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Spontaneous and Evoked EEG Changes in Perinatal Rats Following in Utero Exposure to Baygon: A Preliminary Investigation

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Propoxur (2-Isopropoxyphenyl-N-Methylcarbamate) or Baygon, an insecticidal carbamate, is a reversible cholinesterase inhibitor (MATSUMURA 1975) that has an acute, oral LD_{50} of 125 mg/Kg in rats. Like other carbamate compounds, Baygon exhibits parasympathomimetic activity resulting from regional accumulations of acetylcholine, has a relatively short biological half life, but unlike many of the organophosphates, generally does not cause delayed peripheral neuropathies.

Baygon is found in many commercially available household insecticides. Since home and garden use of this insecticide is quite extensive, there exists a potential risk to the general population and more specifically to pregnant females and their conceptuses and young. There have been no studies related to possible central nervous system (CNS) effects on the perinate. Much of the Baygon CNS toxicity data acquired has been obtained from older animals (DESI et al. 1974).

This investigation focused upon potential perinatal neurological alterations that may result from continuous maternal exposure to low levels of Baygon. Both simple reflex development and EEG changes were investigated.

MATERIALS AND METHODS

Time pregnant, random bred albino rats (CD strain) were used (1). Twenty-five rats were divided into a control (8 rats) and a treated group (17 rats). Baygon was administered in the diet from day 6 of gestation through day 15 postpartum. Treated rats received ground laboratory chow mixed with technical grade (97%

[1]Time pregnant females were obtained from the Charles River Breeding Laboratories, Inc.

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pure) Baygon (2), using corn oil as the vehicle. The diet contained 1000 ppm Baygon. Control animals received only the laboratory chow and corn oil mixture. Food and water were given ad libitum. Treated animals were returned to the control diet at 15 days postpartum. Rats were maintained in a temperature (20°C) and photoperiod (12 hour light-dark cycle) controlled room.

Immediately after birth the dams were weighed and the maternal weight gain or loss determined as the difference between the day 3 and the parturition weights. Litters were normalized to 4 males and 4 females. Throughout lactation, litters were examined for body weight differences, percent survival, and reflex development. The reflexes examined were righting and startle. The righting reflex was defined as a swift torsion of the body after quickly releasing a neonate being held upside down approximately 24 cm over a thick layer of animal bedding. The neonate testing for this reflex began on day 13 after birth. The startle reflex test consisted of grasping the neonate lightly behind the shoulders, allowing it to hang limp, and generating a sudden noise. A positive response was recorded when a full body jerk was noted immediately following the sound. Testing for this reflex began on day 9 after birth.

On day 24 postpartum, electroencephalograms (EEG's) were obtained. Rats were lightly anesthetized by i.p. injection of pentobarbital sodium (Nembutal). Platinum needle electrodes were placed s.c. in the midfrontal (MF), right (RO), and left (LO) lateral occipitoparietal area of the scalp. Three channels of EEG were recorded: RO X MF, LO X MF, RO X LO. Visual evoked potentials (VER's) also were obtained using a PS-2 photostimulator (3). The flash unit was maintained at a distance of 30.5 cm from the head. Photic stimuli were delivered at regular intervals (0.3 Hz). Three channels of electrophysiological data were obtained as above. All bioelectric potential data were initially recorded on analog tape and subsequently transferred to a PDP 8I computer (4) for analysis.

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[2]Baygon was supplied by Chemagro, Inc., a division of Baychem Corp., Kansas City, Mo.

[3]Grass Medical Instruments, Quincy, Ma.

[4]Digital Equipment Corporation, Maynard, Ma.

Electroencephalograms were initially screened using time interval analysis followed by a determination of the power spectral characteristics. Visual evoked responses were classified according to the nomenclature cited by DONCHIN and LINDSLEY (1969) and statistically evaluated using Stepwise Discriminant Analysis (DIXON, 1975). Statistical comparisons were made using Analysis of Variance, Student's t test and Chi-square. Differences between groups were regarded as significant if P values were less than or equal to 0.05.

RESULTS

Maternal Weight Gain

Animals on Baygon-containing diet gained significantly less ($P < 0.05$) than controls (93 g as compared to 102 g).

Neonatal Growth and Survival

On days 2, 9 and 15 postpartum, weights were obtained. Litters of Baygon-exposed dams consistently weighed less than did controls. At day 15 their weight was significantly ($P < 0.01$) reduced as treated animals weighed an average of 31.6 g as compared to 36.1 g for controls. There was no significant treatment-related reduction in percent survival during the course of the experiment.

Reflex Development

Pups born to Baygon-treated dams showed a significant ($P < 0.01$) delay in the development of the startle reflex. Treated litters averaged 4.1% exhibiting this reflex on day 11 as compared to 26.1% in controls. By day 12 the percentages had increased to 27.4 and 60.1 respectively. Contrary to the startle reflex, the righting reflex showed no significant differences in onset or development.

Electroencephalograms

Time interval analysis of the electroencephalogram indicated selected differences existed (Table I). When compared to controls, the abundance of waveforms in the delta and alpha frequency class in both sexes were significantly altered as a consequence of treatment. Delta waves in the treated groups were significantly decreased whereas alpha waves were significantly increased. No significant effects were noted in theta or beta waves.

TABLE I

The Effects of Baygon on the EEG of the Developing Rat[a]
LO X MF Derivation

GROUP	SEX	FREQUENCY CLASS	% WAVE ABUND.	% SPECTRAL POWER	ABS. POWER (μV^2)	TOTAL POWER (μV^2)
Control	Male	δ	31.1 ± 8.0	50.5 ± 4.7	84 ± 19	162 ± 20
		θ	34.2 ± 7.9	27.1 ± 2.0	43 ± 2	
		α	14.9 ± 2.7	12.1 ± 1.1	19 ± 1	
		β	19.6 ± 3.5	9.7 ± 1.5	15 ± 2	
1000 ppm	Male	δ	17.4 ± 2.2[b]	37.3 ± 3.3[b]	42 ± 6[b]	114 ± 2[b]
		θ	40.2 ± 3.4	32.8 ± 2.5	38 ± 6	
		α	21.4 ± 1.8[b]	16.8 ± 0.6[b]	18 ± 1	
		β	21.0 ± 5.4	12.2 ± 1.1	14 ± 2	
Control	Female	δ	39.5 ± 4.4	55.9 ± 2.0	100 ± 17	167 ± 26
		θ	30.7 ± 9.6	25.4 ± 4.3	38 ± 7	
		α	14.4 ± 2.0	10.7 ± 1.7	16 ± 1	
		β	15.3 ± 2.3	7.6 ± 1.2	12 ± 2	
1000 ppm	Female	δ	27.8 ± 3.8[b]	57.4 ± 2.5	74 ± 6[b]	126 ± 10[b]
		θ	36.7 ± 3.4	23.3 ± 1.0	28 ± 4	
		α	17.4 ± 1.9[b]	11.1 ± 1.0	14 ± 2	
		β	18.1 ± 2.4	7.6 ± 0.6	10 ± 2	

[a]Mean ± SEM

[b]Significant treatment effect, ($P < 0.05$)

Analysis based on % spectral power for the treated male LO X MF derivation reflected significantly lower percent power in the delta wave region along with a significantly higher percentage of alpha wave power. No differences, however, were noted in females. When spectral power was placed on an absolute basis, both male and female rats exhibited significantly less total power than controls. Separating total power into the classical frequency bands, no differences in absolute power were noted for the theta, alpha and beta bands. However, in the delta frequency class treated males and females had significantly less spectral power than controls.

The results for the RO X LO derivation are shown in Table II. No significant treatment-related differences in % wave abundance, % spectral power, or total EEG power were found for either sex.

When the spectral power was divided into component bands, both treated male and female rats reflected somewhat higher power levels than controls. This was particularly true in the alpha and theta bands where the difference was significant ($P < 0.05$).

Visual Evoked Response

The average VER for control and treated male rats is shown in Figure 1. Compared to controls, Baygon treatment resulted in a reduction in wave amplitude. The first negative peak (N_{3b}) appeared slightly later in treated animals. This particular effect was not as pronounced for both the first and second positive peaks (P_{3a} and P_{4a}). The quiescent phase (> 240 msec) was not altered.

Trends in the VER for the female rats appeared similar, but more pronounced than those seen in the males (Figure 2).

Statistical analysis (DIXON, 1975) of the VER for the female rats suggested a very poor discrimination between controls and treated groups in that only one time interval was selected. This suggested therefore, that there was no real basis for establishing a significant difference between control and treated female rats (see DONCHIN and LINDSLEY, 1969). Conversely, four variables were selected for male rats at time points ranging from the first to the 22nd variable (0 to 200 msec). A discriminant was thus established for the male rats indicating the possibility of a significant difference between treated and control animals.

TABLE II

The Effects of Baygon on the EEG of the Developing Rat[a]
RO X LO Derivation

GROUP	SEX	FREQUENCY CLASS	% WAVE ABUND.	% SPECTRAL POWER	ABS. POWER (μV^2)	TOTAL POWER (μV^2)
Control	Male	δ	7.6 ± 2.4	39.1 ± 5.3	49 ± 10	121 ± 10
		θ	41.0 ± 3.9	22.9 ± 3.8	28 ± 4	
		α	23.7 ± 6.2	17.4 ± 1.5	21 ± 1	
		β	32.0 ± 4.1	18.9 ± 3.1	23 ± 3	
1000 ppm	Male	δ	37.0 ± 10.6	30.9 ± 1.5	46 ± 13[b]	152 ± 47
		θ	26.9 ± 5.0	24.9 ± 2.4	38 ± 12[b]	
		α	16.6 ± 4.0	21.5 ± 1.9	24 ± 10	
		β	19.5 ± 1.8	20.6 ± 1.5	32 ± 11	
Control	Female	δ	70.2 ± 2.6	45.7 ± 1.0	30 ± 3	66 ± 8
		θ	10.4 ± 1.7	21.2 ± 3.3	15 ± 4	
		α	6.4 ± 1.0	14.7 ± 2.2	10 ± 2	
		β	13.0 ± 3.1	17.0 ± 2.1	11 ± 0.2	
1000 ppm	Female	δ	30.0 ± 2.8	46.2 ± 5.6	69 ± 15[b]	145 ± 18
		θ	30.7 ± 0.8	23.8 ± 3.2	34 ± 4[b]	
		α	15.5 ± 1.9	14.5 ± 1.2	21 ± 2[b]	
		β	23.8 ± 1.5	14.0 ± 1.2	20 ± 1	

[a]Mean ± SEM

[b]Significant treatment effect, ($P < 0.05$)

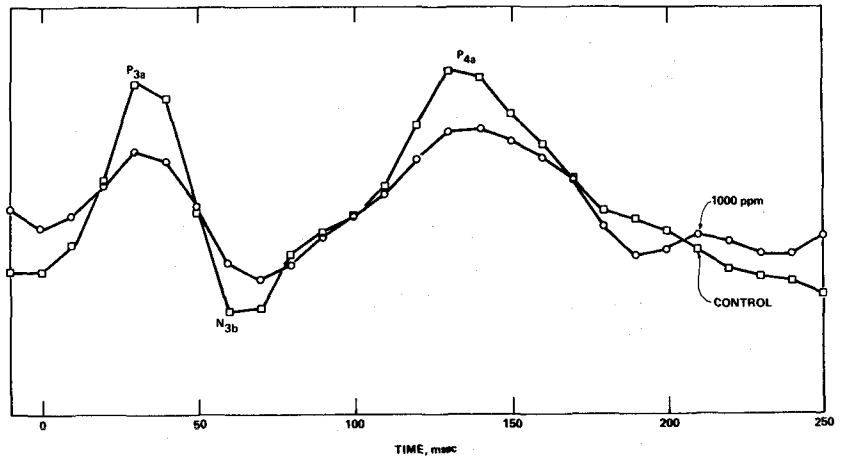

Figure 1. Visual evoked responses obtained from control and treated male perinates.

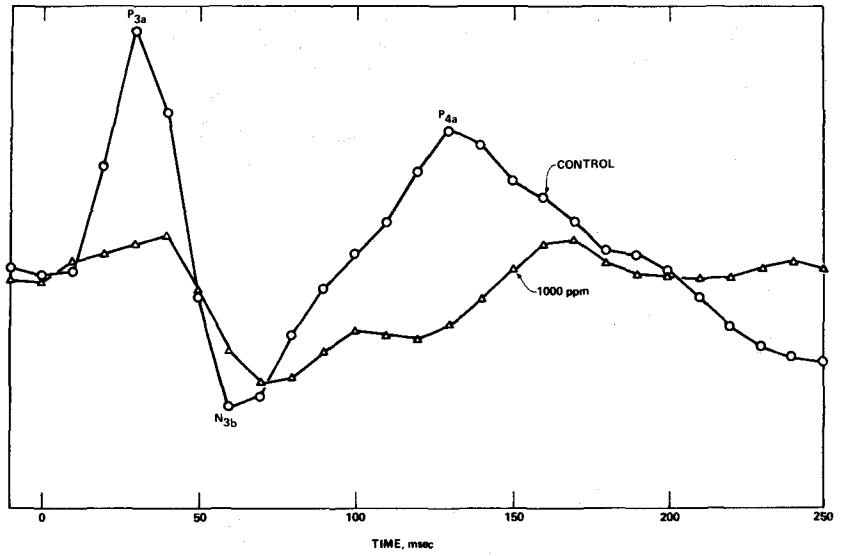

Figure 2. Visual evoked responses obtained from control and treated female perinates.

DISCUSSION

Perinatal exposure to high levels of Baygon resulted in significant reductions in maternal weight gain and neonatal growth, and a significant delay in the ontogeny of the startle reflex. A delay in startle and righting reflex development after perinatal exposure to parathion, also a parasympathomimetic pesticide, was noted by TALENS and WOOLEY (1968).

Based upon their observations they concluded that a possible cause for this effect was inadequate maternal care. Frequent observations of the dams in the experiment currently reported would suggest that this was not an important factor in the Baygon animals. The data obtained in these experiments would indicate a direct Baygon-induced perinatal toxic effect.

In a previous study a reduction in total electrical activity (LO X MF derivation) was noted in Baygon-treated older rats (DESI et al., 1974). This is in agreement with our results in which both treated male and female rats showed a decrease in electrical activity. DESI et al. (1974) also found a dose related decrease in electrical activity in the delta, theta, alpha and beta frequencies using the above derivation. In our study, lower power amplitudes were also found in treated animals, specifically in the delta, theta, and alpha bands. The power spectral relationship described above also was observed by VAJDA et al. (1974) in a study in which rats were treated with parathion.

VAJDA et al. (1974) in their study also obtained visual evoked responses from parathion treated rats. They observed reduced amplitudes with an increase in peak latency, effects similar to those noted in our study.

From the data presented, it is possible to conclude that Baygon administered to female rats at high dose levels during gestation and lactation can produce alterations in the EEG of their offspring. The cause of these effects, their relationship(s), if any, to delays in reflex development, and possible long-term sequelae are presently unknown. The data obtained in these experiments demonstrate that it is possible to produce neurological changes in the perinatal animal similar to those seen in adults.

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