S D Murphy Conditioned taste aversion induced by the organophosphorus insecticide dichlorvos in rats *Soc Neurosci Abstr* **9·** 422, 1983
11 Costa, L G and S. D Murphy Antinociceptive effect of the GABA mimetic 4,5,6,7-tetrahydroisoxazolo [5,4-C] pyridin-3-ol(THIP) chronic treatment increases alpha$_2$-adrenoceptors in the mouse brain *J Pharmacol Exp Ther* **229:** 386–390, 1984
12 Dantzer, R Conditioned taste aversion as an index of lead toxicity *Pharmacol Biochem Behav* **13** 133–135, 1980
13 Deutsch, R Effects of atropine on conditioned taste aversion *Pharmacol Biochem Behav* **8.** 685–694, 1978
14 Ellins, S R and S M Catalano Field application of the conditioned taste aversion paradigm to the control of coyote predation on sheep and turkeys *Behav Neurol Biol* **29** 532–536, 1980
15 Ellman, G R , K D Courtney, V Andres and R M Featherstone A new and rapid colorimetric determination of acetylcholinesterase activity *Biochem Pharmacol* **7:** 88–95, 1961
16 Garcia, J , D J Kimeldorf and R A Koelling Conditioned aversion to saccharin resulting from exposure to gamma radiation *Science* **122.** 157–158, 1955
17 Garcia, J , W G Hanlins and K W Rusiniak Behavioral regulation of milieu interne in man and rat *Science* **185:** 824–831, 1974
18 Gaston, K E Brain mechanisms of conditioned taste aversion learning a review of the literature *Physiol Psychol* **6·** 340–353, 1978
19 Grote, G W and R T Brown Conditioned taste aversions two stimulus tests are more sensitive than one stimulus test *Behav Res Meth Instru* **3·** 311–313, 1971
20 Kallman, M J , R R Lynch and M R Landauer Taste aversions to several halogenated hydrocarbons *Neurobehav Toxicol Teratol* **5** 23–27, 1983
21 Kenley, R A , R A Howd and E T Uyeno Effect of PAM, proPAM and DFP on behavior, thermoregulation and brain AChE in rats *Pharmacol Biochem Behav* **17·** 1001–1008, 1982
22 Kumar, R , J A Pratt and I P Stolerman Characteristics of conditioned taste aversion produced by nicotine in rats *Br J Pharmacol* **79:** 245–253, 1983
23 Landauer, M R and J A Romano The acute behavioral toxicity of Tabun in rats *Soc Neurosci Abstr* **10·** 1070, 1984
24 Landauer, M R , J A Romano, R M Alvarez and J M McDonough Behavioral effects of Sarin in rats *Soc Neurosci Abstr* **9·** 135, 1983
25 MacPhail, R C Studies on flavor aversions induced by N-substituted carbamates and by alkyltin compounds *Toxicologist* **1.** 44, 1981
26 MacPhail, R C Studies on the flavor aversions induced by trialkyltin compounds *Neurobehav Toxicol Teratol* **4:** 225–230, 1982
27 MacPhail, R C and J D Leander Flavor aversions induced by chlordimeform *Neurobehav Toxicol* **2:** 363–365, 1980

28 Miyagawa, M Conditioned taste aversion induced by inhalation exposure to methyl bromide in rats *Toxicol Lett* **10** 411–416 1982
29 Miyagawa, M T Honma, M Sato and H Hasegawa Conditioned taste aversion induced by toluene administration in rats *Neurobehav Toxicol Teratol* **6** 33–37, 1984
30 Myers, R H and J M Decastro Learned aversion to intracerebral carbachol *Physiol Behav* **19**· 467–472, 1977
31 Namba, T , C T Nolte, J Jackrel and D Grob Poisoning due to organophosphorus insecticides *Am J Med* **50**· 475–492, 1971
32 Nicolaus L K , J F Cassel, R B Carlson and C R Gustavson Taste-aversion conditioning of crows to control predation on eggs *Science* **220** 212–214, 1983
33 Ossenkopp, K P Taste aversion conditioned with gamma radiation attenuation by area postrema lesions in rats *Behav Brain Res* **7.** 297–305, 1983
34 Parker L A , S Hutchison and A L Riley Conditioned flavor aversions a toxicity test of the anticholinesterase agent physostigime *Neurobehav Toxicol Teratol* **4** 93–98, 1982
35 Pedigo, N W and K R Brizzee Muscarinic cholinergic receptors in brainstem areas regulating emesis *Brain Res Bull* **14** 169–178 1985
36 Preston K L and C R Schuster Conditioned gustatory avoidance induced by three cholinergic agents *Pharmacol Biochem Behav* **15** 827–828, 1981
37 Riley, A L and C M Clarke Conditioned taste aversion a bibliography In *Learning Mechanisms in Food Selection*, edited by L M Barker, M R Best and M Domjan Waco, TX Baylor University Press, 1977, pp 593–610
38 Ritter, S , J J McGlone and K W Kelley Absence of lithium-induced taste aversion after area postrema lesion *Brain Res* **201.** 501–506, 1980
39 Romano, J A , J M King and D M Penetar A comparison of physostigime and soman on taste aversion and antinociception *J Am Coll Toxicol* **2** 248, 1983
40 Rondeau, D B , F B Jolicoeur A D Merkel and M J Wayner Drugs and taste aversion *Neurosci Biobehav Rev* 5 279–294, 1981
41 Roney, P L G Costa and S D Murphy Conditioned taste aversion induced by organophosphate compounds in rats *Toxicologist* **4** 19 1984
42 Snedecor, G W and W G Cochran *Statistical Methods* Ames, IA Iowa State University Press, 1974
43 Sjoden P O T Archer and N Carter Conditioned taste aversion induced by 2,4,5-trichlorophenoxyacetic acid dose-response and preexposure effects *Physiol Psychol* 7 93–96, 1979
44 Vrbovsky L , R R Sellecky and S Roxwall Toxikogesche und pharmakologische studien der phosphorsaureester insecticidien *Naunyn Schmiedebergs Arch Pharmacol* **236** 202–204, 1959
45 Walsh, R N and R A Cummins The open field test a critical review *Psychol Bull* **83** 482–504 1976