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The IPCS Collaborative Study on Neurobehavioral Screening Methods: III. Results of Proficiency Studies

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Abstract: *The goal of the IPCS Collaborative Study on Neurobehavioral Screening Methods was to determine the intra- and inter-laboratory reliability of a functional observational battery (FOB) and an automated assessment of motor activity in eight laboratories world-wide. The first phase of the Collaborative Study involved training the participants: evidence of training was then evaluated using positive-control compounds. The positive-control studies required the laboratories to identify, using the FOB, specific neurotoxic syndromes produced by acute exposure to p,p'-DDT, parathion, and by short-term repeated dosing with acrylamide. For the sake of expediency, only one dose of each chemical was used instead of collecting dose-response data. Motor activity test chambers were not of uniform design. The laboratories were therefore required to demonstrate adequate sensitivity by the ability to detect statistically-significant activity increases and decreases produced by triadimefon and chlorpromazine, respectively, following acute administration of a range of doses. The resulting FOB and motor activity data showed variability in the magnitude of effects obtained: some of these differences were attributed to miscommunications, difficulties with the techniques or protocol, or the limitations of having only one dose. All laboratories, however, successfully met the criteria set forth by the Study Steering Committee. ©1997 Intox Press, Inc.*

Key Words: Functional Observational Battery, Motor Activity, DDT, Parathion, Acrylamide, Triadimefon, Chlorpromazine

INTRODUCTION

The International Programme on Chemical Safety (IPCS) sponsored a collaborative study to evaluate the utility of neurobehavioral test methods for identifying neurotoxic chemicals. The goal of the IPCS Collaborative Study on Neurobehavioral Screening Methods was to determine the intra- and inter-laboratory reliability of a functional observational battery (FOB) and an automated assessment of motor activity (Moser *et al.*, 1997a, 1997b). This study included four laboratories in the U.S. and four in Europe, each of which evaluated the effects of seven prototypic chemicals. Training and proficiency studies were the first phase of this collaborative effort. These pro-

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ficiency studies provided an opportunity for the laboratories to become more familiar with the test protocol, and identified specific problems or misunderstandings before formal data collection began. This manuscript presents details regarding the training procedures, and data from the motor activity and FOB studies.

In this Collaborative Study the type of motor activity device was not standardized, but specific requirements were placed on its operating characteristics. Participating laboratories used either photocell devices or video-based monitoring systems, of different sizes and configurations (listed in Moser *et al.*, 1997a). Each laboratory was required to demonstrate the ability to detect a statistically-significant change in activity level of at least 40% from baseline values (both increases and decreases). The test session was to be of sufficient length to show habituation within the session.

Each laboratory also conducted proficiency studies to show they could detect specific chemical-induced syndromes using their available equipment, techniques, and personnel. The criteria for demonstrating proficiency were set by the Study Steering Committee, and represented the cardinal signs of toxicity for the selected chemicals. The participants were to demonstrate most, if not all, of these cardinal signs to show that they could detect and discriminate the effects of these compounds.

Training Procedures

Training is a critical aspect to the proper conduct of these tests since, for most measures, the observer is the instrument which collects the data. The Study Steering Committee strongly felt that standardized hands-on training was critical for transferring the techniques to others, with practice being essential.

All laboratories participating in this Collaborative Study had previous experience with neurobehavioral testing in rats; however, they differed considerably in the amount of experience with these specific FOB and motor activity test measures. To facilitate training, one or two representatives from each laboratory attended two-day sessions held in Research Triangle Park, NC. Participants reviewed a videotape and training manual specifically prepared for this project, which were provided to them to take back to their laboratories for further training purposes. The Study protocol was reviewed in detail, with emphasis on standardizing terminology and procedures. In addition, hands-on training was provided using both treated and untreated rats. Information on receipt and safe handling of the chemicals was also provided.

MATERIALS AND METHODS

STUDY 1: Motor Activity Proficiency Studies

Dose-response studies using chlorpromazine and triadimefon were conducted to show activity decreases and increases, respectively. Chlorpromazine, a phenothiazine antipsychotic agent, is known to lower activity levels and has been used as a standard treatment in behavioral pharmacology studies. Triadimefon, a fungicide, was chosen in place of the typically-used stimulant, *d*-amphetamine (which presented legal problems for international shipment), since recent work had shown that it possesses stimulant properties that are very similar to other psychomotor stimulants (Crofton *et al.*, 1988; Moser and MacPhail, 1989).

A dose-response determination for each compound was required from each laboratory. Chlorpromazine was dissolved in isotonic saline and administered in doses of 0, 1, 2, or 4 mg/kg intraperitoneally (i.p.) 30 minutes before testing. Triadimefon was suspended in corn oil (Mazola®) and given in doses of 0, 50, 100, or 200 mg/kg orally (p.o.) one hour before testing.

Motor activity counts were square-root transformed (to better approximate a normal distribution), then subjected to a one-way analysis of variance (ANOVA) using dose as the grouping factor. When a significant dose effect was obtained ($p \leq 0.05$), group means significantly different from control were determined using Dunnett's t-test (SAS, 1990).

STUDY 2: FOB Proficiency Studies

Three positive-control studies were designed to show: 1) tremorigenic effects of *p,p'*-DDT, six hours after a single p.o. dose of 75 mg/kg; 2) autonomic effects of parathion, with both the dose (p.o.) and time of evaluation (peak effect) determined by the laboratory (using the methods specified in the protocol for the formal tests); and 3) peripheral neuropathic effects evaluated one to three days following short-term repeated dosing (one to two weeks) with acrylamide 40 mg/kg/day (i.p.). DDT and parathion were suspended in corn oil, and acrylamide was dissolved in isotonic saline; concurrent vehicle-treated control groups were included in each study. All rats were tested with the FOB (without the motor activity assessment) before dosing, and at the specified time after dosing.

Analyses of the individual FOB measures as well as the physiological measures (body weight and body temperature) were conducted using a two-way ANOVA (GLM; SAS, 1990), with dose as a grouping factor and repeated testing as a within-group factor (time-0 and the single test time after dosing). Descriptive and ordered response (rank) data were analyzed using a categorical data modelling procedure (CATMOD; SAS, 1990). Since only two dose groups were used (control and treatment), no post-hoc analyses were necessary. Significance was concluded when either the dose factor or dose-by-time interaction had a probability value ≤ 0.05; slightly higher p-values ($p<0.10$) were, however, considered a trend that approached significance.

RESULTS

STUDY 1: Motor Activity Proficiency Studies

Figure 1A shows the individual laboratory data for the chlorpromazine dose-response determination. One laboratory included a lower dose than specified (0.5 mg/kg), and another included a higher dose (10 mg/kg). Regardless of the type of activity chamber, all laboratories achieved significant decreases of at least 40% at one or more doses. The data for triadimefon are presented in Figure 1B. Again, significant increases were obtained in all laboratories at one or more doses. There was, however, a wide range in the magnitude of activity increases. For example, one laboratory obtained a maximum 40% increase while another obtained a 335% increase over control levels. Triadimefon produces an array of stereotypies and tremors at high doses, and it is possible that this range of apparent effectiveness was partly due to differences in photobeam and device configurations, which varied in the detection of non-ambulatory (e.g., stereotypical) movements (Crofton *et al.*, 1991).

Activity data were collected in five epochs within the session, regardless of the actual session length. The control activity data for these five intervals within the session are shown in Figure 2 (control data from the chlorpromazine and triadimefon experiments). From these data, the laboratories chose session lengths which ranged from 30 to 62.5 minutes for the formal study (Moser *et al.*, 1997a).

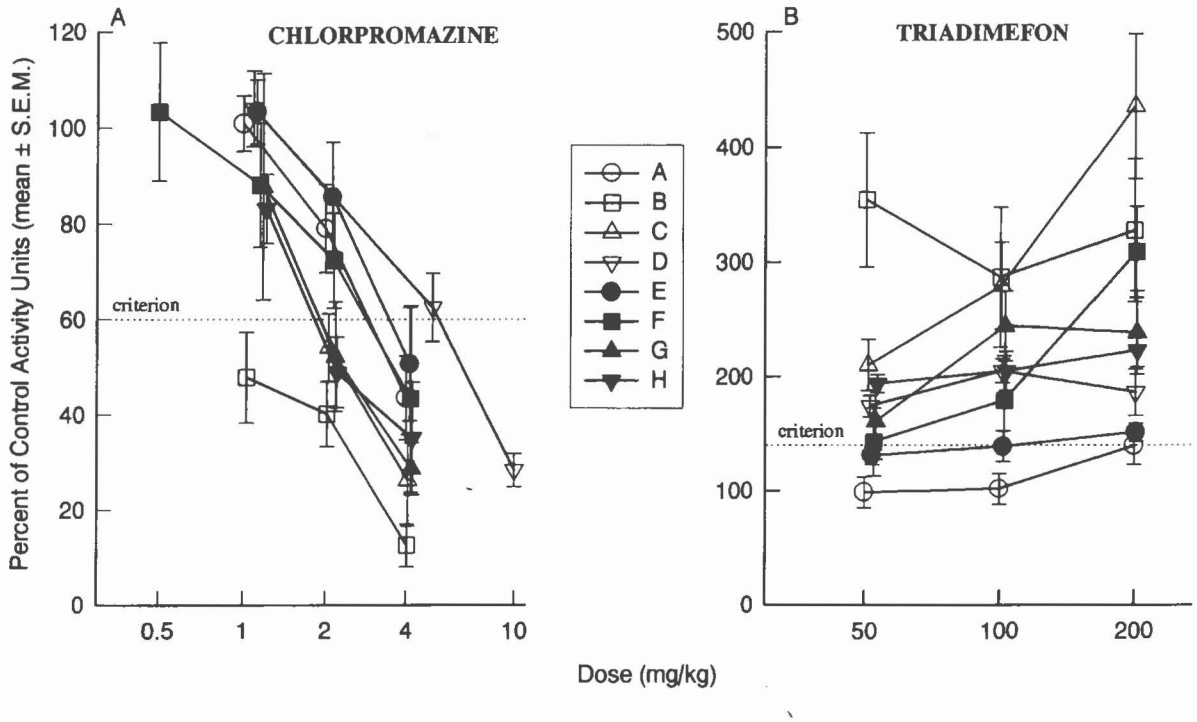

FIG. 1. Motor activity data from the proficiency studies using A) chlorpromazine and B) triadimefon. Data are presented as the mean (± S.E.M.) percent of the respective vehicle control values. The dotted line indicates the criteria for determining a 40% change in activity. Laboratories are referred to as A-H.

TABLE 1. Cardinal Signs of Toxicity for Acrylamide Established by the Study Steering Committee, and the Data from each Laboratory.

Acrylamide Cardinal Signs		Laboratory (Total Acrylamide Dose Administered, mg/kg)							
Magnitude of Effects[1]	Treatment	A (200)	B (400)	C (400)	D (400)	E (320)	F (200)	G (200)	H (400)
Gait Changes	Control	1.0	1.0	1.0	1.0	1.0	1.2	1.0	1.0
Mean Score	Acrylamide	**2.6**	**3.0**	**3.4**	**2.0**	**2.1**	**2.0**	**1.9**	**3.9**
Increased Foot Splay	Control	86%	92%	115%	117%	92%	112%	102%	99%
% of Baseline Value	Acrylamide	**114%**	**189%**	**187%**	**140%**	**174%**	125%[a]	114%	**191%**
Decreased Hindlimb Grip Strength	Control	92%	127%	116%	134%	86%	93%	120%	119%
% of Baseline Value	Acrylamide	93%	90%	**94%**	118%	**70%**	**50%**	**95%**	**84%**
Decreased Body Weight	Control	106%	111%	122%	169%	110%	101%	102%	111%
% of Baseline Value	Acrylamide	**100%**	**102%**	**104%**	**157%**	**98%**	**97%**	97%[a]	**88%**

[1] Bold numbers indicate those data that were significantly different from control ($p \leq 0.05$). Laboratories are referred to as A-H.

[a] data shows trend, p-value < 0.10

TABLE 2. Cardinal Signs of Toxicity for DDT Established by the Study Steering Committee, and the Data from each Laboratory.

DDT Cardinal Signs		Laboratory (DDT 75 mg/kg)							
Magnitude of Effects[1]	Treatment	A	B	C	D	E	F	G	H
Gait Changes	Control	1.0	1.0	1.0	1.0	1.0	1.3	1.0	1.0
Mean Score	DDT	1.3	**2.4**	**1.7**	**2.3**	**1.9**	1.4	1.4	**2.9**
Tremors and/or Myoclonus	Control	0%	0%	0%	0%	0%	25%	0%	0%
Incidence	DDT	**100%**	**100%**	**90%**	**80%**	**100%**	**63%**	**100%**	**100%**
Increased Click Response	Control	-0.5	-0.2	0.0	-0.2	-0.3	-0.8	-0.6	-0.2
Mean Change from Baseline Score	DDT	0.1[a]	**1.0**	**0.9**	**0.6**	**1.8**	**0.4**	**0.4**	**1.0**
Increased Body Temperature	Control	38.1	37.9	38.3	37.5	38.7	—[b]	38.1	38.7
Mean Temperature	DDT	**39.0**	**38.9**	**39.0**	**39.6**	**39.7**		**38.9**	**39.6**

[1] Bold numbers indicate those data that were significantly different from control ($p \leq 0.05$). Laboratories are referred to as A-H.

[a] data shows trend, p-value < 0.10

[b] incorrect procedure used, data invalid

TABLE 3. Cardinal Signs of Toxicity for Parathion Established by the Study Steering Committee, and the Data from each Laboratory.

Parathion Cardinal Signs		Laboratory (Parathion Dose, mg/kg)							
Magnitude of Effects[1]	Treatment	A (4.5)	B (6.75)	C (6.75)	D (15.19)	E (6.75)	F (4.5)	G (6.75)	H (10.13)
Salivation	Control	0%	0%	0%	0%	0%	0%	0%	0%
Incidence	Parathion	0%	22%	**50%**	29%[a]	**50%**	**50%**	13%	**50%**
Tremors and/or Mouth Smacking	Control	0%	0%	0%	0%	13%	38%	0%	0%
Incidence	Parathion	**67%**	**44%**	**70%**	**43%**	**88%**	**88%**	**38%**	**100%**
No Pupil Response, Miosis	Control	0%	—[b]	0%	0%	0%	0%	0%	0%
Incidence	Parathion	**50%**		**40%**	**71%**	**88%**	**38%**	25%[a]	**50%**
Gait Changes	Control	1.0	1.1	1.0	1.0	1.1	1.3	1.0	1.0
Mean Score	Parathion	**2.7**	**2.7**	**2.9**	**1.9**	**2.8**	**1.9**	**2.0**	**2.9**
Decreased Arousal	Control	3.0	3.3	3.3	3.2	3.4	4.1	3.6	4.0
Mean Score	Parathion	**2.7**	**2.7**	**2.8**	2.7	**2.8**	**2.8**	**2.8**	**3.4**
Decreased Tail-Pinch Response	Control	0.0	0.2	-0.5	-0.6	-0.1	0.6	-0.6	-1.0
Mean Change from Baseline Score	Parathion	**-1.2**	-0.9[a]	-1.0[a]	**-2.0**	**-3.1**	**-0.8**	-1.3	**-2.6**
Decreased Body Temperature	Control	38.2	37.5	38.3	38.4	38.2	—[b]	38.0	38.3
Mean Temperature	Parathion	**34.4**	**36.2**	**35.4**	**36.4**	**36.5**		**36.7**	**35.0**

[1] Bold numbers indicate those data that were significantly different from control ($p \leq 0.05$). Laboratories are referred to as A-H.

[a] data shows trend, p-value < 0.10

[b] incorrect procedure used, data invalid

STUDY 2: FOB Proficiency Studies

The proficiency criteria set by the Study Steering Committee represented the cardinal signs of toxicity for each chemical. That is, based on the committee's experience, a list of expected effects was compiled which should be obtained with the different test compounds. These effects and the results from each laboratory are provided in Tables 1-3.

Some difficulties became apparent during this portion of the study, which provided additional learning experiences for the laboratories. When data were submitted which appeared aberrant, the laboratory was contacted for details regarding their techniques. As a result, some misunderstandings and misinterpretations of the protocol were corrected before the formal testing began. Furthermore, the use of a single dose of each chemical precluded the establishment of a dose-response function. It became clear that a single dose was not always sufficient to produce the full anticipated syndrome, given the differences in strain of rat and testing conditions. In many instances, the magnitude of effect of a specific dose varied considerably across the laboratories. Despite these hindrances, all laboratories detected most (all, or all but one or two) of the cardinal signs for each compound.

With acrylamide, five of the laboratories dosed their rats for two weeks, whereas the others ended dosing after one to 1½ weeks. As would be expected based on the cumulative nature of acrylamide's effects, the laboratories which continued dosing for a longer period of time obtained more signs of neurotoxicity than did those that ended dosing earlier. The acrylamide cardinal signs and results from each laboratory are presented in Table 1. All laboratories observed gait changes, and most obtained increased foot splay, decreased hindlimb grip strength, and body weight deficits. The magnitude of these effects, however, differed across laboratories. The landing foot splay data are presented in Figure 3: significant changes were obtained in six laboratories, and the data from one laboratory showed a trend ($p<0.09$). It is evident from Table 1 that the laboratories which reached a cumulative dose of only 200 mg/kg (laboratories A, F, and G) generally obtained less effects than did those which dosed for a longer period of time.

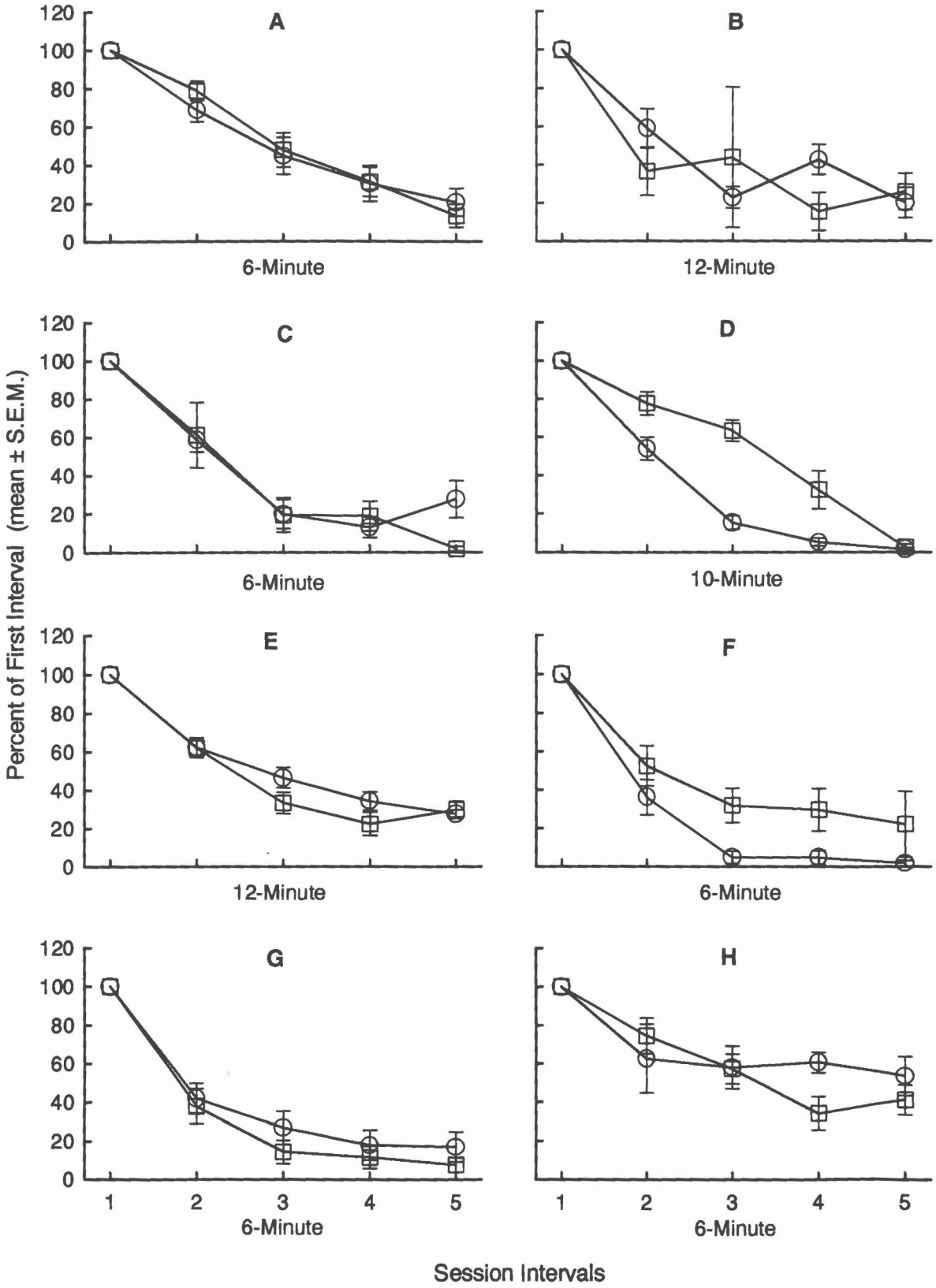

FIG. 2. Habituation, or change in activity during the session, of the motor activity data from control groups in the proficiency studies (circles, chlorpromazine study; squares, triadimefon study). Data are calculated separately for each control rat as the percent of that rat's first interval; data are then averaged for the control group and mean (± S.E.M.) are plotted. The interval length is indicated for each laboratory. Laboratories are referred to as A-H.

The DDT syndrome of tremors, myoclonus, hyperresponsiveness (increased reaction to the click stimulus), and hyperthermia was detected in almost all laboratories (data were excluded from one laboratory due to an incorrect technique in recording body temperature). The cardinal signs and data from each laboratory are presented in Table 2, and the data for increased click response are shown in Figure 4. Every laboratory obtained significantly higher body temperature, and a high incidence of tremors and/or myoclonus, and all but one detected increased click responsiveness (the eighth laboratory showed a trend in the data, $p<0.09$). Three laboratories, however, did not report significant gait changes.

The proficiency data for parathion were somewhat more problematic, due partly to the steep dose-response characteristics of this compound and the use of only one dose. The actual doses determined by the top dose procedure ranged from 4.5 to 15.2 mg/kg, and unfortunately lethality at this dose ranged from none to 38%. The data from each laboratory are shown in Table 3. The data for a few endpoints showed a convincing trend towards an effect, although in the absence of statistical significance. Tremors and/or mouth smacking, gait changes, and hypothermia were reported in all eight laboratories. Fewer laboratories reported salivation, miosis (and resultant inhibited pupil response), lowered arousal, and decreased tail-pinch response. Data were excluded from one laboratory due to an incorrect technique in recording body temperature, and from another due to an inability to observe the pupil response. As with the other chemicals, the magnitude of the cardinal signs differed considerably between laboratories.

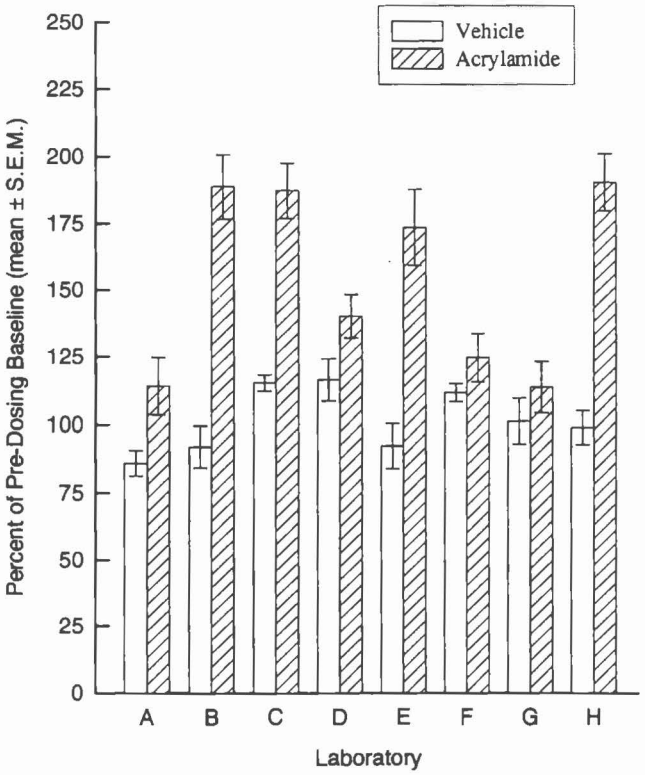

FIG. 3. Landing foot splay data from the acrylamide proficiency study. Data are presented as the mean (± S.E.M.) of the pre-dosing value for each dose group (vehicle and acrylamide 40 mg/kg/day). Dosing regimens varied somewhat: laboratory A, F, and G dosed for 1 week (total cumulative dose, 200 mg/kg), laboratory E for 1½ weeks (total dose, 320 mg/kg), and laboratories B, C, D, and H for 2 weeks (total dose, 400 mg/kg). Changes in foot splay values were significantly different from control ($p\leq0.05$) in all laboratories except F and G.

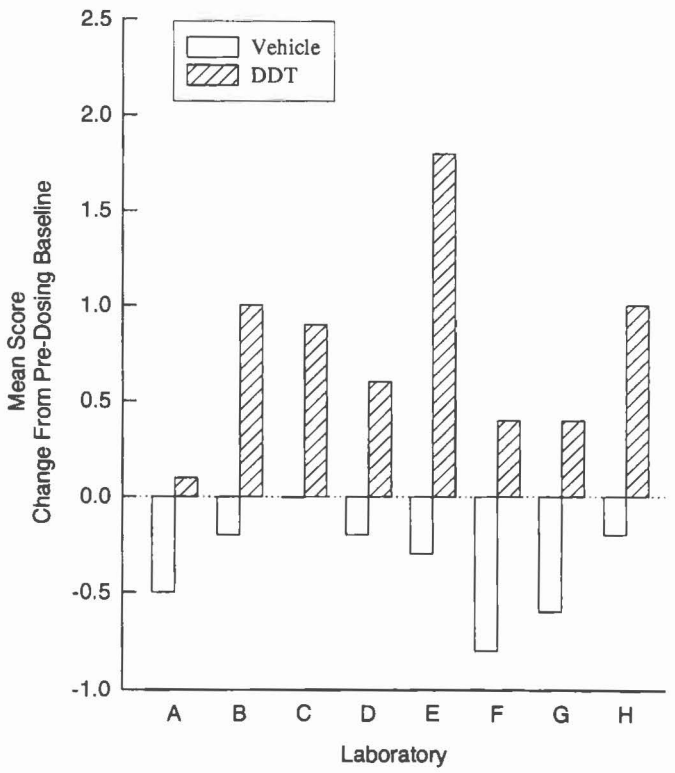

FIG. 4. Click response data from the DDT proficiency study. Data are presented as the mean change in response scores from the pre-dosing value for each dose group (vehicle and DDT 75 mg/kg). Changes in click response values were significantly different from control ($p\leq0.05$) in all laboratories except A.

DISCUSSION

Considerable interactions took place between the Study Coordinator and the participating laboratories at this preliminary phase of the Collaborative Study. Variations became evident in the interpretation of certain aspects of the Study protocol, some of which may have been due to language differences. In some laboratories, the protocol was translated into the native language, whereas in others the English version was used. Another unanticipated factor was a difference in who attended the training and who actually conducted the tests: in three laboratories, the same person attended the training and conducted all the studies, in two, the attendee was one of several observers who conducted the tests, yet in three laboratories, no one who attended the training actually collected the data. The Study Steering Committee took all these considerations into account when reviewing the proficiency data.

For each of the participating laboratories, the shapes of the dose-response curves for chlorpromazine and triadimefon were similar, and a 40% change in activity level was detected regardless of motor activity device, actual activity units, or session length. The FOB evaluations detected neuromuscular changes following acrylamide dosing, tremors and hyperreactivity from DDT, and autonomic changes and tremors following parathion; these effects were evident despite some lethality and the lack of dose-response data that precluded a complete characterization of the effects. All laboratories completed the proficiency phase of the Collaborative Study and proceeded to test the formal set of chemicals.

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THE IPCS COLLABORATIVE STUDY ON NEUROBEHAVIORAL SCREENING METHODS

Continued