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The IPCS Collaborative Study on Neurobehavioral Screening Methods: V. Results of Chemical Testing

VIRGINIA C. MOSER[1§†], GEORGE C. BECKING[2§], VINCENZO CUOMO[3§†], EMIL FRANTÍK[4§†], BEVERLY M. KULIG[5§†], ROBERT C. MACPHAIL[1§], HUGH A. TILSON[1§], GERHARD WINNEKE[6§], W. STEPHEN BRIGHTWELL[7†], MARIA A. DE SALVIA[3†], MICHAEL W. GILL[8†], GILLIAN C. HAGGERTY[9†], MIROSLAVA HORNYCHOVÁ[4†], JAN LAMMERS[5†], JENS-JØRGEN LARSEN[10†], KATHERINE L. MCDANIEL[1†], B.K. NELSON[7†] AND GRETE ØSTERGAARD[10†]

§ Members of the IPCS-sponsored Steering Group for the Collaborative Study on Neurobehavioral Screening Methods † Principal investigators and key personnel from the participating laboratories.

[1]Neurotoxicology Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, U.S.A.; [2]International Programme on Chemical Safety, World Health Organization, Research Triangle Park, North Carolina, U.S.A.; [3]Institute of Pharmacology, University of Bari, Bari, Italy; [4]National Institute of Public Health, Prague, Czech Republic; [5]TNO Nutrition and Food Research, Zeist, The Netherlands; [6]Institute of Environmental Hygiene, Dusseldorf, Germany; [7]National Institute for Occupational Safety and Health, Cincinnati, Ohio, U.S.A; [8]Bushy Run Research Center, Union Carbide Corp., Export, Pennsylvania, U.S.A.; current address: Toxicology Regulatory Service, Inc., Charlottesville, Virginia, U.S.A.; [9]G.D. Searle and Co., Skokie, Illinois, U.S.A.; [10]Ministry of Health, National Food Agency, Soborg, Denmark.

Abstract: *The IPCS Collaborative Study on Neurobehavioral Screening Methods was undertaken 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. Following the training phase and the conduct of proficiency studies in all laboratories, participants proceeded to test the effects of seven chemicals in both single dose and four-week repeated dosing scenarios. The chemicals studied were acrylamide, bis-acrylamide, p,p'-DDT, lead acetate, parathion, toluene, and triethyl tin. Participants received coded samples from a common source. In order to judge the general utility of these procedures in a diversity of testing situations, laboratories conducted the studies under their standard conditions, using their choice of rat strain and test equipment. Chemical doses and time of peak effect for acute testing were determined by each laboratory: these parameters were quite similar for some chemicals, but varied greatly for others. The results of the chemical tests indicated that while there was some variability in the data on specific endpoints, all laboratories detected and characterized the effects of all but one of the known neurotoxicants. The one exception (toluene) was probably due to other factors (e.g., dose level, route of administration) rather than lack of sensitivity of the test methods. This study provides extensive data regarding the use of neurobehavioral screening methods over a range of laboratory conditions as well as the reliability, sensitivity, and robustness of the tests to detect neurotoxic potential of chemicals. ©1997 Intox Press, Inc.*

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INTRODUCTION

The International Programme on Chemical Safety (IPCS) sponsored a Collaborative Study on Neurobehavioral Screening Methods to evaluate the utility of neurobehavioral test methods for identifying neurotoxic chemicals (see Moser *et al.*, 1997a). The objectives of this collaborative study were: 1) to evaluate the reliability, sensitivity and specificity of a standardized set of neurobehavioral screening tests designed to detect potential human neurotoxicants; 2) to develop a data-base of neurotoxic effects with a known set of chemical agents and to compare those effects with compounds having little or no reported neurotoxicity; and 3) to analyze data from

Please send requests for reprints to Dr. V.C. Moser, NTD, (MD-74B), U.S. EPA, Research Triangle Park, NC 27711.

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participating laboratories to determine the dose- and time-dependent effects of chemical exposure and determine the influence of laboratory, if any, on such treatment effects (Moser *et al.*, 1997a). The neurobehavioral procedures chosen were the functional observational battery (FOB) and an automated test of motor activity. The FOB is a type of neurological examination in that a wide range of neurobiological functions are assessed, including measures of sensory, motor and autonomic function. An automated assessment of motor activity was included because of its widespread use in evaluating the behavioral effects of chemicals (Gad, 1982; Irwin, 1968; Moser *et al.*, 1988; O'Donoghue, 1989; Tilson and Moser, 1992). It was hypothesized that the FOB and motor activity measurements would identify potential neurotoxic agents, detect dose- and time-dependent effects following single and repeated dosing, and distinguish these from non-neurotoxic compounds. This study included four laboratories in the U.S. and four in Europe, each of which evaluated the effects of seven prototypic chemicals.

Training of the participants and the completion of proficiency studies were the first phase of this collaborative effort (see Moser *et al.*, 1997b). Following completion of that phase, data collection then began using the coded chemicals. This portion of the Collaborative Study was projected to span 18 months: actually, it took over three years before all the data were submitted. For a variety of reasons, some laboratories had delays exceeding six months in data collection. Data were sent to the Study Coordinator (Dr. V.C. Moser) shortly after completion of each study. The Study Coordinator maintained contact with the participating laboratories, and analyzed and summarized all the data. These data were presented and discussed at a 1995 meeting of all the participants, the Study Steering Committee, and expert peer reviewers. The results of the chemical tests are presented in this and accompanying manuscripts (Moser *et al.*, 1997b, 1997c).

METHODS

Animals

Animals used in this Collaborative Study were adult (60-90 days of age) male rats of the strain or stock used regularly by the individual participating laboratory. Wistar and Sprague-Dawley rats were used in three laboratories each, with the remaining laboratories using Long-Evans or an inbred strain (WAG/Rij/MBL). Laboratories A, B, D, E, H and G (repeated-dose studies only) used 10 rats/treatment group, whereas Laboratories C, F, and G (single-dose studies) used 8 rats/group.

There was no attempt to standardize housing conditions across laboratories. Because of potential interactions between social variables, dosing and testing, however, animals were individually housed. Food and water in the home cage were available at all times during the course of the experiment. Details of laboratory conditions, cages, feed, animals, etc., are found in Moser *et al.* (1997a).

Chemicals

The chemicals provided to the participants were of pure or known composition, supplied by the National Toxicology Program (through the repository located at Radian Corporation, Dallas TX, at that time under contract to the U.S. National Institute of Environmental Health Sciences) and coded before distribution to investigators. Chemicals were referenced only by the code number assigned by Radian, which was unique for each chemical sent to each laboratory. Only the Study Coordinator was aware of the chemical identity for each laboratory. Safety handling sheets on the compounds were provided to each participating laboratory. Steps were taken to ensure that information on each chemical was provided to a non-participating official in each laboratory in case of accident.

The seven chemicals were acrylamide (CAS #79-06-1), N,N'-methylene bisacrylamide (bis-acrylamide, CAS #110-26-9), 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (*p,p'*-DDT, or DDT, CAS #50-29-3), lead acetate trihydrate (CAS #6080-56-4), parathion ethyl (CAS #56-38-2), toluene (CAS #108-88-3), and triethyl tin bromide (TET; CAS #2767-54-6). Three chemicals were administered by oral gavage (p.o.; parathion, *p,p'*-DDT, and toluene) while the others were delivered intraperitoneally (i.p.). Water-soluble chemicals were dissolved in isotonic saline (acrylamide, triethyl tin) or distilled water (bis-acrylamide, lead acetate), and insoluble chemicals (DDT, parathion, toluene) were dissolved/suspended in corn oil (Mazola®).

Behavioral Procedures: FunctionalObservational Battery (FOB)

The FOB consisted of 30 measures of sensory, motor and autonomic function (listed in Moser *et al.*, 1997a). The evaluation of these endpoints and the scoring criteria used in this Collaborative Study have been described in

detail elsewhere (McDaniel and Moser, 1993). Briefly, the first measurements were made while the animal was in the home cage. The observer evaluated each animal's posture, palpebral closure, and the presence or absence of convulsions. If convulsions were present, they were categorized further. Following observations in the home cage, the animals were removed. The presence or absence of spontaneous vocalization, piloerection, and other fur and skin abnormalities were then noted, as well as the ease of removal and handling. Lacrimation, salivation, and ptosis were also noted and scored. Other signs such as exophthalmus, crustiness around the eyes, or emaciation were recorded.

The rat was then placed on a flat surface which served as the open field (e.g., cart top with a perimeter barrier) covered with a clean absorbent pad. A timer was started for three minutes, during which time the frequency of rearing responses was recorded. At the same time, gait characteristics were noted and ranked; the ease with which the rat moved about was also ranked, and arousal, tremor, convulsions and abnormal postures were evaluated. At the end of the three minutes, the number of fecal boluses and urine pools on the absorbent pad were recorded. Reflex testing followed next and consisted of recording each rat's responses to the frontal approach of a blunt object such as a pencil, a touch of an object to the posterior flank, and an auditory click stimulus. Reactivity to a pinch on the tail and the ability of the pupil to constrict in response to light was also assessed. These measures were followed by a test for the righting reflex, then by measures of forelimb and hindlimb grip strength, body weight and rectal temperature, and finally hindlimb foot splay. The entire battery of tests required approximately six to eight minutes per rat.

Behavioral Procedures: Motor Activity Assessment

Many methods are available for the evaluation of chemical-induced changes in motor activity, and different apparatuses were used in the participating laboratories (Moser *et al.*, 1997a). Six of the eight laboratories chose photocell-based chambers, which tabulate photocell interruptions as a measure of locomotor activity. The remaining two used a video-based system which recorded actual distance traveled. Testing was conducted in a location isolated from the animal housing area and in a space that controlled for extraneous changes in light and sound. Different treatment groups were counterbalanced for multiple devices and time of day. When testing was necessarily conducted over several days, treatment groups were also to be counterbalanced across time of day and days of testing. Both FOB and motor activity were assessed in the same rats, with motor activity following the FOB within a short period of time.

Experimental Design

The protocol specified that participating laboratories would determine the neurobehavioral effects of all seven compounds following both single and repeated exposures in order to identify differential time-course and dose-response characteristics. In the single-dose experiments, testing was conducted one to seven days (actual number of days to be held constant) prior to exposure (time-0), at the time of peak effects (TOPE) on the day of dosing, and at one and seven days following exposure. In the repeated dosing studies, animals were dosed five days per week for four weeks. Testing was conducted one to seven days before dosing began (time-0), at the end of weeks two and four of dosing, and two weeks after the end of dosing. Previously-trained personnel were required for participation in the study. For any one chemical, it was highly preferred that all FOB testing be conducted by the same person.

Dose Range-Finding

Because of the number of variables that were not standardized (e.g., strain of rat), each laboratory determined the range of doses and acute testing times to be used for each chemical. This process was intended to assure that doses and times would be biologically similar, despite the different strains and laboratory conditions.

Dose range-finding studies by each of the participating laboratories were conducted before final dose selection. For each chemical, laboratories were given a starting dose (which was loosely based on published values of the LD50), as well as information on vehicle, route of administration, and preparation instructions. One rat received the starting dose, while three higher and three lower doses were each given to individual rats. Spacing of the doses was based on a constant factor (1.5 for doses higher than the starting dose, and 0.67 for lower doses). A "top dose" (TD) was defined as the highest non-lethal dose based on seven-day survival data. In all cases, the maximum dose could not exceed 2000 mg/kg.

Each laboratory next performed a determination of the acute time of peak effect (TOPE) for each chemical.

Four rats received the previously-determined top dose while four other rats received vehicle. Gait score and arousal, as described in the FOB protocol, were evaluated at 30-minute intervals for the first two hours, then at three, four, five and six hours post-dosing, and again at 24 hours. If more than one rat died within seven days after dosing, the top dose was lowered one step, and the TOPE determination was repeated. The results of this experiment defined the appropriate time to evaluate the animals in the single-dose experiments.

For the single-dose determinations, the doses were 100%, 50%, 25%, 12.5% and 0% of the previously-determined top dose. For the repeated-dosing determinations, the doses were 50%, 25%, 12.5%, 6.25% and 0% of the top dose, and were given five days/week for four weeks. During the dosing period, body weights were recorded at least twice per week and used to adjust dosing volumes. For each study, treatment groups consisted of either 8 (laboratories C, F, and G-acute studies) or 10 (laboratories A, B, D, E, H, and G-repeated dose studies) rats/dose.

Data Custody

The raw data (either pre-printed data sheets or computer files) were sent to the Study Coordinator (Dr. V.C. Moser) as soon as possible after the completion of each study. Each study was assigned a unique experiment number, and entered into the EPA computer network. All data entries were subjected to several checks against the original data, and are retained on the EPA VAX cluster. All hardcopies of raw data, printouts, documentation of data checks, and statistical analyses are currently retained in the office of Dr. Moser (US EPA). During the data-collection portion of the Study, the Steering Committee occasionally met to review its progress. These data are the property of IPCS and the World Health Organization, and will be made available to interested individuals upon request.

Data Analysis

Data collected with the FOB and motor activity assessment include categorical, ordinal, and continuous values. Measures were collected from the same animals at multiple time points. Analysis of the individual FOB measures as well as the physiological measures (body weight and body temperature) was conducted as previously described (Creason, 1989; Moser *et al.*, 1988). Continuous data were analyzed by a linear model (GLM; SAS, 1990); a square-root transformation was applied to motor activity units and to the count of rears in the open field (to approximate better a normal distribution). To correct for response differences due to pre-existing differences, a two-step "covariate" analysis was followed. First, regression analyses were conducted between the initial (time-0) value and the average subsequent values for all rats, regardless of treatment. Predicted values were derived based on initial values and were subtracted from the observed values at each time point to yield "adjusted" values; the analysis of variance (ANOVA) was then conducted on these adjusted values with dose as a grouping factor and repeated test times as a within-subject factor.

Descriptive and ordered response (rank) data were analyzed using a categorical data modelling procedure (CATMOD; SAS, 1990), which converts response frequencies to linear functions for which estimation of parameters can be analyzed using techniques analogous to that used for general linear models. For ordinally-scaled variables the response functions are mean scores, and for descriptive data they are marginal probabilities. These methods are based on the procedure described by Grizzle *et al.* (1969), with the special case of repeated measures by Koch *et al.* (1977). The CATMOD analyses also included dose as a grouping factor and repeated test times (time-0 was included). In certain situations, however, another analytical approach was employed for test measures such as lacrimation and salivation. For these evaluations, scores were rarely greater than '1' (normal); when this does occur it is especially noteworthy even though it may not attain statistical significance. Occasionally a dose group would show an apparent effect, typically at only one time point, which would be obscured using traditional ANOVA techniques (i.e., overall dose and dose-by-time effects would not be significant). In these cases, the Kruskal-Wallis non-parametric analysis was used for the data at that time point. These methods are summarized in Moser *et al.* (1997a).

Regardless of method used, significant overall ANOVAs prompted additional tests to identify significant effects of dose and time. At each significant time point, each dose group was compared to control using either Dunnett's t-test for continuous data, or simple contrasts in CATMOD (SAS, 1990). In all analyses, resulting probability values $<.05$ were considered significant.

The sheer amount of data generated with the screening battery necessitated that additional steps be taken to integrate and reduce the data. For example, because of

the large number of tests carried out in individual rats and at varying times relative to exposure, it is possible that one or a few measures would be significantly affected purely by chance. In order to minimize the likelihood of excessive false-positive results, the individual tests were grouped into several domains of neurobiological function: Activity (which included automated motor activity data), Autonomic, Convulsive, Excitability, Neuromuscular, and Sensorimotor. To facilitate comparison of each of the domain-related tests, which have different types of data (ordinal, continuous, etc.), a severity-scoring scheme was used to normalize individual data for all measures to a single 1-to-4 scale, wherein a score of '1' reflected what was often observed in control rats and a score of '4' reflected a rare occurrence in control rats. Severity scores were always positive regardless of the direction of effects of the individual tests.

The conversion of individual data to a severity score was based on the data for control rats at each time point. For continuous measures (e.g., motor activity counts, grip strength), the transformation was based on the mean of the control sample with ranges defined by multiples of the standard deviation of the sample. For some ranked data (e.g., arousal, handling reactivity), score assignments were based on the frequency of occurrence of each score in the control group. The mode of the sample distribution (the score which occurred with the greatest frequency) was determined and the transformation was based on the position on the scale relative to that mode (i.e., the further away on the scale from the mode, the higher the severity score). For some ranked measures, no conversion was necessary since the scale itself was a severity score of sorts (e.g., righting reflex and gait score: 1=no abnormality, 4=severe abnormality). The conversion to severity scores took place in sequence. First, for each control sample, the range defining each score was established. Then each observed value was evaluated: those values that met the criteria for a '1' were transformed, then those remaining that met the appropriate criteria became a '2', and so forth until any data not already converted became a '4'. Control rats typically received scores of '1's (mostly) and '2's.

For each functional domain, the severity scores in individual rats were combined into a domain-related score. The composite scores were averaged across treatment groups and analyzed across time using a repeated-measures ANOVA, followed by Dunnett's test to compare treatment and control groups. This approach, therefore, allowed subsequent calculation of treatment-group statistics (mean, standard error), as well as tests of significance and comparisons across laboratories. The approach also has the advantage of not placing a disproportionate emphasis in interpreting data on any one test measure.

Across-laboratory analysis of domain effects was accomplished by subjecting the group means from each laboratory to two-way ANOVA with the grouping factor of dose and repeated measures across time, i.e., each laboratory mean represented one observation. The analytical method described above was also applied to the across-laboratory domain data.

RESULTS

Data Exclusions

As described before (Moser *et al.*, 1997a), the Study Steering Committee had drafted and extensively reviewed a protocol which detailed many specific requirements for conducting these tests. The final document was believed to contain adequate detail for the conduct of these experiments, yet not impose undue burden on the participating laboratories in conforming to an exact protocol. Despite these efforts, many deviations from the protocol became apparent as the data sets were reviewed. The Steering Committee extensively discussed these issues, which ranged from minor differences in procedures to those that seriously jeopardized the outcome of the study. In the end, only a few of these deviations were deemed serious enough to result in the data actually being excluded from the formal comparisons: these are listed below. In addition, it became apparent that a few endpoints of the FOB were generally misunderstood by participants, or else had no specific value: these measures were excluded from the summaries presented here, and are listed below. All data, however, are available upon request.

In many cases, questions arose during the data review process: these were submitted back to the laboratories for additional information. For example, one laboratory used five different observers to conduct these studies, none of which had attended the initial training session. Upon request, that laboratory provided extensive details of the training procedures for their observers and the number of other studies that each had performed. These were judged adequate explanations and the data were accepted by the Steering Committee, noting that sufficient training is crucial for conducting these subjective evaluations.

Procedures used in some laboratories did not strictly adhere to the protocol, but most variances were deemed to be relatively minor. Some variations that were relevant only to the Collaborative Study protocol were considered trivial, such as the difference between testing seven or eight days after dosing (the protocol stated seven days). The protocol also stated that the pre-dosing test interval should be held constant within a laboratory, since changes occur with repeated testing that are dependent on the time interval between testing. Thus, comparisons of data within each laboratory is more consistent when the pre-dosing interval is the same. Across laboratories this interval covered the entire allowed range, one to seven days, so that between-laboratory comparisons were difficult. Within a study, the pre-dosing interval should also be held constant, even if testing is staggered over days. In the instances where this time was not constant for all rats in a study, however, the difference was no more than one day and the Steering Committee felt that the data were not irrevocably compromised.

Other items were not specifically addressed in the protocol, and in fact were not considered until the differences surfaced. For instance, technicians in some laboratories dosed rats in the middle of the night so that testing at the time of peak effect could begin at a standard time in the morning. While chemical effects on behavior may be markedly different when administration and behavioral measurements take place during the night phase, the Steering Committee knew of no data on the influence of interrupting the night phase to dose rats for subsequent testing during the light period.

Excluded Studies. There were several circumstances which violated general scientific principles of study design, experimental control, and biological feasibility; these are listed in Table 1. The Steering Committee felt that these items were sufficiently serious to warrant excluding the data from formal analyses.

Sound experimental design calls for counterbalancing treatment groups across subsets of rats whenever it is necessary to stagger dosing and testing. In one laboratory, however, all of a single treatment group were dosed and tested at one time, with as many as three months intervening between dose groups. The datasets in which this occurred were eliminated from the across-laboratory analyses.

Experimental control also dictates that all rats be tested in the same order and manner, as testing on one measure may influence the results on another measure. For one laboratory, there were two rats out of the total 50 for each study that were evaluated with the FOB but not tested in the motor activity chambers (which occurred after the FOB), and thus those rats missed a significant portion of the handling and experimental history experienced by the others. Interestingly, when those rats were excluded from the studies and the data re-analyzed, the apparent treatment effects on the excitability measures (handling reactivity, ease of removal, arousal) were sometimes eliminated; i.e., the rats which were not tested in the motor activity chambers tended to show much higher reactivity levels.

Differences in experimental history were also the reason for rejecting a study in which the TOPE testing occurred at 24 hours; since those rats were not tested on the day of dosing, there was no same-day data with which to compare to the other laboratories, and the 24-hour data could not be compared to the others where 24 hours was the second test after dosing.

Finally, one laboratory reported various clonic movements (mild to severe tremors, myoclonus) in a significant number of rats, including controls, across all studies, and the incidence of these movements increased considerably with repeated testing. In examining all control data for this laboratory, on average two of 10 control rats showed myoclonus, and at some time points this incidence was as great as 90%. Likewise, an average of 41% of control rats at any observation time showed mild tremors. The Steering Committee felt that it was not likely that this many healthy, young adult vehicle-treated rats would show these clonic abnormalities spontaneously. The apparent discrepancies in these observations could not be resolved with that particular laboratory, and may have been due to the observer being overly sensitive to minor spontaneous movements displayed by the rats, i.e., they may have erred by being too liberal in labeling slight movements as clonic abnormalities. Since we could determine no justifiable method to correct the observations, their data for clonic and tonic movements were excluded from all analyses.

Excluded Measures. While reviewing data from the various laboratories, it became apparent that the ranking of mobility score was too closely associated with the gait score measure. Mobility score was defined as the ability of the rat to move about in the open field, regardless of the degree of gait impairment (which is assessed with gait score). For more than half of the laboratories, however, the score for mobility was either always the exact same as the gait score, or else always closely related. In these cases, mobility score was not providing an evaluation of a different aspect of behavior; in fact it was duplicating information from the gait score. For this reason the data for mobility score were eliminated from the chemical summaries.

TABLE 1. Excluded Data From The IPCS Collaborative Study on Neurobehavioral Screening Methods Protocol.[1]

Deviation	Comment	Data Affected	Laboratory
Testing of dose groups was not counterbalanced; entire dose groups were tested several months apart	When testing must be staggered, all conditions including treatment must be counterbalanced across days. All testing for a single study must be conducted as closely in time as possible	Entire datasets for TET, toluene, lead acetate, DDT, and repeated-dosing parathion study	C
Not all rats that were evaluated with the FOB were tested in the motor activity chambers	All subjects within a study must be treated identically, especially with regard to testing procedures	2 rats for each study: DDT, acrylamide, bis-acrylamide, parathion	B
The time of peak effect was determined to be 24 hours, and therefore rats were only tested three times	The number of times rats were tested had to be the same across laboratories for comparisons; subjects in this study were not treated the same as in other studies	Bis-acrylamide acute	G
High incidence of myoclonus, tremors, and clonic convulsions were recorded in all dose groups, including controls	This incidence of occurrence was not biologically feasible	Clonic movements for all datasets	A

[1] These deviations were determined by the Study Steering Committee to violate accepted scientific principles and therefore the data were unacceptable for inclusion in the study analyses.

Two other measures provided data which were essentially uninterpretable. The importance of recording vocalizations was questioned, since most laboratories either reported "present" for the vast majority of rats across all treatment conditions, or else "absent" in all cases. For similar reasons, the reporting of home-cage palpebral closure was questioned. The palpebral closure observed often depends on the status of the rat at the time of observation. Many rats sit in their cages apparently half-asleep, i.e., with eyelids drooping, or else fully asleep with eyes completely shut. But, some laboratories reported that eyes were open in all studies, regardless of whether "sleep" or any other posture was recorded. Since ptosis, *per se*, is better evaluated when the rat has been aroused by being handled, the home-cage measure was probably not contributing meaningful data. For these reasons, the vocalizations and home-cage palpebral closure measures were eliminated from the chemical summaries. To reiterate, these measures were excluded only because the data across laboratories were uninterpretable, not because they did not show the expected treatment effects.

Time of Peak Effect and Top Dose Determinations

Each laboratory determined their own testing times to be used for the single-dose studies of each chemical; the results are listed in Table 2. In general, acrylamide, bis-acrylamide, and DDT had a longer time frame of effect (median TOPEs were five to six hours) than the remaining chemicals (two to three hours). There was good agreement and more consistent TOPEs for the compounds which produced well-defined observable acute syndromes; for example, with parathion seven of eight laboratories chose either two or three hours. For other chemicals, however, the TOPE was harder to discern; for example, with bis-acrylamide and toluene, no more than two laboratories chose the same time point.

Each laboratory also determined their own top dose for each chemical, which was the basis for subsequent dose selection; these doses are listed in Table 3. There was generally good agreement among the laboratories in the TD determinations. For acrylamide, bis-acrylamide,

TABLE 2. Time of Peak Effect (hours) Determined by Each Laboratory.

Laboratory	Chemical						
	Acrylamide	Bis-Acrylamide	*p,p'*-DDT	Lead Acetate	Parathion	Triethyl Tin	Toluene
A	3	4	3	3	3	2	3
B	5	3	6	2	3	1	1.5
C	6	6	-[1]	-	2	-	-
D	6	6	4	4	2	3	4
E	5	5	6	4	2	2	3
F	8	4	6	1.5	3	1.5	2
G	5	-	1	1	1.5	1	1
H	5	5	6	3	2	2	4

[1] Data excluded from analyses (see Data Exclusions).

lead acetate, and toluene, six to seven laboratories arrived at the same TD values. The range of TDs covered only two "steps" (out of the series of seven doses) for those four chemicals, whereas the range covered three to four "steps" for the remaining three chemicals. DDT and parathion showed the widest spread in values, covering a three-fold range (58-195.8 mg/kg and 3-10.13 mg/kg, respectively). Even with this range of values, however, there was a similar number of laboratories selecting each dose.

For comparison purposes, the published LD50 values for these chemicals are listed in Table 3, as well as the strains of rat used in each laboratory. Lethality obtained in each study are presented in Table 4. For most chemicals, the TDs derived in these studies were close to half of the LD50 values. In the single-dose studies, most laboratories reported few or no deaths at the 100%-TD dose level. In fact, out of the total 51 acute studies, there was no lethality in 33 of them, and only slight lethality (one rat in a dose group) in an additional nine studies. Of the remaining nine studies, lethality occurred in less than half of the high-dose group except for lead acetate (one laboratory had 60% deaths), parathion (one laboratory had 50%), and TET (two laboratories had 70-90%; these used the highest TD). Thus this approach of determining and using a maximally-tolerated dose as the high dose for acute studies worked reasonably well in this Collaborative Study.

The repeated-dosing studies were also based on the TD, with the highest dose specified to be 50% of the TD. Unfortunately, this method of setting doses in a four-week dosing paradigm did not work well, and considerable lethality occurred in the 50%-TD dose group for four chemicals (acrylamide, lead acetate, parathion, and TET; see Table 4). Furthermore, for three of these chemicals deaths also occurred at the 25%-TD level, sometimes leaving only two dose groups with which to assess dose-response characteristics. These results indicate that the algorithm was not appropriate for setting dose levels for repeated-dosing studies.

Acrylamide

There was good agreement among the laboratories in the TD determinations, with 113.3 mg/kg selected as the TD in seven laboratories and 75.5 mg/kg selected in the remaining laboratory (see Table 3). The TOPE values were generally long, with only one laboratory choosing a time as short as three hours (see Table 2). The remaining laboratories chose either five hours (four laboratories) or six hours (two laboratories) as the TOPE; one laboratory chose eight hours even though the protocol specified test times only up to six hours.

Acute Acrylamide Dosing. The TD was tolerated in the acute studies, with only two laboratories reporting 12.5% and 20% lethality following single-dose administration (see Table 4). In the remaining laboratories, no deaths or moribund animals were reported.

Across-laboratory analysis of functional domain effects was accomplished by subjecting the treatment group means from each laboratory to two-way ANOVA with the grouping factor of dose and repeated measures across time. The effects on functional domains are shown as mean severity scores in Figure 1, whereas data for the physiolog-

TABLE 3. Top Doses (mg/kg) Determined by Each Laboratory.

Laboratory	Chemical						
	Acrylamide	Bis-Acrylamide	*p,p'*-DDT	Lead Acetate	Parathion	Triethyl Tin	Toluene
A[1]	113.3	147	195.8	200	4.5	4	2000[b]
B[2]	75.5	147	58	200	4.5	4	2000
C[1]	113.3	220	-[a]	-	4.5	-	-
D[2]	113.3	147	87	200	6.75	9	2000
E[3]	113.3	147	87	133.3	6.75	6	2000
F[1]	113.3	147	130.5	200	3	4	2000
G[2]	113.3	220	87	133.3	6.75	6	1333
H[4]	113.3	147	58	200	10.13	9	2000
LD_{50}(mg/kg)	170[5]	390[6]	113-450[7]	286[8]	13-30[9]	≈6[10]	5000[6]

[a] Data excluded from analyses (see Data Exclusions). [b] Limit dose. [1] Wistar rats. [2] Sprague-Dawley rats. [3] Long-Evans hooded rats. [4] WAG/Rij/MBL inbred rats. [5] Windholz, 1976. [6] Sweet, 1988. [7] Hayes, 1982a. [8] Pryor *et al.*, 1983. [9] Hayes, 1982b. [10] Barnes and Stoner, 1958

ical measures (body weight and temperature) are expressed as mean change from baseline (time-0) values in Figure 2. At this overall level of analysis, statistical results indicated that a single dose of acrylamide produced mild but statistically significant effects in the Activity, Convulsive, Excitability, Neuromuscular, and Sensorimotor domains, while Autonomic function was unaffected. The greatest magnitude of change was detected in the Activity domain. Subsequent one-way ANOVAs conducted at each time point indicated that the time course of effects was different for the different domains. Significant effects on the Activity, Convulsive, and Excitability domains could be detected at both the TOPE and 24 hours post-exposure; on the Neuromuscular domain, at the TOPE and one week post-exposure; and on the Sensorimotor domain, at 24 hours and one week post-exposure. In addition, body weight and body temperature were affected. A significant decrease in body temperature was detected at the TOPE. Even though the overall treatment-by-time interaction for body weight was significant, the apparent loss at 24 hours did not reach statistical significance ($p<0.061$).

In general, this pattern of effect was not mirrored in any single laboratory. Individual laboratory profiles ranged from no significant domains (laboratory G) to significant effects in all domains (laboratory E). Some effects on individual measures, however, were more consistent than the profiles may indicate. Table 5 presents the results of statistical analyses of both the domains and individual test measures for each laboratory. The Activity domain was most consistently altered: seven of the eight laboratories obtained significant effects at the TOPE, although one of the laboratories had no individual endpoint significantly altered at that time. Activity domain effects were due primarily to decreased motor activity seen at the TOPE in six laboratories, accompanied by decreased rearing in three laboratories. In most instances where there was lowered motor activity, the activity levels were decreased to 50% or less than that of vehicle controls; these data are presented in Figure 3. Furthermore, effects on motor activity could be detected 24 hours post-exposure in four laboratories (Figure 3). The only residual effect on activity at the one-week time point was increased rearing, reported in one laboratory.

Excitability domain effects were evident in four laboratories at the TOPE, which persisted to the 24-hour time point in three cases (see Table 5). Examination of the individual measures comprising this domain revealed that increased resistance to handling and/or home-cage removal in the high-dose group were the most prominent effects. Figure 4 shows the magnitude of change, where present, for the excitability measures (ease of removal and handling reactivity) in contrast to those laboratories which did not obtain effects. The differences between laboratories may be due to the strain of rat, since none of

TABLE 4. Lethality Occurring in Each Laboratory, During Single and Repeated Dosing With Each Chemical.

	Percent Lethality For Each Chemical (Single and Repeated Dosing)																				
	Acrylamide			Bis-Acrylamide			*p,p'*-DDT			Lead Acetate			Parathion			Toluene			Triethyl Tin		
	Single	Repeated		Single	Repeated		Single	Repeated		Single	Repeated		Single	Repeated		Single	Repeated		Single	Repeated	
	100%[a]	50%[b]	25%	100%	50%	25%	100%	50%	25%	100%	50%	25%	100%	50%	25%	100%	50%	25%	100%	50%	25%
Lab																					
A	0%	100%	(10%)[2]	0%	(0%)[3]	0%	10%	(0%)[4]	0%	20%	90%	60%	10%	(0%)[5]	0%	0%	10%	0%	0%	100%	70%
B	0	100	0	0	10	0	10	0	0	60	100	50	0	0	0	0	0	0	0	100	100
C	12.5	100	50	0	37.5	12.5	-[c]	-	-	-	-	-	12.5	-	-	-	-	-	-	-	-
D	0	(20)[1]	0	0	10	0	40	0	0	0	100	70	20	100	0	0	0	0	70	100	100
E	20	100	70	0	10	0	0	0	0	40	80	40	0	30	0	0	30	0	10	100	100
F	0	100	12.5	0	0	0	0	0	0	12.5	100	37.5	0	12.5	12.5	0	0	0	0	100	100
G	0	100	40	-[c]	10	0	0	0	0	0	90	50	50	70	0	0	0	0	0	100	100
H	0	100	30	0	10	0	10	10	0	10	100	100	0	40	0	0	0	0	90	100	100

[a] The highest dose in the single-dose studies was 100% of the Top Dose (TD). [b] The highest dose in the repeated-dosing studies was 50% of the TD. [c] Data excluded from analyses (see Data Exclusions). [1] Stopped dosing after 2 weeks. [2] Stopped dosing after 17 doses. [3] All rats were sacrificed after 2 weeks. [4] All rats were sacrificed after 2 doses. [5] All rats were sacrificed after 1 week.

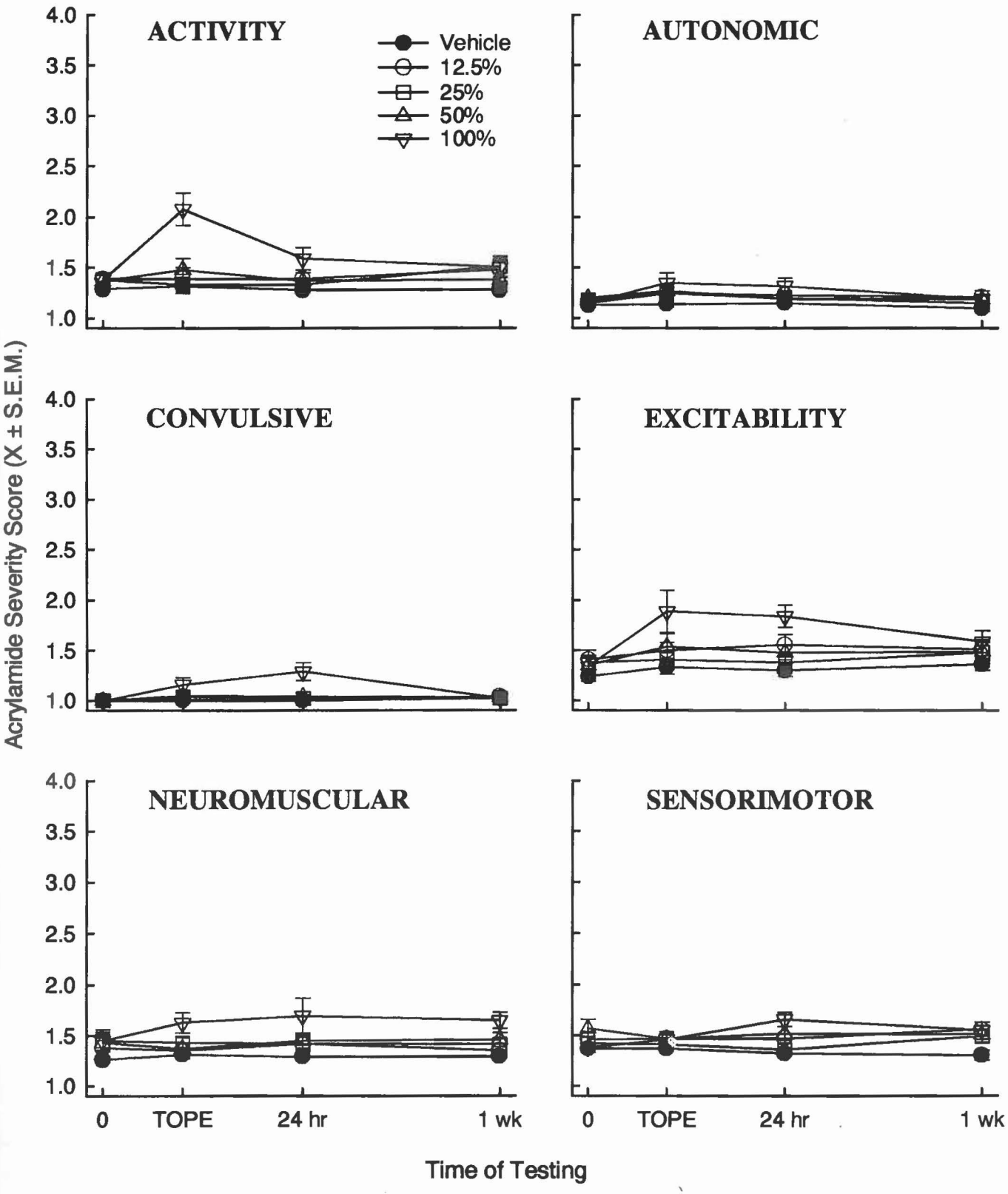


FIG. 1. Effects of acute acrylamide on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects in the Activity, Convulsive, Excitability, Neuromuscular, and Sensorimotor Domains.

the laboratories using Sprague-Dawleys reported increased excitability. Otherwise, there are no obvious differences, e.g., higher control baseline scores, which might prevent the detection of such an effect.

Significant effects were also detected in the Convulsive domain in four of the seven laboratories for which data were available (excluding the data from laboratory A; see Data Exclusions, above). In two laboratories, significant domain effects were detected at the TOPE, and in three laboratories at the 24-hour time point. In almost all instances, the reported observation was mild tremors in the high-dose group (100% TD), the incidence of which ranged from 30%-100%.

Treatment effects were detected for the Neuromuscular domain in five laboratories, although the affected time points varied across laboratories. In one laboratory, significant effects were only obtained at the one-week time point, in another only at 24 hours, and in yet another the domain was significantly affected at all test times. Examination of the individual measures comprising this domain indicated that the most consistent alterations were 1) gait disturbances at both the TOPE and 24 hours post-exposure, and 2) increased landing foot splay observed mostly at one week. The changes in these two measures, however, were generally mild. The ranking of gait abnormalities mostly indicated slight to moderate changes described as ataxia, with uncoordinated placement of the hindlimbs. In the four laboratories which detected increased foot splay at one week, the magnitude of the increase was 21-38% over control values.

There was little consistency in the effects reported for the measures comprising the Sensorimotor domain, although significant overall domain effects were found in three of the eight laboratories. The measures affected as well as the time-course and dose-response were quite variable among the laboratories, and the magnitude of effects were generally slight. Changes in the auditory click response were the most frequently reported, but some laboratories reported increases and others decreases. Similar results were evident with the tail-pinch response.

Only two laboratories obtained significant Autonomic domain effects; lacrimation and a lack of the pupil response were reported in these laboratories only. Effects on defecation and urination were more often reported, but the direction of change and time-course was not consistent among the laboratories.

Body temperature was lowered at the high dose in all laboratories at the TOPE, and the magnitude of the hypothermia ranged from 0.5° to 1.6°C lower than controls. Two laboratories still showed effects at 24 hours (hypothermia in one laboratory, hyperthermia in the other). Body weight loss at 24 hours was reported in all laboratories, with group means for the 100%-TD treatment ranging from 90-98% of controls at that time. In five laboratories, body weight had not returned to control levels at one week. Piloerection was only reported in one laboratory.

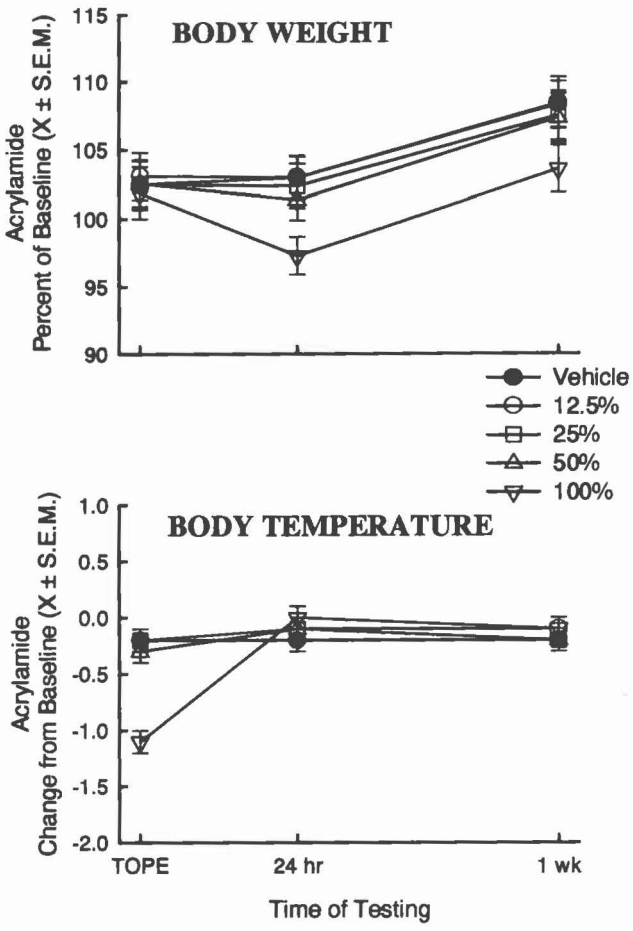


FIG. 2. Effects of acute acrylamide on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects on both measures.

Repeated Acrylamide Exposures. Repeated dosing of acrylamide produced clear cumulative lethality (see

TABLE 5. Acrylamide: Effects[a,b] of a Single Dose in Each Laboratory, Presented in Descending Order of Top Dose and TOPE.

Lab	TD	TOPE	Time of Testing		
			TOPE	24 hr	1 wk
Activity					
F	113.3	8	˅ MA, ˅ REAR	˅ ma	-
C	113.3	6	-*	-	-
D	113.3	6	˅ MA	-	-*
E	113.3	5	˅ MA, ˅ REAR	˅ MA	-
G	113.3	5	-	-	^ rear
H	113.3	5	˅ MA, ˅ REAR	˅ MA	-
A	113.3	3	˅ MA	-	-
B	75.5	5	˅ MA	˅ ma	-
Autonomic					
F	113.3	8	-	-	^ def, ^ urin
C	113.3	6	˅ def	-	˅ def'
D	113.3	6	^ urin	^ urin'	-
E	113.3	5	˅ DEF, PUPIL, LACRIM	˅ DEF, PTOSIS, LACRIM	-
G	113.3	5	-	˅ urin'	-
H	113.3	5	-	-	˅ urin'
A	113.3	3	-	-	-
B	75.5	5	˅ def, lacrim	PUPIL	-
Convulsive					
F	113.3	8	TREM	-	-
C	113.3	6	-	-	-
D	113.3	6	-	TREM	-
E	113.3	5	TREM, MYOC	TREM	-
G	113.3	5	-	myoc	-
H	113.3	5	-	TREM	-
B	75.5	5	trem	trem	-
Excitability					
F	113.3	8	^ REM	^ rem	-
C	113.3	6	-	-	-
D	113.3	6	-	-	-
E	113.3	5	^ REM, ^ HAND	^ REM, ^ AR	-
G	113.3	5	-	-	-
H	113.3	5	^ HAND	^ HAND	-
A	113.3	3	^ REM, ˅ AR	^ HAND	-
B	75.5	5	-	˅ rem	-
Neuromuscular					
F	113.3	8	gait	-	right'
C	113.3	6	GAIT	GAIT, RIGHT, ˅ HGRIP	gait, ^ splay
D	113.3	6	^ FGRIP	gait	^ SPLAY
E	113.3	5	GAIT	GAIT, ˅ HGRIP, ^ SPLAY	-*
G	113.3	5	-	-	-
H	113.3	5	gait	GAIT, ˅FGRIP	^ splay, ^ hgrip
A	113.3	3	gait, ˅ splay	gait	^ SPLAY
B	75.5	5	-	-	-
Sensorimotor					
F	113.3	8	˅ touch'	˅ click'	^ touch'
C	113.3	6	-	-*	^ click
D	113.3	6	^ click'	˅ click'	-
E	113.3	5	^ appr, ^ click'	^ CLICK	-
G	113.3	5	-	^ click	-
H	113.3	5	-	^ tp	^ tp
A	113.3	3	˅ TP	˅ TP	-
B	75.5	5	-	-	-
Physiological Measures					
F	113.3	8	˅ temp	˅ wt	˅ wt
C	113.3	6	˅ temp	˅ wt	-
D	113.3	6	˅ temp	˅ wt, ^ temp	˅ wt
E	113.3	5	˅ temp	˅ wt, ˅ temp	˅ wt
G	113.3	5	˅ temp	˅ wt	-
H	113.3	5	˅ temp, pilo	˅ wt, pilo	˅ wt
A	113.3	3	˅ temp	˅ wt	˅ wt
B	75.5	5	˅ temp	˅ wt	-

[a] Measures significantly affected, at any dose, are listed for each time point. Capital letters indicate that the domain for that laboratory was significant at that time point. Arrows indicate direction of change, where applicable.

[b] Abbreviations are as follows: appr, approach response; ar, arousal; def, defecation; click, click response; conv, convulsions; fgrip, forelimb grip strength; gait, gait changes; hand, handling reactivity; hgrip, hindlimb grip strength; lacrim, lacrimation; pupil, inhibited pupil response; ma, motor activity; myoc, myoclonus; opisth, opisthotonus; pilo, piloerection; post, abnormal home-cage posture/activity; rear, rearing; rem, removal reactivity; right, altered righting reflex; sal, salivation; smack, mouth-smacking; splay, landing foot splay; temp, body temperature; touch, touch response; tp, tail-pinch response; trem, tremors; urin, urination; wt, body weight.

' data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose

* Domain significant at time point but no individual measure was significant

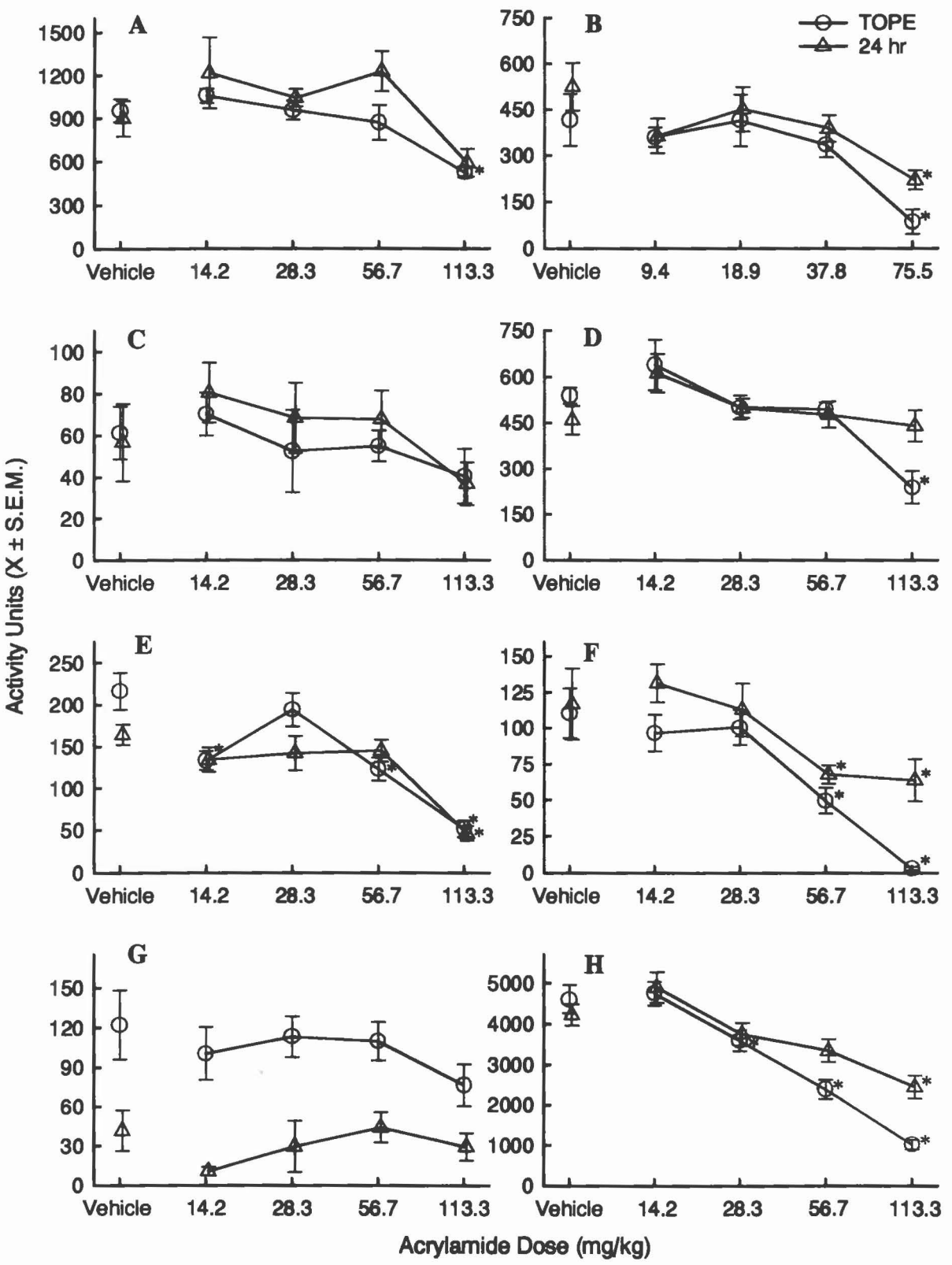


FIG. 3. Effects of acute acrylamide on motor activity (mean total activity units during the session ± S.E.M.) in individual laboratories, at the TOPE (circles) and at 24 hours (triangles). Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

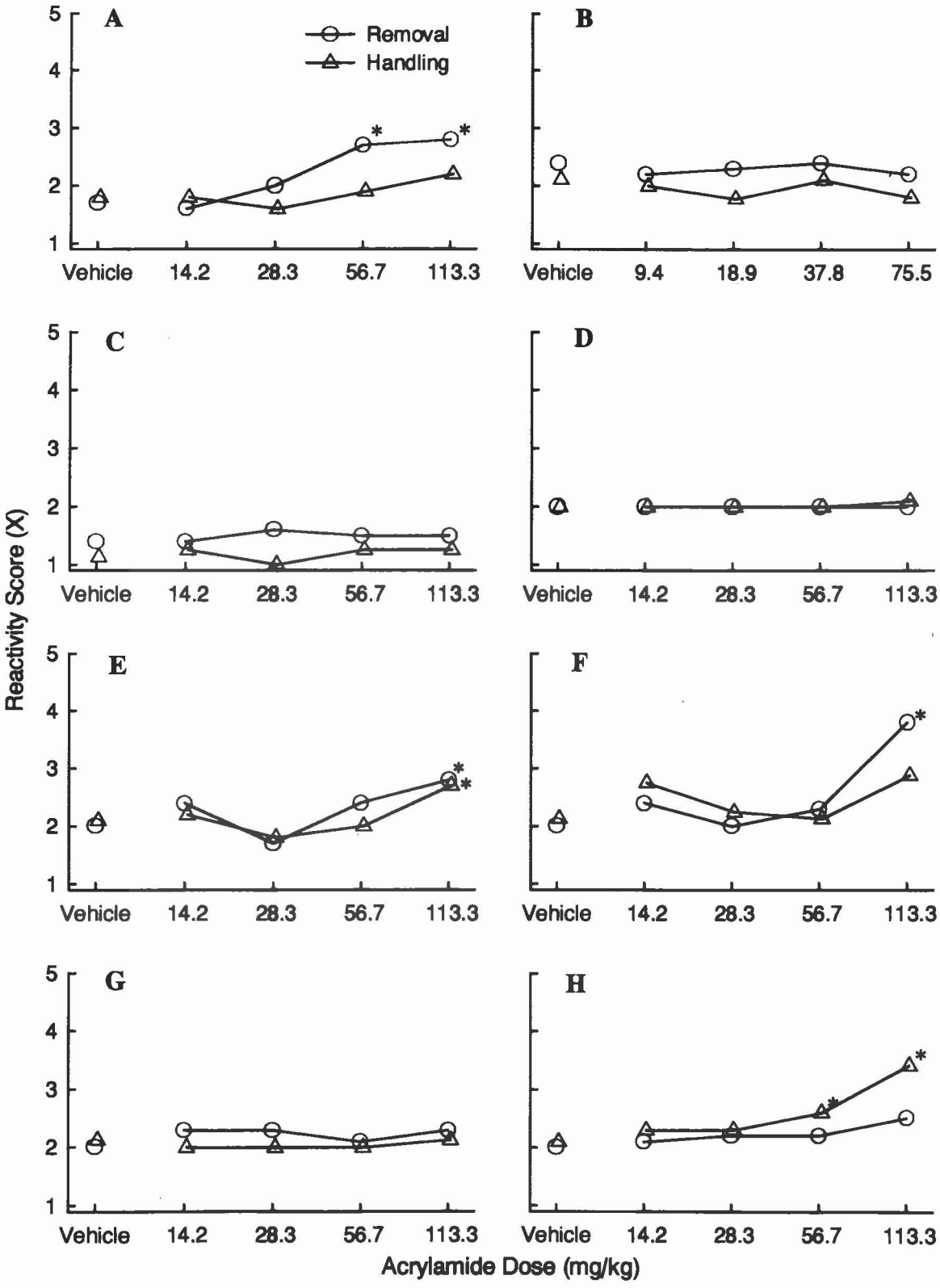


FIG. 4. Effects of acute acrylamide on excitability measures, ease of removal (circles) and handling reactivity (triangles), at the TOPE in individual laboratories. Data are presented as mean score for each treatment group. These endpoints are ranked from '1' (no reactivity) to either '4' for handling reactivity or '5' for ease of removal (extreme reactivity). Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

Table 4). Administration of the high dose (50% of the TD) produced 100% lethality in all laboratories (except in the laboratory that stopped dosing due to morbidity). At the next-highest dose (25% TD), six laboratories reported mortality which ranged from 12.5% to 70%; these deaths typically occurred in the last two weeks of dosing. Furthermore, three laboratories reported 10-25% mortality at the next-lower dose (12.5% TD). In the repeated-measures analyses, data for the high-dose group were eliminated as well as the next-highest dose group for laboratory E, which obtained 70% lethality at that dose. In addition, the data at two weeks and four weeks (only for laboratory E) were analyzed separately with all doses included, in order to assess the effects of acrylamide at those time points.

The composite severity scores calculated across laboratories for the effects of repeated acrylamide are presented in Figure 5. One-way ANOVA of the two-week data indicated that the high-dose group (50% TD) produced significant effects on all domains. This illustrates the magnitude of the toxicity produced by that dose level. Repeated-measures analyses of the lower dose groups revealed significant effects, mostly at four weeks, for all except the Convulsive domain. Only the Neuromuscular domain was significant at the lower dose levels at two weeks, and recovery was not complete in the Autonomic, Activity, and Neuromuscular domains at six weeks. The Neuromuscular domain clearly displayed the greatest magnitude of effects, followed by the Activity domain. At two weeks, weight loss and hypothermia were significant across laboratories in the high-dose group, as shown in Figure 6. The data from the lower dose groups showed that body temperature was also decreased at four weeks. Analysis of body weight data in the lower dose groups produced a significant dose-by-time interaction, but no single time-point ANOVA attained statistical significance.

The profiles of effect across domains for the individual laboratories were generally similar to the composite profile, with the Neuromuscular, Activity, and Autonomic domains altered by most if not all of the laboratories. The exceptions were Excitability and Sensorimotor function, where the overall composite analysis indicated slight effects but only one to two laboratories detected significant changes. At two weeks, which included the highest dose in the analysis, significant effects were obtained on the Neuromuscular and Activity domains (seven laboratories each). Only two or fewer laboratories showed effects on the remaining domains. The seven laboratories for which data were available for the next-highest dose showed significant Neuromuscular domain effects at four and six weeks; values for the eighth laboratory approached significance ($p<0.056$), even though only the two lowest doses were included in the analysis (due to the 70% lethality at 25%-TD). Thus, neuromuscular changes clearly dominated the profile in terms of magnitude of effect as well as effective doses. Activity domain effects were obtained at four and six weeks in half of the laboratories, and Autonomic effects were evident more at six weeks (five laboratories showed significant effects) than at four weeks (one laboratory). Overall effects were not reflected in the Excitability domain (two laboratories significant) or the Sensorimotor domain (one laboratory significant, but no endpoint affected at that time point).

The effects of acrylamide for each laboratory on the different domains and on the measures comprising those domains are presented in Table 6. For many endpoints, changes that were evident at two weeks in the high dose group (indicated in brackets) were produced at four weeks by lower, non-lethal doses. Within the Neuromuscular domain, both gait score and landing foot splay were the most sensitive measures. These were the only two test measures affected by doses lower than 50%-TD after only two weeks of dosing in most laboratories. Data for the dose-response and time-course of ranking these gait changes are shown in Figure 7. The gait changes generally emerged within two weeks at both the 50%- and 25%-TD dose levels, and persisted out to six weeks. Ataxia and uncoordinated placement of the hindlimbs were descriptions of early abnormalities, progressing to splaying of the hind feet, knuckling of the paws, or dragging the hindlimbs as indications of more severe neurotoxicity. The magnitude of effect (average score) observed at four weeks in the 25%-TD dose group was remarkably similar to the effects of the 50%-TD dose at two weeks. In addition, the least alterations were seen in laboratory B which used the lowest dose range (50%-TD=37.8 mg/kg). Increased landing foot splay, presented in Figure 8, was also evident at two weeks, at which time all laboratories displayed significant effects. Furthermore, in all but one laboratory the effects of the 25%-TD dose group were significantly different from control at four weeks. As with gait score, foot splay alterations persisted to six weeks in some laboratories.

Grip strength (which is included in the Neuromuscular domain) was altered in all laboratories, with hindlimb grip strength showing somewhat more effects than forelimb. Lowered hindlimb grip strength emerged in the 25%-TD dose group at four weeks in all but one laboratory (B); in five laboratories this effect persisted at the six-

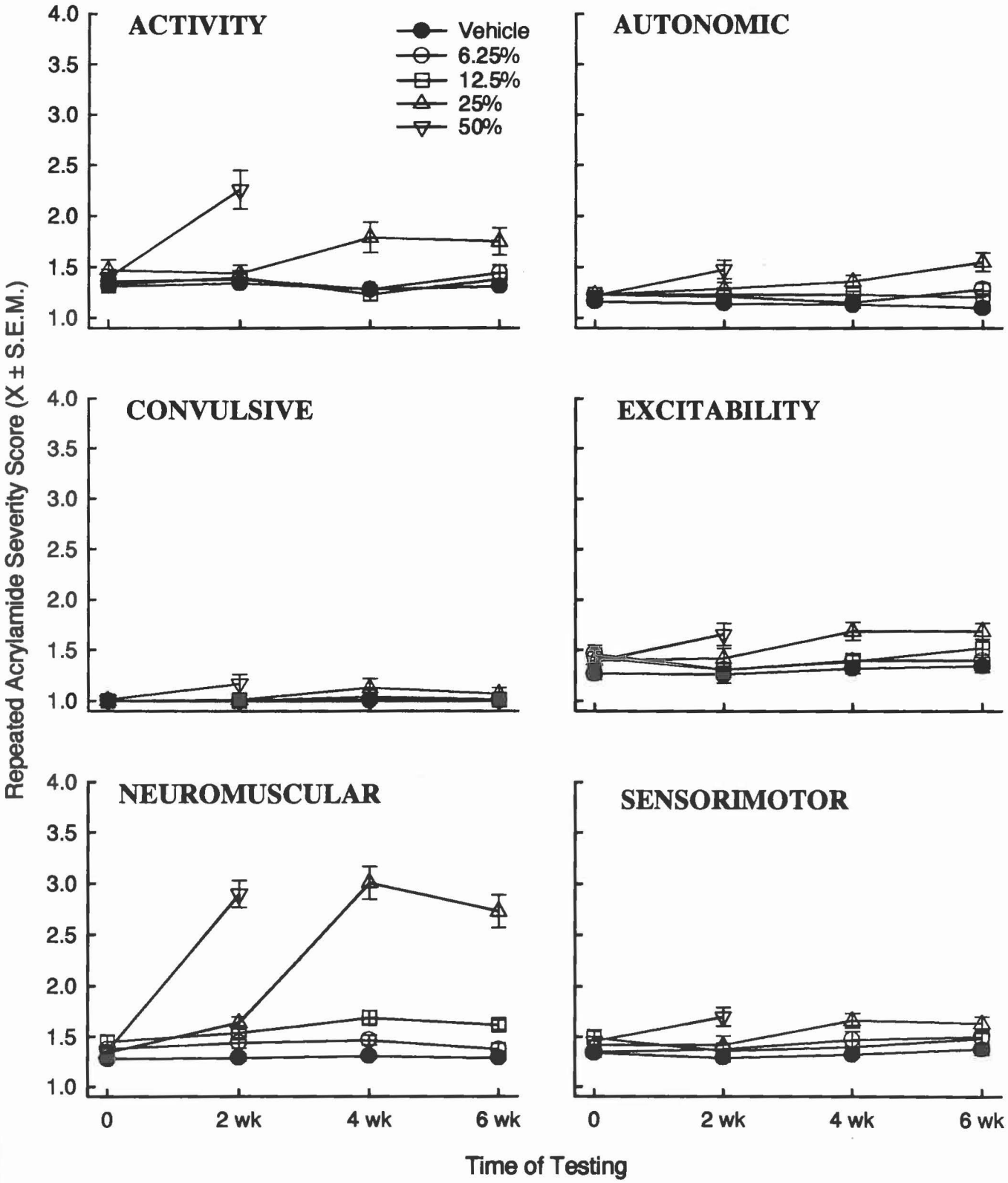


FIG. 5. Effects of repeated administration of acrylamide on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Data for the 50%-TD treatment are shown at two weeks only, due to lethality in that dose group; all domains were altered by that dose. Two-way ANOVAs of the lower dose groups revealed significant overall effects in the Activity, Autonomic, Excitability, Neuromuscular, and Sensorimotor Domains.

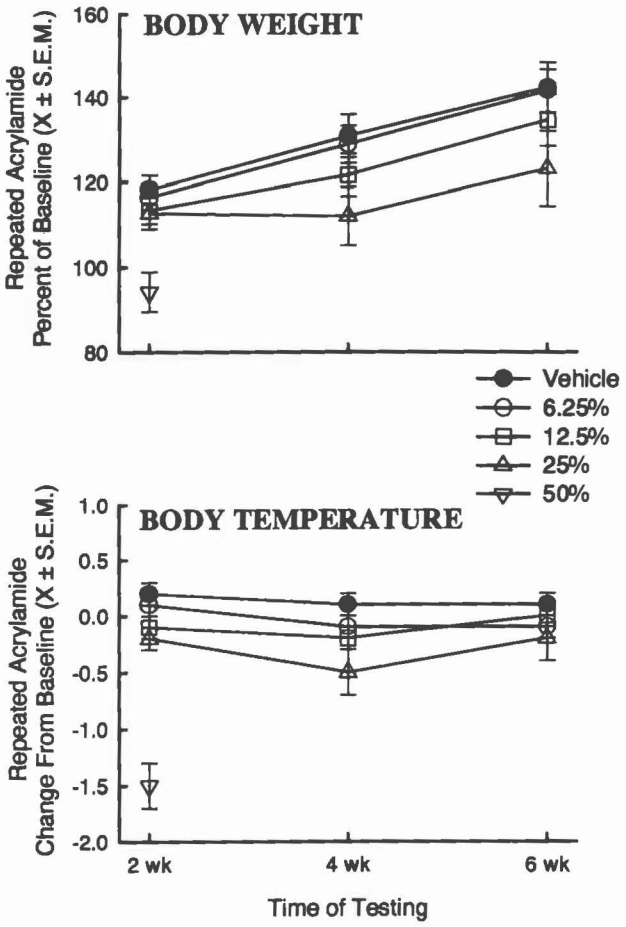


FIG. 6. Effects of repeated administration of acrylamide on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Data for the 50%-TD treatment are shown at two weeks only, due to lethality. Two-way ANOVAs revealed significant overall effects on both measures.

week test. Decreased forelimb grip strength followed a pattern similar to hindlimb grip, except that recovery was more rapid such that by six weeks, effects were no longer evident in six laboratories. At four weeks, the 25%-TD dose group had hindlimb grip strength values ranging from 38% to 71% of control, whereas for forelimb grip the values ranged from 48% to 84% of control; i.e., hindlimb grip tended to be more affected than forelimb. The righting reflex was affected in about half of the laboratories, but the dose-response and time-course were not as consistent as the other measures.

The Activity domain also showed fairly consistent changes. Motor activity was decreased in six of the eight laboratories at two weeks, and in all but one of these the effects were seen only at the highest dose. At four weeks, lower doses (≤25% of the TD) decreased activity in five laboratories. In one laboratory, however, a lower dose (12.5% TD) increased activity levels (33-40% above control levels) at both two and four weeks. Rearing was also depressed in a majority of laboratories in the high-dose group at two weeks, but was not as consistently altered at four or six weeks. Home-cage activity measures indicated that in some laboratories, rats were lying prostrate, or flattened in the cage.

Autonomic function was altered at two weeks, mostly in the high-dose group, but no measure was consistently affected in even half of the laboratories. In contrast, a clear effect of increased defecation emerged at six weeks in six laboratories. Diarrhea or soft stools, however, were not observed at any time. Reports of tremors (20-80%) or myoclonus (20%) were listed in four laboratories. Alterations in the Sensorimotor and Excitability domains displayed no consistent pattern in terms of time-course, effective doses, or direction of change on the different measures, and the effects were minor in terms of magnitude.

Body weight and growth were clearly affected by acrylamide. Weight loss was generally reported in the 50%-TD dose group at two weeks, and in the 25%-TD group at four weeks. Average weight in the 50%-TD group at two weeks was 72-90% of the control values, with the highest value (90%) obtained in the laboratory which used the lower dose range. Weights in the 25%-TD group at four weeks ranged from 67% to 95% of control, again with the highest value obtained in the laboratory which used lower doses, and the lowest value from the laboratory which reported 70% lethality at that dose; the remaining laboratories ranged from 79% to 87% of control. Depressed weight gain was evident in the lower dose groups, but group means were usually >90% of control values at the end of four weeks. Hypothermia, ranging from 0.7° to 2.4°C lower than controls, was also reported in all laboratories and was usually, but not always, confined to the high-dose group. Piloerection was recorded in half of the laboratories.

N,N′-Methylene Bis-Acrylamide

Six laboratories selected 147 mg/kg as the TD for bis-acrylamide, and the remaining two reported 220 mg/kg (Table 3). There was more disagreement on the

TABLE 6. Acrylamide: Effects[a,b] of Repeated Doses in Each Laboratory, Presented in Descending Order of Top Dose.

Lab	TD	Time of Testing		
		2 wk	4 wk	6 wk
Activity				
F	56.7	-, [↓ MA, ↓ REAR]	↓ MA, ↓ REAR	↓ MA, ↓ REAR
C	56.7	-, [↓ MA, ↓ REAR, POST]	-	-
D	56.7	↑ ma[1]	↓ ma, ↑ ma[1]	-*
E	56.7	-, [↓ MA]	-, [↓ MA]	-
G	56.7	-, [↓ REAR]	-	-
H	56.7	-, [↓ MA]	↓ MA	↓ MA
A	56.7	↓ MA, [↓ REAR, POST]	POST	↓ REAR, POST
B	37.8	-, [↓ MA, ↓ REAR]	↓ MA, ↓ REAR, POST	-
Autonomic				
F	56.7	-, [↓ DEF, SAL]	-	-
C	56.7	↓ DEF, ↓ URIN[1]	↓ urin	↓ DEF
D	56.7	-, [↑ urin]	↑ DEF	↑ DEF, ↑ URIN
E	56.7	-, [ptosis]	-, [ptosis]	↑ def
G	56.7	-, [↓ DEF]	-	↑ def
H	56.7	-, [pupil, ptosis]	↑ def	↑ DEF, ↑ URIN
A	56.7	-, [PUPIL]	-	↑ DEF
B	37.8	-	-	↑ DEF
Convulsive				
F	56.7	-	-	-
C	56.7	-, [TREM]	-	-
D	56.7	-	-	-
E	56.7	-	-, [TREM, MYOC]	-
G	56.7	-, [TREM]	-	-
H	56.7	-	-	-
B	37.8	-	TREM	TREM
Excitability				
F	56.7	-	↑ HAND	-
C	56.7	-	-	↓ ar[1]
D	56.7	-	↓ ar	-
E	56.7	-	-	-
G	56.7	-, [↑ HAND]	-	-*
H	56.7	↓ ar[1]	↑ REM, ↓ AR[1]	↑ REM, ↓ AR[1]
A	56.7	-, [↓ ar]	↓ rem, ↓ hand	-
B	37.8	-	↓ hand	-
Neuromuscular				
F	56.7	GAIT, ↑ SPLAY, [↓ FGRIP, ↓ HGRIP, RIGHT]	GAIT, RIGHT, ↑ SPLAY, ↓ FGRIP, ↓ HGRIP	GAIT, RIGHT, ↑ SPLAY
C	56.7	GAIT, [↑ SPLAY, ↓ FGRIP, ↓ HGRIP]	GAIT, ↓ HGRIP	GAIT, ↓ FGRIP, ↓ HGRIP
D	56.7	-, [GAIT, ↑ SPLAY, ↓ FGRIP, ↓ HGRIP]	GAIT, ↑ SPLAY, ↓ FGRIP, ↓ HGRIP	GAIT, ↑ SPLAY, ↓ HGRIP
E	56.7	-, [GAIT, ↑ SPLAY, ↓ FGRIP, ↓ HGRIP, RIGHT]	↑ splay, [GAIT, ↓ FGRIP, ↓ HGRIP]	-
G	56.7	gait, right, [↑ SPLAY, ↓ HGRIP]	GAIT, RIGHT, ↑ SPLAY, ↓ FGRIP, ↓ HGRIP	GAIT, RIGHT, ↑ SPLAY, ↓ HGRIP
H	56.7	gait, ↑ splay, [↓ FGRIP, ↓ HGRIP, RIGHT]	GAIT, RIGHT, ↑ SPLAY, ↓ FGRIP, ↓ HGRIP	GAIT, RIGHT, ↑ SPLAY, ↓ FGRIP, ↓ HGRIP
A	56.7	gait, ↑ splay, [↓ FGRIP, ↓ HGRIP]	GAIT, ↑ SPLAY, ↓ FGRIP, ↓ HGRIP	GAIT, RIGHT, ↑ SPLAY, ↓ HGRIP, ↑ FGRIP
B	37.8	-, [gait, ↑ splay, ↓ hgrip]	GAIT, RIGHT, ↑ SPLAY	GAIT, RIGHT, ↑ SPLAY
Sensorimotor				
F	56.7	↓ click[1], ↓ touch[1]	-	-
C	56.7	↑ appr, [↑ tp]	-	↑ appr
D	56.7	↓ click[1]	-	↓ tp
E	56.7	-, [↑ tp, ↑ click, ↑ touch[1]]	-, [↓ tp, ↓ touch]	-
G	56.7	-, [↑ tp]	↓ appr	-*
H	56.7	-	-	↓ tp[1], ↓ touch
A	56.7	↓ tp	↓ tp	-
B	37.8	-	-	-
Physiological Measures				
F	56.7	↓ wt, [↓ temp]	↓ wt	↓ wt
C	56.7	↓ wt, pilo, [↓ temp]	↓ wt, ↓ temp, pilo	↓ wt, ↓ temp, pilo
D	56.7	↓ wt	↓ wt	↓ wt
E	56.7	↓ wt, [↓ temp, pilo]	↓ wt, [↓ temp, pilo]	-
G	56.7	↓ wt, [↓ temp]	↓ wt	↓ wt
H	56.7	↓ wt, ↓ temp, pilo	↓ wt, ↓ temp, pilo	↓ wt, ↓ temp
A	56.7	-, [↓ wt, ↓ temp]	↓ wt, ↓ temp	↓ wt
B	37.8	-, [↓ wt, ↓ temp, pilo]	↓ wt, pilo	-

[a] See Table 5 for key.
[b] Effects listed in brackets are those that were produced only by the doses which were excluded from analyses due to high (>50%) lethality (50%-TD at two weeks in all laboratories, also 25%-TD at four weeks in laboratory E).
[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose.
* Domain significant at time point but no individual measure was significant.

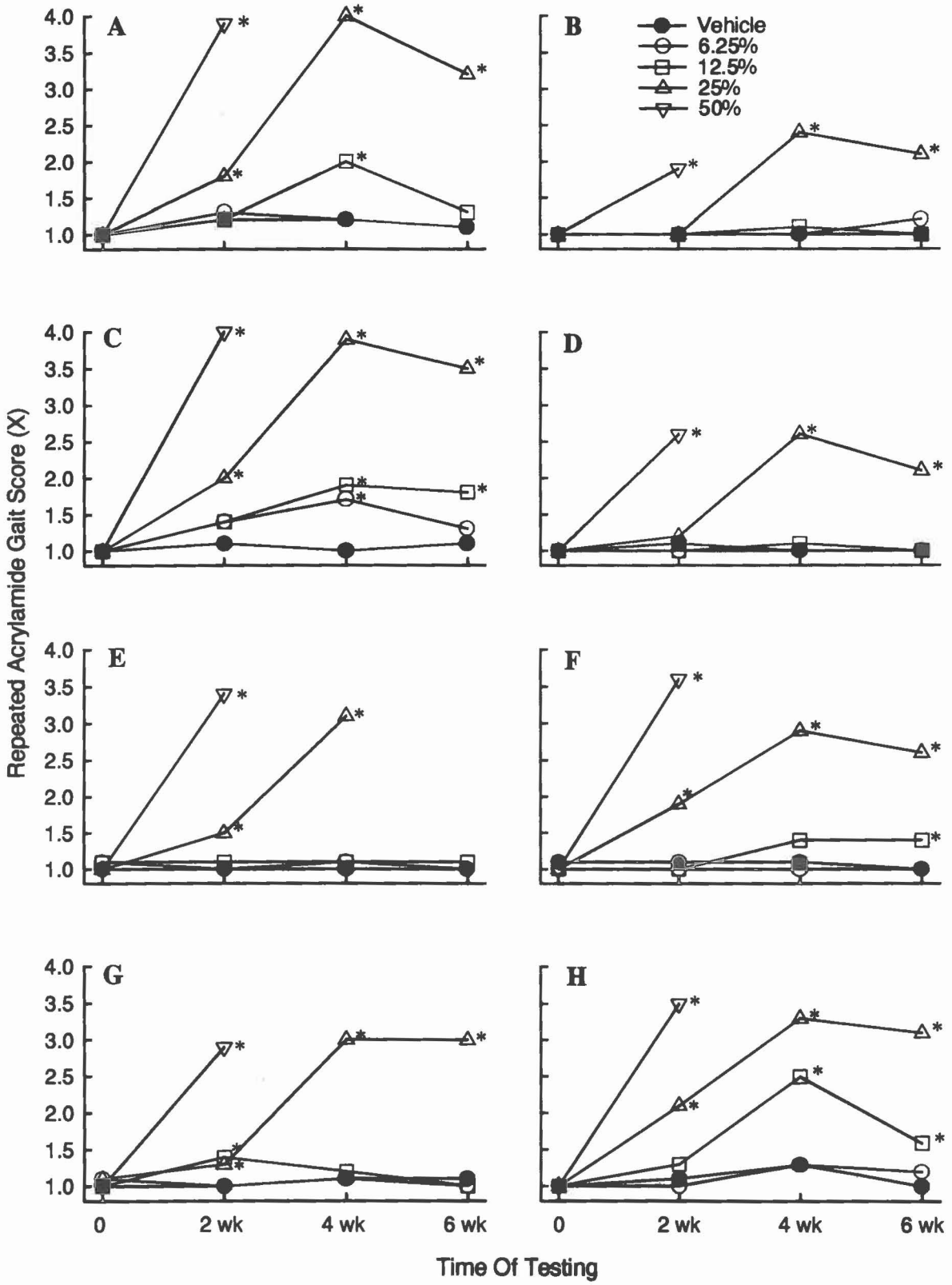


FIG. 7. Effects of repeated administration of acrylamide on the ranking of gait abnormalities in individual laboratories. Gait is scored as '1' (normal), '2' (slightly abnormal), '3' (moderately abnormal), or '4' (severely abnormal). Data are presented as the group means for each dose group (vehicle control or percentages of the top dose) at each time point. Data for the 50%-TD treatment are shown to two weeks only, due to lethality in that dose group. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

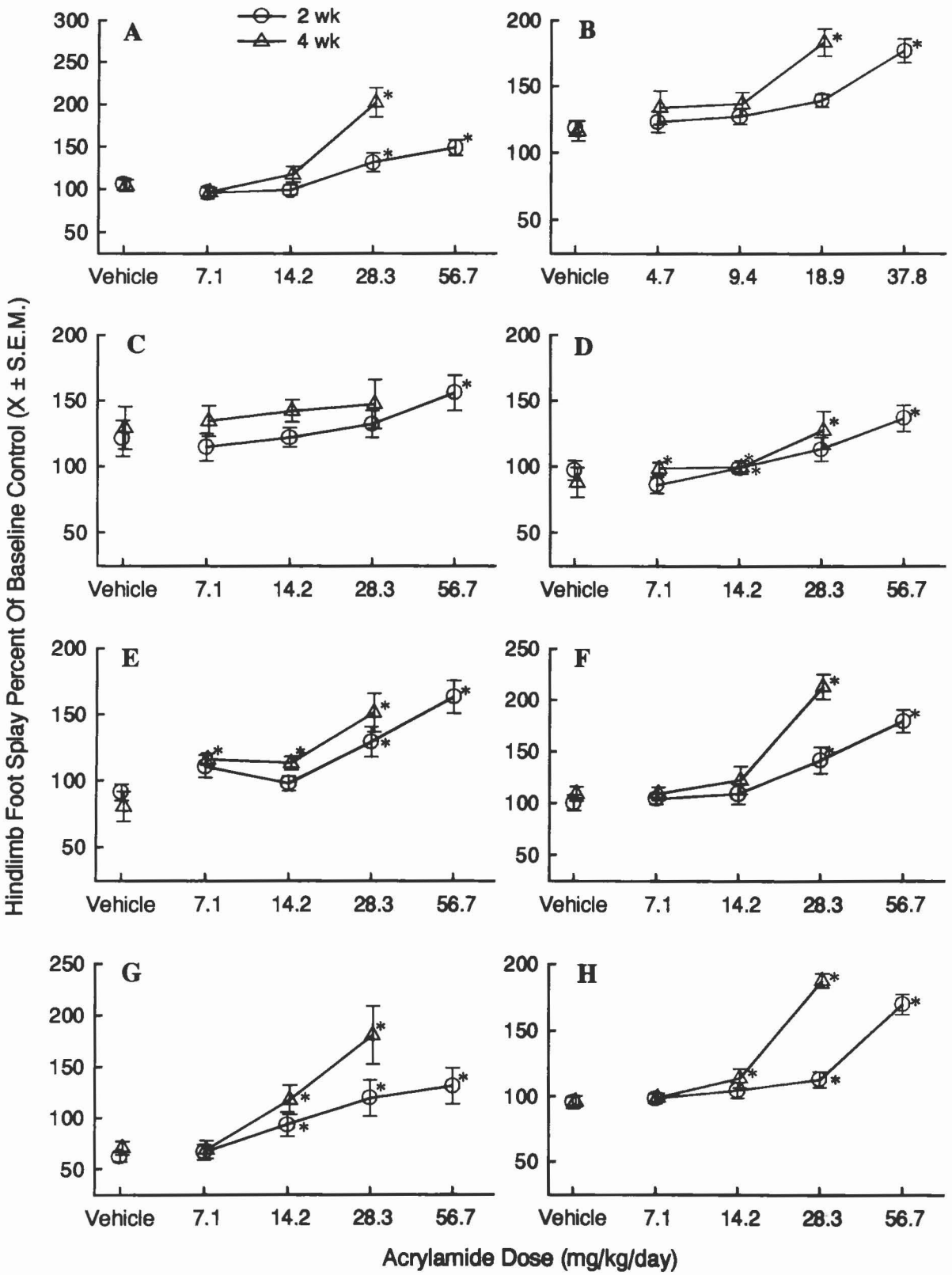


FIG. 8. Effects of repeated administration of acrylamide on landing foot splay, expressed as mean percent (± S.E.M.) of each rat's baseline value, in individual laboratories at two and four weeks. Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

range of times selected as the TOPE, ranging from three to six hours with generally equal distribution across these times (Table 2). No strain-related associations were evident that might explain the discrepant values. Several laboratories reported having a difficult time distinguishing any differences between control and treated rats; this lack of pronounced effects following a single dose obviously made it harder to observe a defined time-course.

Acute Bis-Acrylamide Dosing. The data for only seven laboratories could be used in the acute study, since the eighth laboratory did not examine the rats on the day of dosing (see Data Exclusions, above). There was no lethality at any dose in the evaluation of bis-acrylamide.

When the data for all laboratories were combined in the analysis of functional domains, only the Activity and Neuromuscular domains were significantly affected. The data for all functional domains are presented in Figure 9. Subsequent ANOVAs revealed that these effects were detected for both domains at the TOPE, with residual significant changes at 24 hours (Activity) or one week (Neuromuscular) after dosing. The changes in Activity function were clearly the most pronounced. Body temperature was lowered in the overall-laboratory analysis at both the TOPE and 24 hours, and weight loss was evident at 24 hours and one week. These data are displayed in Figure 10.

The prominent Activity changes which were revealed in the overall laboratory analysis were evident in all individual laboratories as well; these are listed in Table 7. The Activity domain was significant in six laboratories; in the seventh laboratory, the overall analysis was significant but baseline differences between the control and certain dose groups precluded significance in the univariate analyses. These activity changes mostly consisted of decreased motor activity in all laboratories at the TOPE, and in all but one at 24 hours. In most laboratories only the highest dose (100% TD) was effective, but in three laboratories the next-highest dose (50% TD) was significant as well. These data are presented in Figure 11. The magnitude of effect across time varied somewhat: the day after dosing revealed either more depression (laboratory C, which used the highest TD), the same or slightly less effect (five laboratories), or else full recovery (laboratory F). Decreases in rearing responses, however, were only obtained in one to two laboratories at any one time point, and in addition one laboratory reported crouched, motionless home-cage posture in most of the high-dose treatment group.

No individual laboratory obtained significant effects in the Neuromuscular domain at the TOPE, even though the overall-laboratory analysis did indicate significant effects at that time. Only three laboratories obtained significance at 24 hours, and one laboratory at one week. Four laboratories reported gait alterations on the day of dosing; these were described as ataxia and uncoordinated hindlimbs, as well as crouched, or hunched, posture. The relatively few effects on the other measures in this domain at any time point were less consistent.

Although only two laboratories obtained significant Autonomic domain effects at any time, decreased defecation was reported in five laboratories. This effect was recorded at the TOPE in five laboratories, as well as at 24 hours in four laboratories. One laboratory reported lacrimation in the high-dose group. The other reported effects were minor alterations.

Bis-acrylamide produced very few effects on the other measures of the FOB. Arousal was variously affected, being decreased at different times in about half of the laboratories, and showing increases in lower dose groups or at one week. Only one laboratory reported mild tremors, which occurred in 40-60% of the high-dose group. In the sensorimotor measures, there were only small, inconsistent effects.

There was much more agreement in reporting the general toxic sequelae of bis-acrylamide exposure (see Figure 10). All laboratories reported significant hypothermia at the TOPE, with the decreases ranging from 1.6° to 3.0°C lower than controls. Four laboratories reported hypothermia at 24 hours, and also at one week in one laboratory. All rats in the 100%-TD group lost weight at 24 hours, at which time average weights were 90-95% of controls; a few laboratories obtained detectable effects on the day of dosing. In all laboratories, rats showed either continued weight loss, or else depressed weight gain, that was still evident at one week. At that time, average body weights of the high-dose groups ranged from 77-95% of control levels. Six laboratories also reported piloerection in the high dose group, mostly at the TOPE and 24 hours. Thus, although there was no lethality resulting from bis-acrylamide exposure, rats in the high-dose group showed clear signs of compromised general health.

Repeated Bis-Acrylamide Exposures. Four weeks of exposure to bis-acrylamide produced only moderate mortality: one laboratory reported 37.5% lethality at the high dose (50% TD), and four others reported only 10% at that dose level. In this respect, bis-acrylamide was well-tolerated. Laboratory A, however, sacrificed all the rats in the high-dose group after two weeks of dosing due to

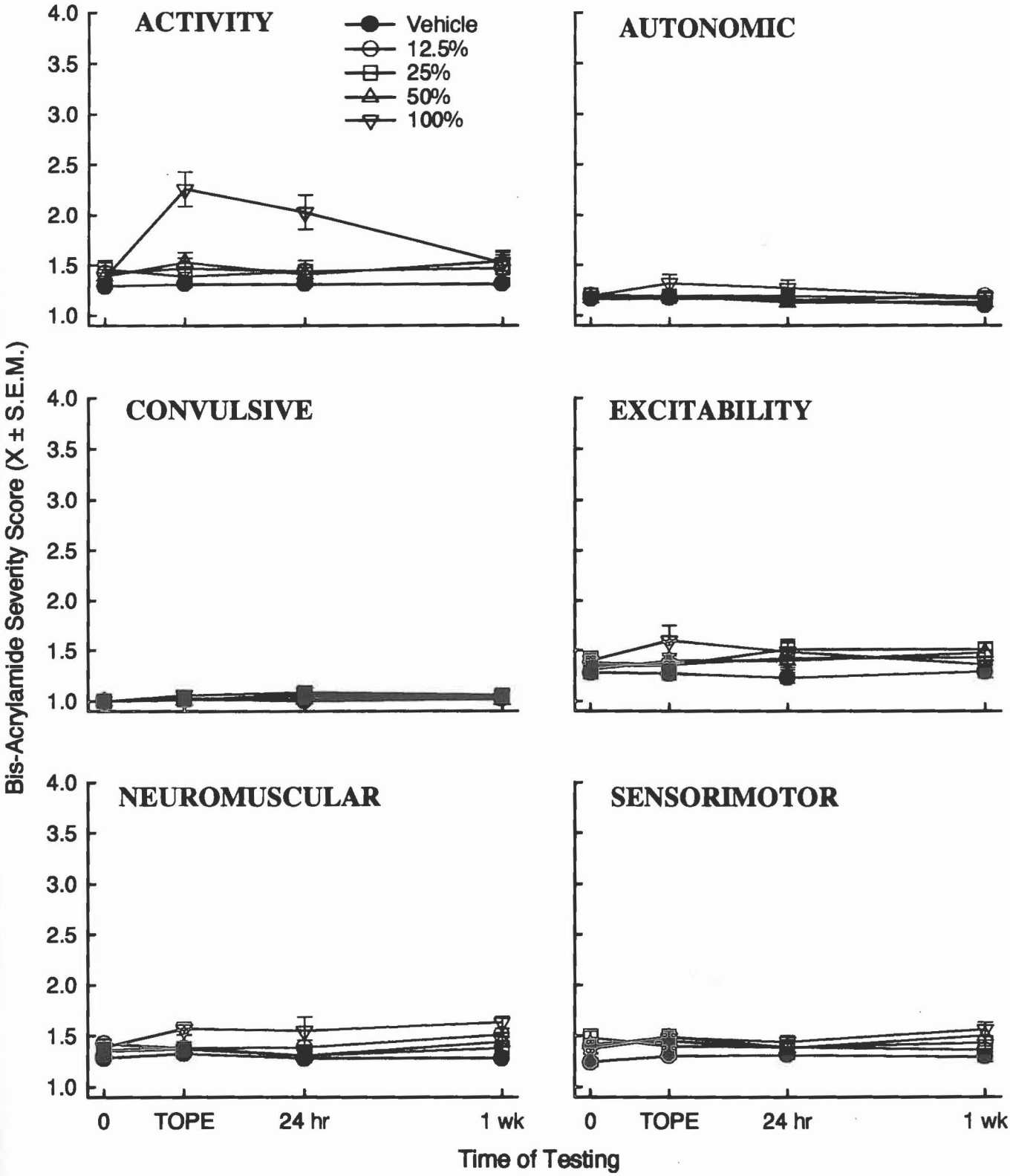


FIG. 9. Effects of acute bis-acrylamide on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects in the Activity and Neuromuscular Domains.

TABLE 7. Bis-Acrylamide: Effects[a] of a Single Dose in Each Laboratory, Presented in Descending Order of Top Dose and TOPE.

Lab	TD	TOPE	Time of Testing		
			TOPE	24 hr	1 wk
Activity					
C	220	6	↓ MA	↓ MA	↓ ma
D	147	6	↓ MA	↓ MA	↓ REAR[1]
E	147	5	↓ MA	↓ MA	↓ rear[1]
H	147	5	↓ MA, ↓ REAR	↓ MA	-
A	147	4	↓ MA, ↓ REAR, POST	↓ MA	-
F	147	4	↓ MA	-	-
B	147	3	↓ ma	↓ ma, ↓ rear	-
Autonomic					
C	220	6	↓ def	↓ def	-
D	147	6	↓ def, pupil	-	-
E	147	5	↓ def	↓ def	-
H	147	5	-	-	-
A	147	4	-	-	-
F	147	4	↓ DEF, ↑ URIN	↓ def	-
B	147	3	↓ def, ptosis, lacrim	↓ DEF, ↓ URIN, LACRIM	-
Convulsive					
C	220	6	-	-	-
D	147	6	-	-	-
E	147	5	-	-	-
H	147	5	-	-	-
F	147	4	-	-	-
B	147	3	-	TREM	trem
Excitability					
C	220	6	-	-	-
D	147	6	↓ AR	-	↓ ar
E	147	5	-	↓ HAND	↑ rem
H	147	5	↓ ar, ↑ ar[1]	↓ ar	↑ ar, ↑ hand[1]
A	147	4	↓ AR, ↓ HAND, ↓ REM	-	-
F	147	4	↑ ar[1]	-	↓ hand[1]
B	147	3	↓ ar	↓ ar	-
Neuromuscular					
C	220	6	gait, ↓ splay	GAIT, ↓ HGRIP	GAIT, ↓ HGRIP
D	147	6	-	-	-
E	147	5	gait	gait	↓ splay
H	147	5	-	-*	-
A	147	4	gait, ↓ splay	-	-
F	147	4	-	-	-
B	147	3	gait, ↑ hgrip[1]	↑ HGRIP[1]	-
Sensorimotor					
C	220	6	↓ touch	-	-
D	147	6	-	↓ tp[1]	-
E	147	5	-	-	-
H	147	5	-	-	↑ tp, ↑ click[1]
A	147	4	↓ tp[1]	↑ touch	↑ appr
F	147	4	-	-	-
B	147	3	-	↓ appr[1]	-
Physiological Measures					
C	220	6	↓ temp, pilo	↓ wt, ↓ temp	↓ wt
D	147	6	↓ temp, pilo	↓ wt, ↓ temp	↓ wt, ↓ temp
E	147	5	↓ temp	↓ wt, ↓ temp, pilo	↓ wt
H	147	5	↓ temp, pilo	↓ wt, pilo	↓ wt, pilo
A	147	4	↓ temp, ↓ wt, pilo	↓ wt	↓ wt
F	147	4	↓ temp, ↓ wt	↓ wt	↓ wt
B	147	3	↓ temp, pilo	↓ wt, ↓ temp, pilo	↓ wt

[a] See Table 5 for key.

[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose.

* Domain significant at time point but no individual measure was significant.

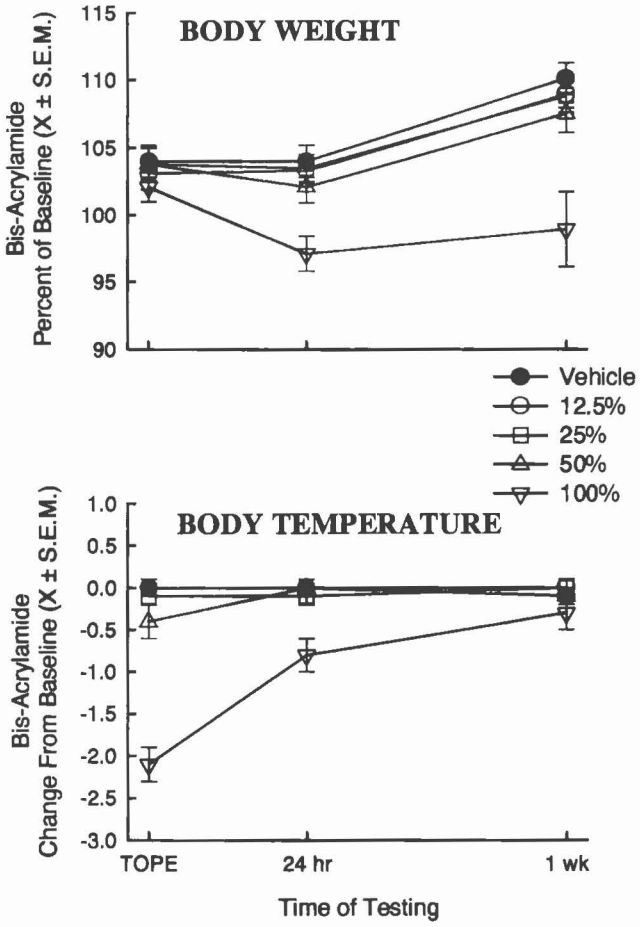


FIG. 10. Effects of acute bis-acrylamide on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects on both measures.

"excessive toxicity." Although the two-week data from that dose group are shown in the figures, repeated-measures statistical analyses excluded those data in the ANOVAs.

Analysis of the functional domain data averaged across laboratories revealed significant effects in the Neuromuscular domain throughout the study, and in the Activity domain at two weeks only. The domain data are presented in Figure 12. Post-hoc tests indicated that these effects were restricted to the high-dose group. The overall-laboratory analysis also showed that body temperature was lowered, and weight gain was suppressed in the two higher dose groups during dosing (two and four weeks). Two weeks after dosing ended, temperature had returned to normal but the weight deficits had not been regained at the high dose. These data are shown in Figure 13.

Data from the individual laboratories are presented in Table 8. Results of the functional domain analysis for the individual laboratories also showed a prominent effect on the Neuromuscular domain, as did the overall laboratory analysis. Six laboratories obtained significant neuromuscular domain effects, mostly at two and/or four weeks. In addition, three laboratories detected significant Activity domain changes, although these effects were not as great in magnitude as was obtained in the Neuromuscular domain. In two of these laboratories the overall analysis was significant at two weeks only, and the third at six weeks. The lack of effects on this domain at four weeks implied that these changes were not cumulative with repeated dosing. The Excitability domain was altered in three laboratories, but in two of these the effects were obtained only at six weeks. Changes in the Autonomic and Convulsive domains were observed only in the laboratories which used the higher dose range (laboratories C and G: 50% TD=110 mg/kg/day).

The most consistent neuromuscular changes were decreased landing foot splay in five laboratories, which was observed more at four weeks than at two weeks, and decreased forelimb and/or hindlimb grip strength, also in five laboratories. Moderate gait changes were reported at four weeks in five laboratories, but the magnitude of effect was much less than that obtained with repeated exposure to acrylamide (average scores ≈ 2, compared to acrylamide scores ≈ 3.5-4, see Figure 7). Three laboratories also reported moderate deficits of the righting reaction.

The most pronounced neuromuscular changes were detected in the two laboratories which used the highest dose range. In these laboratories, at four weeks the high-dose groups showed larger effects (splay values were 53-66% of controls) than the remaining laboratories (60-78% of controls in the three laboratories which obtained significant effects). The landing foot splay data at four weeks are presented in Figure 14. The grip strength endpoints displayed a similar dose-response, in that the laboratories using the higher doses showed average grip strength values of 51-66% and 38-53% of control values for forelimbs and hindlimbs, respectively. Of the remaining six laboratories at four weeks, grip strengths showed smaller and equivalent decreases in two laboratories (75-80% of control

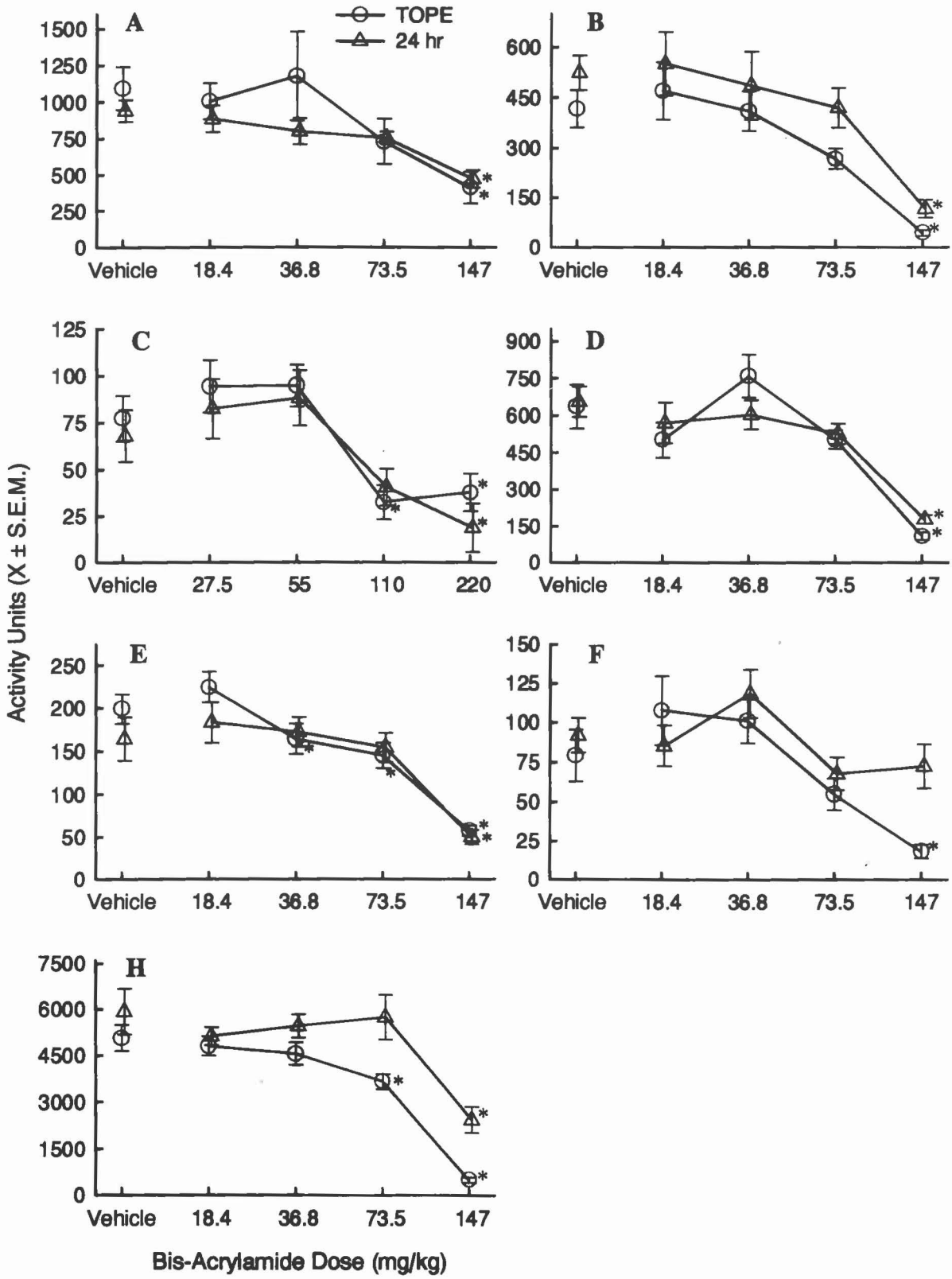


FIG. 11. Effects of acute bis-acrylamide on motor activity (mean total activity units during the session ± S.E.M.) in individual laboratories, at the TOPE (circles) and at 24 hours (triangles). Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

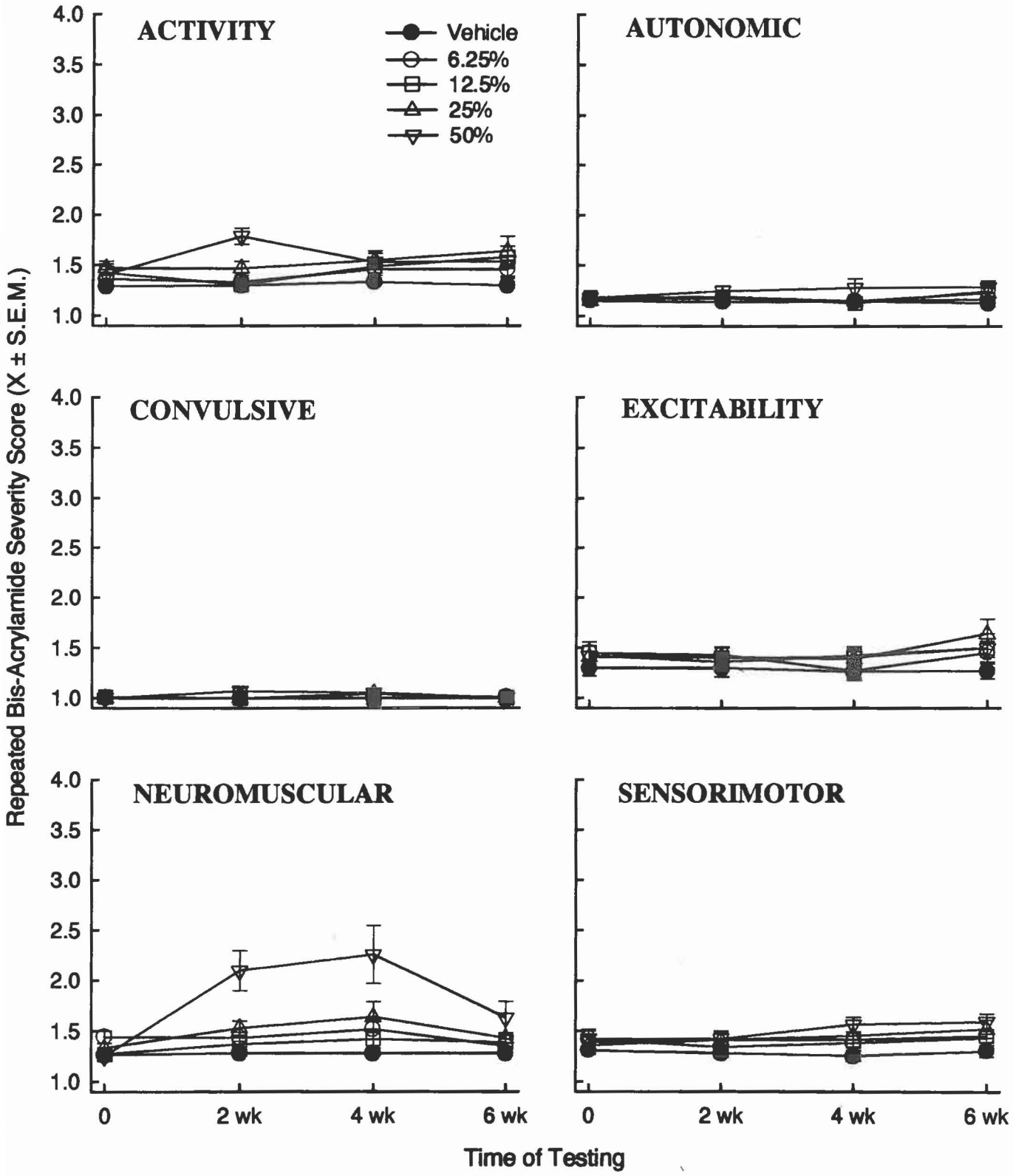


FIG. 12. Effects of repeated administration of bis-acrylamide on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects in the Activity and Neuromuscular Domains.

for both forelimb and hindlimb), and four laboratories reported no changes in these measures. Progressive changes during the course of dosing were not observed in these measures.

Activity domain effects were mostly due to decreases in motor activity which were evident in six laboratories only at two weeks. These effects recovered even while dosing continued. Changes in the numbers of rearing responses were only inconsistently reported.

There was a clear lack of effects in the Convulsive domain. Only two laboratories reported any changes, and these were only a few instances of tremors or smacking (30-38% of the high-dose group). Inconsistent changes in defecation and urination were evident in half of the laboratories, and only one reported lacrimation in the high-dose group. Likewise, the excitability and sensorimotor measures were variously affected; what effects were obtained often did not show a monotonic dose-response (i.e., lower dose or doses, but not the highest dose, were significantly different from controls), or else the magnitude of change was trivial. Even within laboratories, the direction of change for some of these measures changed from one test time to the next; for example, arousal was decreased at two weeks and increased at four weeks (laboratory C). The lack of dose-response was an unexpectedly common finding, in that within the Excitability and Sensorimotor domains, there were 33 significant changes (summed across all time points) and only 14 of these effects were detected in the high-dose group. Whether this is a real phenomenon of bis-acrylamide effects, or simply the result of random statistical significance, is unknown.

Weight loss in the high-dose groups, and depressed weight gain in lower dose groups, was clearly evident in all laboratories. Body temperature was also persistently lowered throughout the four weeks of dosing in most laboratories (hypothermia of -0.7° to -1.7° C). Piloerection (usually 80-100% in the high-dose groups, and somewhat less in lower doses) was also recorded in most laboratories. The weight loss was particularly dramatic, and at the end of dosing the average weight of the high-dose groups ranged from 56-82% of control values, and the next-highest dose group was 77-92% of controls. As with the effects of acute dosing, in all laboratories the rats showed clearly compromised health, yet relatively little lethality occurred.

p,p'-DDT

The data from seven of the eight laboratories were included in the analysis of both single-dose and repeated

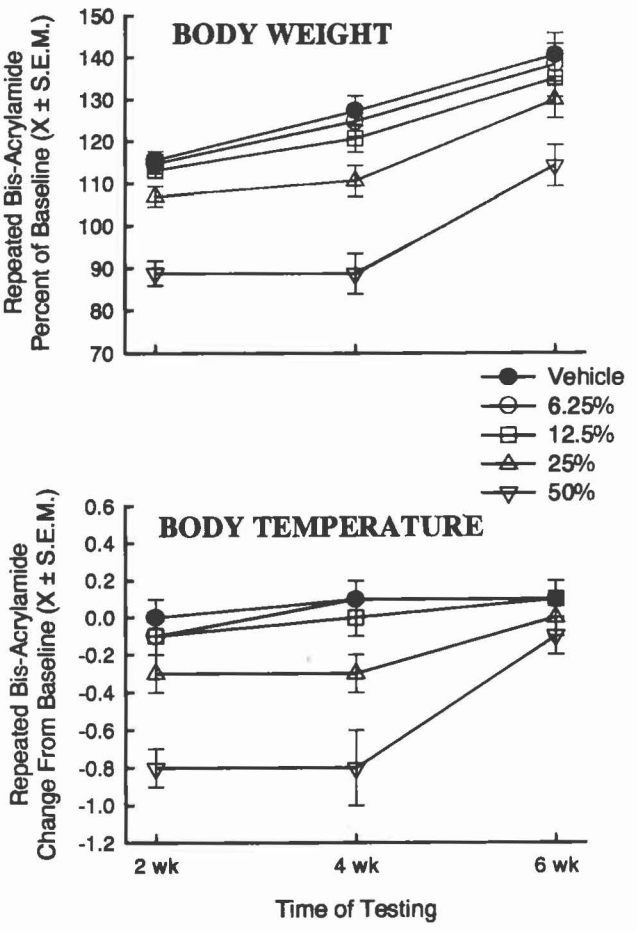


FIG. 13. Effects of repeated administration of bis-acrylamide on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects on both measures.

exposure to DDT (see Data Exclusions, above). There was a range of doses determined as the TD for DDT, from 58.0 to 195.8 mg/kg; the median value was 87 mg/kg (Table 3). The only obvious trend was that Wistar rats appeared less sensitive, since the TD values were highest for the laboratories using that strain of rat (130.5 and 195.8 mg/kg).

There was a fairly wide range of times chosen as TOPE (Table 2). Four laboratories chose six hours, the maximum possible time of testing. A three-hour TOPE was determined in the laboratory which reported the highest TD (195.8 mg/kg). The remaining two laboratories determined the peak times to be either one or four hours.

TABLE 8. Bis-Acrylamide: Effects[a,b] of Repeated Doses in Each Laboratory, Presented in Descending Order of Top Dose.

Lab	TD	Time of Testing		
		2 wk	4 wk	6 wk
Activity				
C	110	↓ma	-	-
G	110	↓rear	-	↑ REAR
D	73.5	↓ma	↓ rear	-
E	73.5	-	-	-
H	73.5	↓MA	↑ rear	↓ ma[1]
A	73.5	↓ma, [post]	-	-
F	73.5	↓ma	-	-*
B	73.5	↓MA	↓ ma	-
Autonomic				
C	110	↓def, ↓urin	-	↓ urin[1]
G	110	↓def	↓ DEF, ↓URIN[1], SAL	↑ URIN
D	73.5	-	-	-
E	73.5	↓def, ↓urin	↓ def	↓ def[1]
H	73.5	-	-	-
A	73.5	-	-	-
F	73.5	-	-	-
B	73.5	lacrim	↑ urin	↑ def[1], ↑ urin
Convulsive				
C	110	SMACK	-*	-
G	110	-	-	-
D	73.5	-	-	-
E	73.5	-	-	-
H	73.5	-	-	-
F	73.5	-	-	-
B	73.5	trem	trem	-
Excitability				
C	110	↓ar, ↑ hand	↑ ar, ↑ rem[1]	↑, ↓ AR[1], ↑ REM[1], ↑ HAND[1]
G	110	-	-*	↑ AR
D	73.5	-	-	-
E	73.5	-	↓ ar[1], ↓ rem, ↑ hand[1]	↓ rem, ↑ hand[1]
H	73.5	↓ rem[1]	↑ ar	↓ AR[1]
A	73.5	↓ hand	-	↓ rem[1]
F	73.5	-	-	-
B	73.5	↓ ar	↓ ar	-
Neuromuscular				
C	110	GAIT, RIGHT, ↓ FGRIP, ↓HGRIP	GAIT, RIGHT, ↓ SPLAY, ↓FGRIP, ↓ HGRIP	GAIT, RIGHT
G	110	↓SPLAY, ↓ FGRIP, ↓ HGRIP	GAIT, RIGHT, ↓ SPLAY, ↓FGRIP, ↓ HGRIP	-
D	73.5	-	gait, ↓ splay	-
E	73.5	-	GAIT	-
H	73.5	↓HGRIP	RIGHT, ↓ SPLAY, ↓ FGRIP, ↓ HGRIP	↓ SPLAY
A	73.5	-, [GAIT, ↓SPLAY, ↓ HGRIP]	gait	-
F	73.5	-	-	-
B	73.5	↓ SPLAY, ↓ FGRIP	↓ SPLAY, ↓ FGRIP, ↓ HGRIP	-
Sensorimotor				
C	110	↑ touch[1]	↓ click[1], ↑ touch[1]	↓ touch
G	110	-	-	-
D	73.5	-	-	-
E	73.5	-	↓ touch[1]	↓ click
H	73.5	-	↓ touch[1]	↓ touch[1], ↓ appr[1]
A	73.5	↑ touch	-	↓ touch[1]
F	73.5	↑ tp[1]	↑ tp	-
B	73.5	-	-	-
Physiological Measures				
C	110	↓wt, ↓temp, pilo	↓ wt, ↓ temp, pilo	↓ wt, pilo
G	110	↓wt, ↓temp	↓ wt, ↓ temp, pilo	↓ wt
D	73.5	↓wt, ↓temp	↓ wt, ↓ temp, pilo	↓ wt
E	73.5	↓wt	↓ wt	↓ wt
H	73.5	↓wt, ↓temp, pilo	↓ wt, ↓ temp, pilo	↓ wt
A	73.5	↓wt, ↓temp, [pilo]	↓ wt, ↓ temp, pilo	↓ wt, ↓ temp[1]
F	73.5	↓wt	↓ wt	↓ wt
B	37.8	↓wt, ↓temp, pilo	↓ wt, pilo	↓ wt

[a] See Table 5 for key
[b] Effects listed in brackets are those that were produced only by high doses which was sacrificed after the two-week test (laboratory A).
[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose
* Domain significant at time point but no individual measure was significant

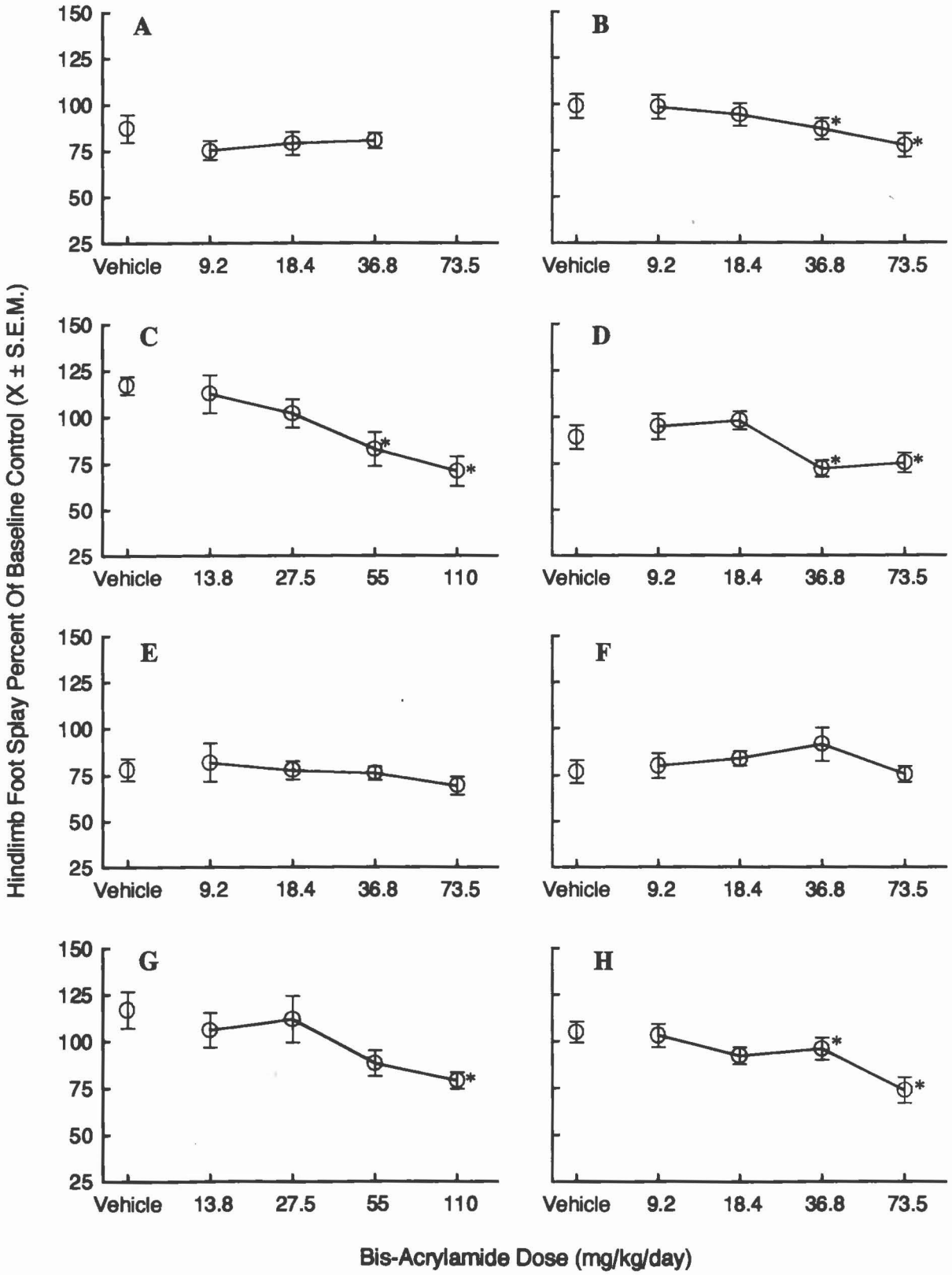


FIG. 14. Effects of repeated administration of bis-acrylamide on landing foot splay, expressed as mean percent of baseline values (± S.E.M.), in individual laboratories at the end of dosing (four weeks). Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

Acute DDT Dosing. Following a single dose, the TD produced little lethality in most laboratories (see Table 4). The exception was one laboratory with 40% mortality at the high dose; the other laboratories lost either one of 10 rats (three laboratories) or none (three laboratories).

Figures 15 and 16 present the effects of a single dose of DDT, averaged across all laboratories, on the functional domains as well as body weight and temperature. Analysis of the laboratory data indicated that all domains except Autonomic function were significantly affected at the TOPE, with no residual effects at either one day or one week after dosing. The Convulsive domain displayed the largest magnitude of effect, followed by Sensorimotor function. In addition, average body temperature showed a marked increase at the TOPE. Mild (non-significant) depression of weight gain was observed at 24 hours and one week.

The profiles of the individual laboratories consisted of selective effects on the Convulsive and Sensorimotor domains. All but one laboratory obtained significant changes in those domains, whereas no more than three laboratories showed effects in any of the other domains. The data for all endpoints are presented in Table 9. Five of the six laboratories for which Convulsive data were available detected significant effects at the TOPE on the Convulsive domain. The principal finding in this domain was the occurrence of clonic movements, ranging from mild to severe tremors in all laboratories, and including myoclonus and clonic convulsions in two (laboratories E and H). The severity of tremors was dose-related, as presented in Figure 17, whereas myoclonus and convulsions were produced only by the highest dose. This finding was reproduced across laboratories, with the exception of the one laboratory which used the shortest TOPE (one hour). Tremors (30% incidence) was still observed in one laboratory 24 hours after dosing.

There was generally good agreement in the Sensorimotor domain at the TOPE, and the most consistent effect within this domain was a change in the click response, as shown in Figure 18. Five laboratories reported an increased click response, mostly at the two higher doses (50% and 100% TD), whereas one laboratory reported a non-dose-related decreased response (this was the laboratory with the short TOPE); the seventh laboratory obtained no effect on this measure. The touch response was also generally increased; while this measure was only statistically significant in two laboratories, there was a non-significant trend (p's<0.06) in an additional three laboratories. The other sensory stimuli responses were not consistently altered; two laboratories showed an increased approach response, and one reported decreased tail-pinch response.

Neuromuscular effects consisted mostly of moderately-altered gait (in five laboratories) on the day of dosing. These gait alterations were described as ataxia and uncoordinated placement of limbs, and two laboratories also reported tiptoe gait and the hind feet pointing out. Gait changes persisted to 24 hours only in two laboratories. Two laboratories reported decreased forelimb or hindlimb grip strength, decreased foot splay, or altered righting reflex. Generally, these were mild effects.

There was less consistency in the Activity and Excitability domains. Although only two laboratories obtained significant domain effects, the Excitability domain indicated generally increased reactivity to removal from the home cage (four laboratories), and increased handling reactivity (two laboratories). There were less consistent effects on arousal, with both increases and decreases reported in just a few laboratories. Some degree of increased excitability was still observed at 24 hours (three laboratories). Three laboratories obtained significant Activity effects, and within that domain, activity levels (automated activity or rearing) were altered by four laboratories at the TOPE; however, the changes were inconsistent. Two laboratories reported decreased activity units, and one showed increased activity. Two laboratories obtained decreases in open-field rearing and two reported that some rats were flattened and not moving in the home cage. No activity changes were evident after the day of dosing.

Essentially no Autonomic measures were affected. At the TOPE, there was only one finding each of decreased urination, inhibited pupil response, and lacrimation; these latter two effects were reported by the same laboratory.

Physiological changes included hyperthermia in all laboratories, except the one that used the shortest TOPE (laboratory G). The magnitude of this effect varied somewhat across laboratories, ranging from 0.4°C above control in laboratory A to 1.5-1.7°C higher in the others, but this difference was possibly due to the shorter post-dosing time used in laboratory A (three hours). Significant effects were obtained in at least two dose groups in four laboratories, and only at the highest dose in two laboratories. Body weight changes included mild weight loss (up to 5%) or depressed weight gain, and were restricted mostly to the TOPE and 24 hours after dosing.

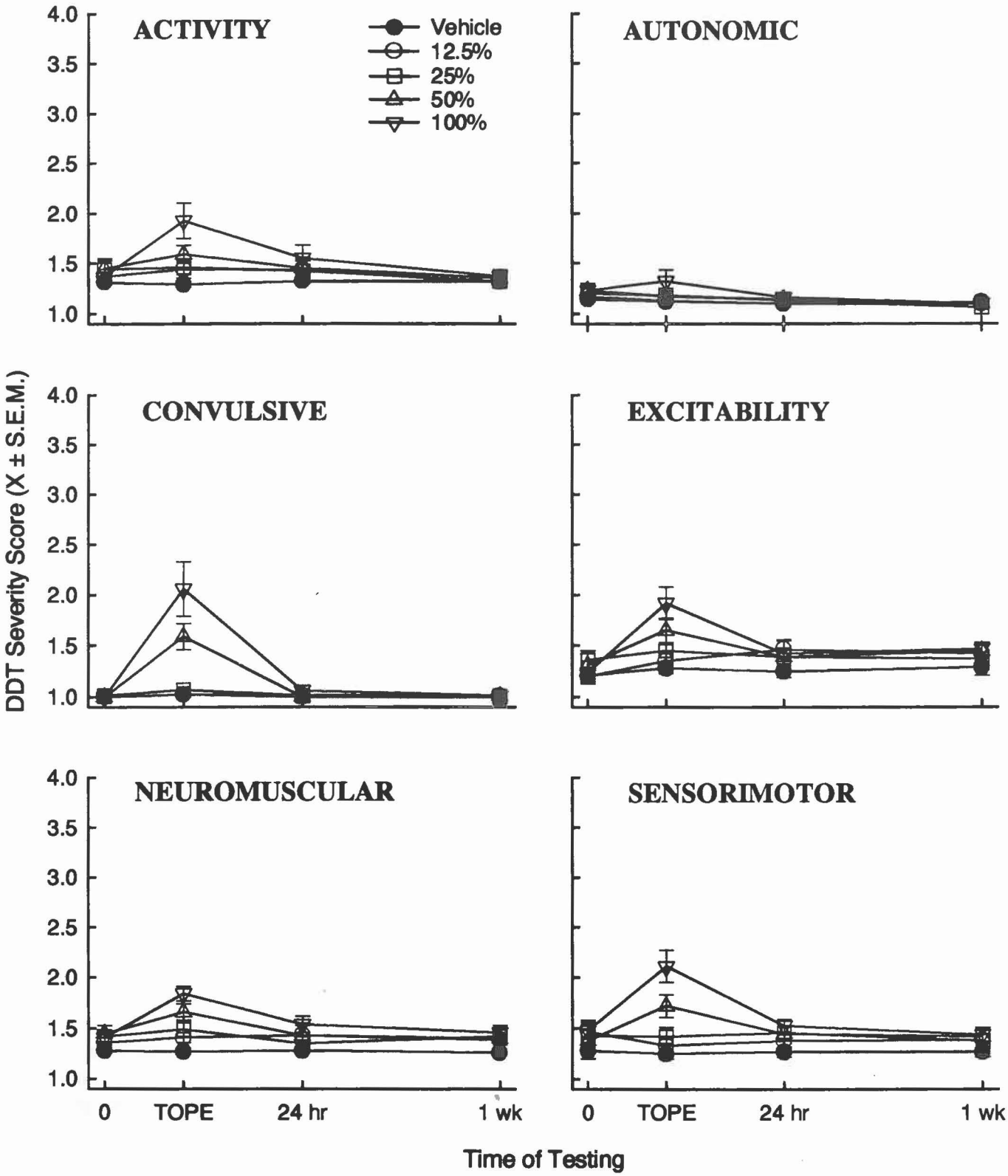


FIG. 15. Effects of acute DDT on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects in the Activity, Convulsive, Excitability, Neuromuscular, and Sensorimotor Domains.

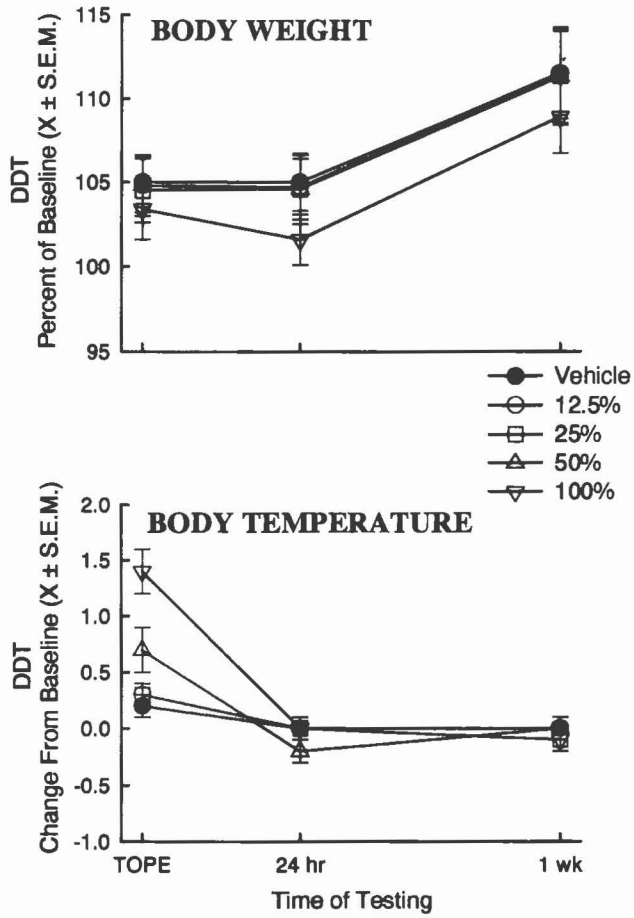


FIG. 16. Effects of acute DDT on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed a significant overall effect on body temperature.

Repeated DDT Exposures. There was almost no lethality reported during repeated dosing of DDT in all but one laboratory (A). Although this laboratory had no mortality due to the DDT, they sacrificed all the rats in the high dose group (50% TD) after only two doses due to "excessive toxicity" (this laboratory used the highest dose, 50% TD = 97.9 mg/kg). Only one laboratory reported that one of 10 high-dose rats died during dosing (see Table 4).

DDT produced very few effects with repeated administration. The analysis of severity scores across laboratories, shown in Figure 19, indicated significance in the Neuromuscular domain at four and six weeks. However, as seen in Figure 19, this effect was trivial. At this overall level of analysis, there were also no effects on body temperature or weight (Figure 20).

DDT effects for each laboratory are listed in Table 10. Although the overall laboratory analysis indicated an effect of DDT on the Neuromuscular domain, only two individual laboratories showed significant Neuromuscular changes (both at four weeks). Two other laboratories obtained no significant domain effects whatsoever, and the remaining laboratories showed only modest changes. For most endpoints, effects were confined to the high-dose group (50% TD).

Within the Neuromuscular domain, the most consistent finding was decreased forelimb grip strength at four weeks; however, this was only reported in three laboratories and the magnitude of change was small (76-81% of vehicle control data). One laboratory reported slight alterations in gait and righting reflex.

Significant changes within the other domains were minor and few in number. A low incidence of mild tremors (20%) or myoclonus (30%) was reported in two laboratories at four weeks. The only other findings reported in more than one laboratory was increased defecation (two laboratories at six weeks), and decreased click response (three laboratories at different testing times). There were few general toxicity signs due to repeated administration of DDT (Table 10). Depressed body weight gain was observed in three laboratories (high dose only), and an unexpected increased weight gain was reported in one laboratory. In one laboratory, a 30% incidence of piloerection was reported, and in another, slight hypothermia.

Lead Acetate

There was good agreement on the choice of top dose for lead acetate, with 200 mg/kg selected in five laboratories, and 133.3 mg/kg in the remaining two (Table 3). There was less consistency in the determination of time of peak effect (TOPE), with times ranging from one to four hours (Table 2). Four laboratories reported either three or four hours as the TOPE, and three reported the TOPE to be one to two hours. A possible explanation for this variability would be that lead acetate did not show a pronounced pattern of onset and offset of acute effects.

Acute Lead Acetate Dosing. Two laboratories obtained no lethality during the single-dose lead acetate

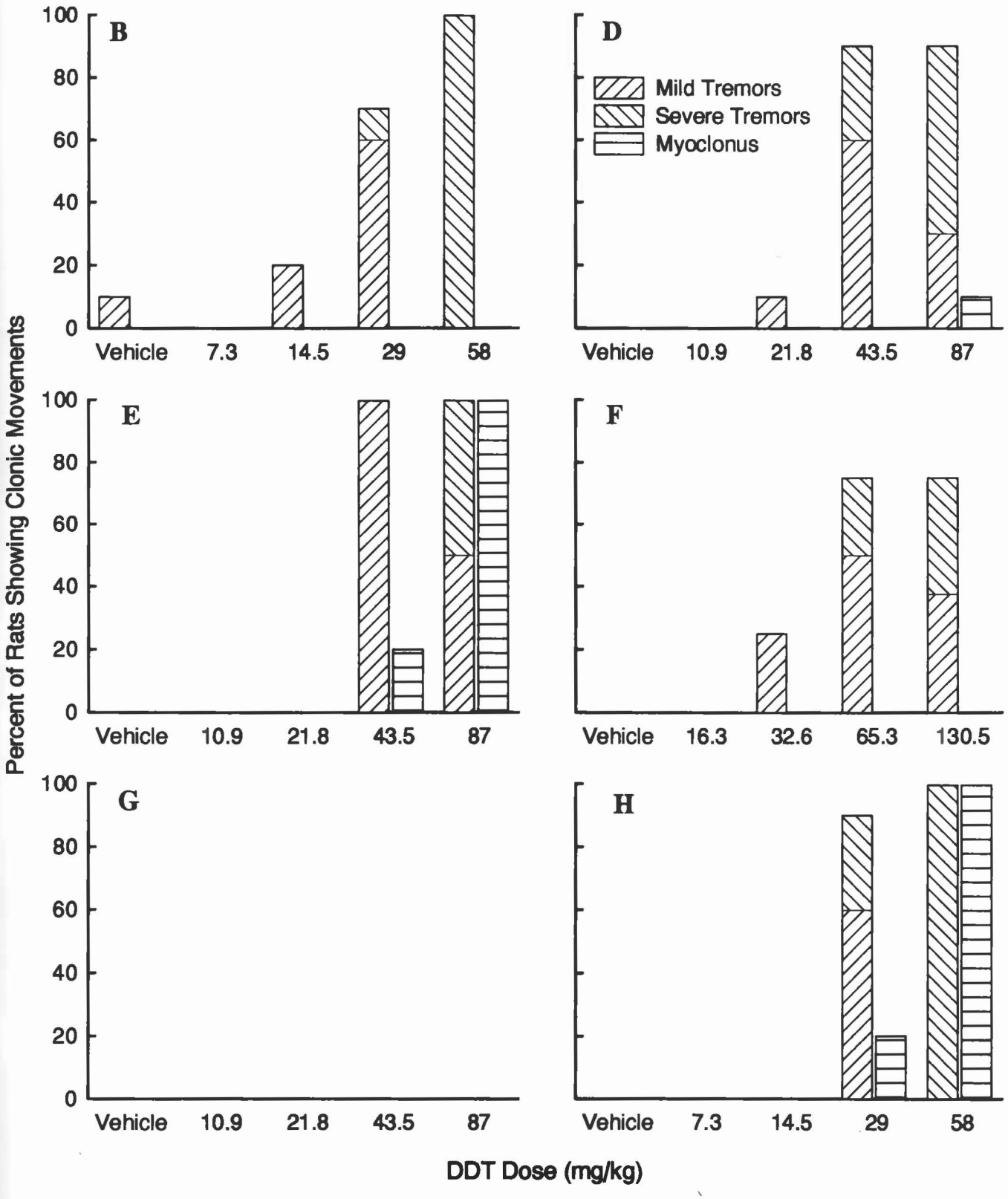
TABLE 9. DDT: Effects[a] of a Single Dose in Each Laboratory, Presented in Descending Order of Top Dose and TOPE.

Lab	TD	TOPE	Time of Testing		
			TOPE	24 hr	1 wk
Activity					
A	195.8	3	-	-	-
F	130.5	6	-	-	-
E	87	6	↓ ma	-	-
D	87	4	↓ REAR, ↑ MA	-	-
G	87	1	-	-	-
B	58	6	↓ MA, POST	-	-
H	58	6	↓ REAR, POST	-*	-
Autonomic					
A	195.8	3	-	-	-
F	130.5	6	-	-	-
E	87	6	-	-	-
D	87	4	-	-	-
G	87	1	-	-	-
B	58	6	PUPIL, LACRIM	-	pupil
H	58	6	↓ urin	-	-
Convulsive					
F	130.5	6	TREM, CONV	-	-
E	87	6	TREM, MYOC	TREM	-
D	87	4	TREM	-	-
G	87	1	-	-	-
B	58	6	TREM	-	-
H	58	6	TREM, MYOC, CONV	-	-
Excitability					
A	195.8	3	↑ ar, ↑ hand	↑ ar	-
F	130.5	6	↑ rem	-	-
E	87	6	↑ hand, ↑ rem	↑ hand	-
D	87	4	-	-	-
G	87	1	↓ rem	↑ rem	↓ rem[1], ↓ hand[1]
B	58	6	-	↓ AR[1], ↓ HAND[1]	-*
H	58	6	↓ AR, ↑ REM	-	-
Neuromuscular					
A	195.8	3	↓ SPLAY	↓ FGRIP	↓ splay[1]
F	130.5	6	gait	-	-
E	87	6	GAIT, ↓ HGRIP	gait	-
D	87	4	↓ hgrip	↓ hgrip	↓ splay[1]
G	87	1	gait	gait	-
B	58	6	gait, right	-	-
H	58	6	GAIT, ↓ FGRIP	-*	-
Sensorimotor					
A	195.8	3	↑ CLICK, ↑ TOUCH, ↑ APPR	↑ appr	↑ touch[1], ↑ appr[1]
F	130.5	6	↑ CLICK	-	-
E	87	6	↑ CLICK, ↑ APPR	↑ touch	-
D	87	4	↑ click	-	-
G	87	1	↓ CLICK[1]	↑ TP	↑ click
B	58	6	↑ CLICK, ↑ TOUCH	-	↑ click
H	58	6	↓ TP	-	-
Physiological Measures					
A	195.8	3	↑ temp	↓ wt, ↑ temp	-
F	130.5	6	↑ temp	-	-
E	87	6	↑ temp, ↓ wt	↓ wt	↓ wt
D	87	4	↑ temp	↓ wt, ↓ temp[1]	-
G	87	1	-	-	-
B	58	6	↑ temp, pilo	pilo[1]	pilo
H	58	6	↑ temp, ↓ wt	↓ wt	-

[a] See Table 5 for key

[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose

* Domain significant at time point but no individual measure was significant



FIG, 17. Effects of acute DDT in individual laboratories at the TOPE on the occurrence of clonic movements: mild or severe tremors, and/or myoclonus. Data are presented as the percent of rats in each dose group showing these signs. Laboratories are indicated by letter.

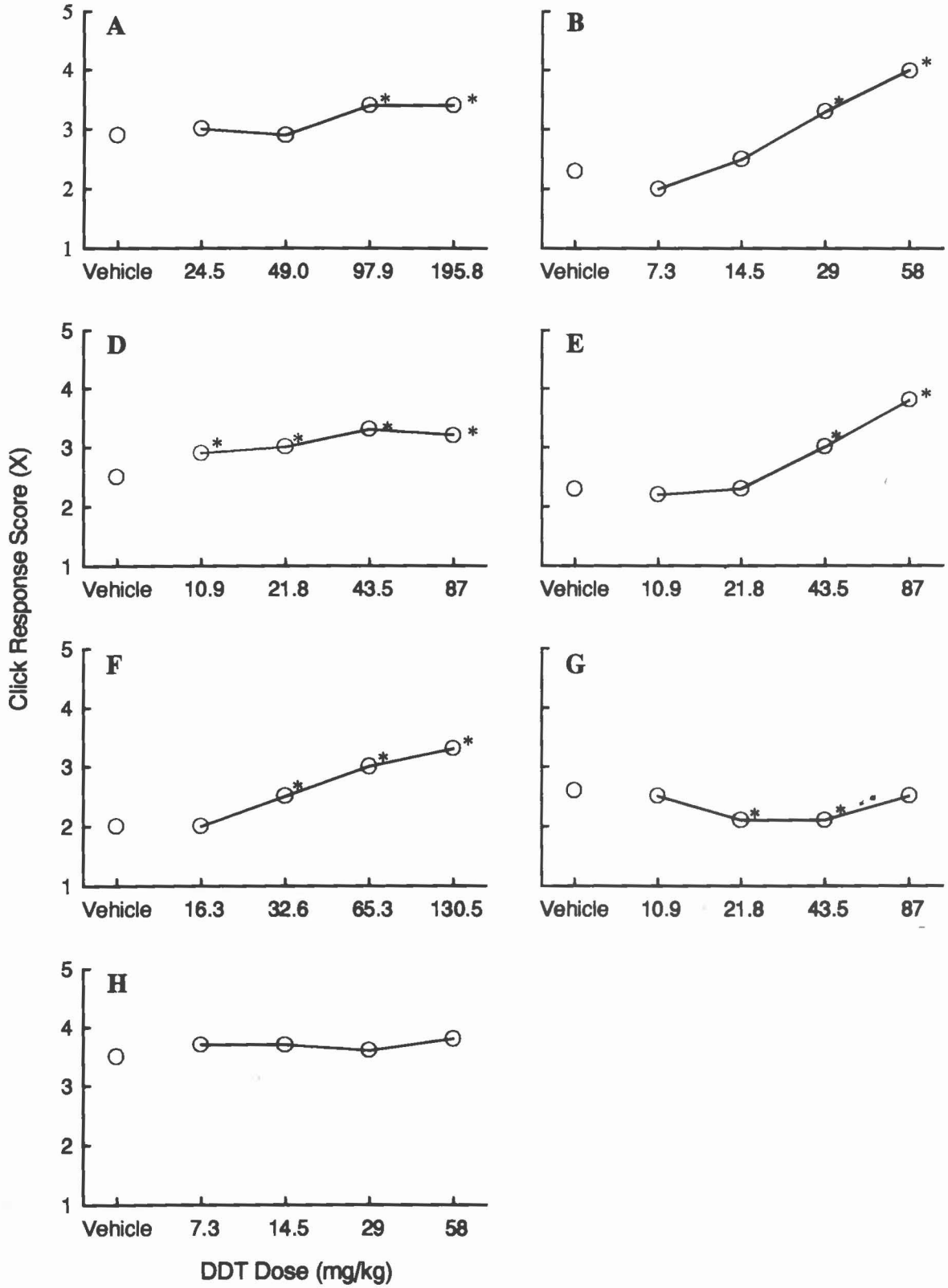


FIG. 18. Effects of acute DDT on the click response of rats in individual laboratories at the TOPE. Click response is ranked on a scale ranging from '1' (no reaction) to '5' (exaggerated reaction). Data are presented as mean score for each dose group. Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

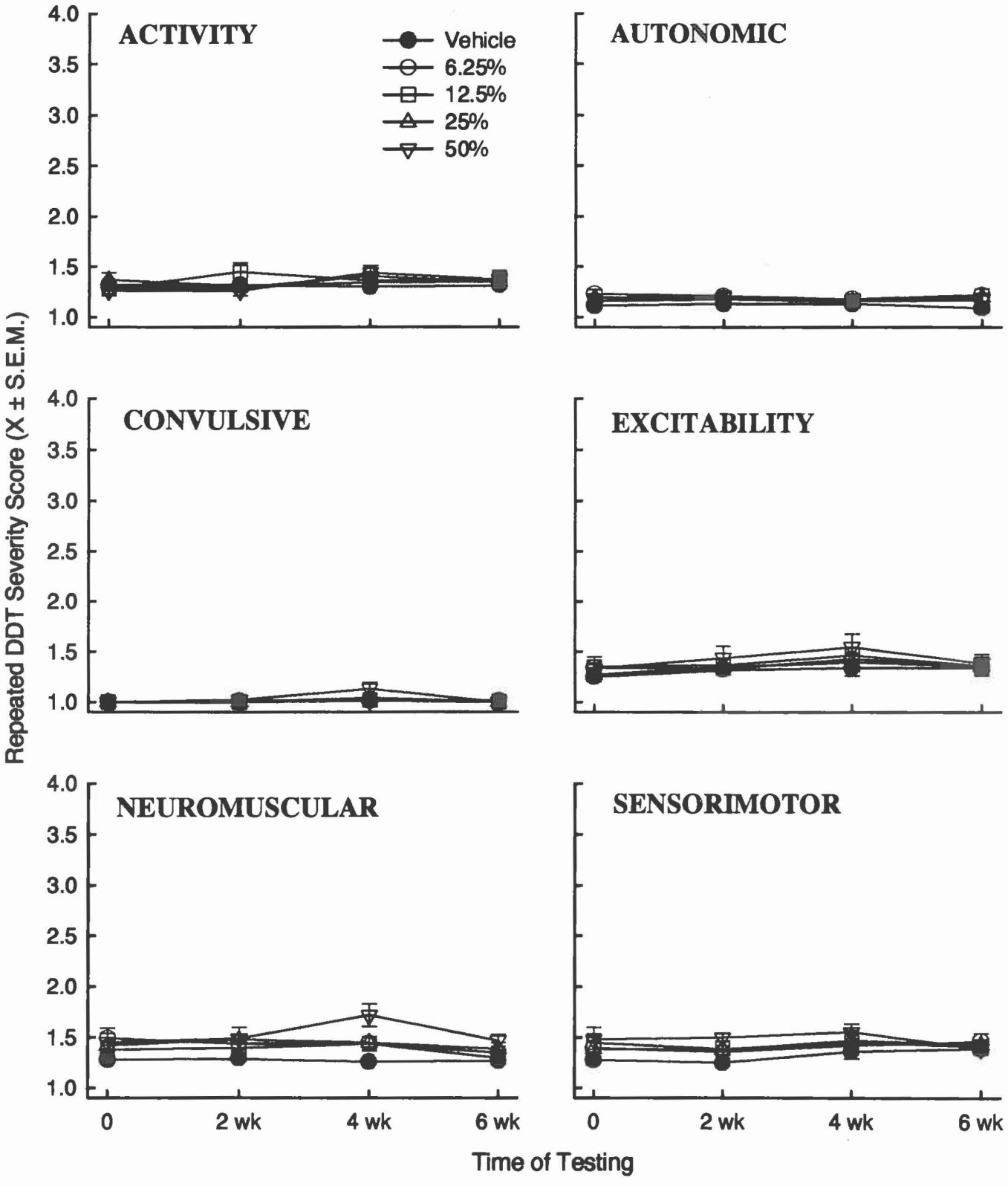


FIG. 19. Effects of repeated administration of DDT on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects only in the Neuromuscular Domain.

TABLE 10. DDT: Effects[a] of Repeated Doses in Each Laboratory, Presented in Descending Order of Top Dose.

Lab	TD	Time of Testing		
		2 wk	4 wk	6 wk
Activity				
A	97.9[b]	-	-	-
F	63.5	-	-	-
E	43.5	-	-	-
D	43.5	-	-	-
G	43.5	-*	-	^ REAR[1]
B	29	-	-	-
H	29	-	-	-
Autonomic				
A	97.9[b]	-	-	^ def
F	63.5	-	^ def[1]	^ DEF
E	43.5	-	-	-
D	43.5	-	-	-
G	43.5	-	-	-
B	29	-	-	-
H	29	-	-	-
Convulsive				
F	63.5	-	-	-
E	43.5	-	TREM	-
D	43.5	-	-	-
G	43.5	-	-	-
B	29	-	-	-
H	29	-	MYOC, TREM	-
Excitability				
A	97.9[b]	-	^ rem	-
F	63.5	-	-	-
E	43.5	⌄ hand[1]	⌄ hand[1]	-
D	43.5	-	-	-
G	43.5	-	-	-
B	29	-	-	-
H	29	-	-	-
Neuromuscular				
A	97.9[b]	-*	-	-
F	63.5	-	⌄ FGRIP	-
E	43.5	⌄ fgrip	⌄ fgrip	-
D	43.5	-	⌄ fgrip	-
G	43.5	-	-	-
B	29	-	-	-
H	29	-	GAIT, RIGHT	right
Sensorimotor				
A	97.9[b]	-	⌄ click	-
F	63.5	⌄ click	-	-
E	43.5	-	-	⌄ tp[1]
D	43.5	-	-	-
G	43.5	-	⌄ tp	⌄ click
B	29	^ appr[1]	-	-
H	29	^ TOUCH	^ touch	-
Physiological measures				
A	97.9[b]	⌄ wt	⌄ wt	-
F	63.5	⌄ wt	⌄ wt	-
E	43.5	⌄ wt	⌄ wt	⌄ wt
D	43.5	-	-	-
G	43.5	-	-	-
B	29	pilo	-	-
H	29	⌄ temp	^ wt[1]	^ wt

[a] See Table 5 for key

[b] All rats in this dose group were sacrificed after 2 doses, therefore no data are available

[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose

* Domain significant at time point but no individual measure was significant

study. Of the remaining five laboratories, lethality ranged from 10-60% (Table 4); the data for the high-dose group with 60% lethality was eliminated from the across-laboratory statistical analyses. All deaths occurred after the 24-hour test, usually between three to five days after dosing.

The severity score analyses across laboratories indicated significant alterations in the Activity, Excitability, Sensorimotor, and Neuromuscular domains; these data are shown in Figure 21. Whereas most effects occurred at 24 hours and one week, Sensorimotor function was significantly affected only at the TOPE. The magnitude of the effects on all domains was relatively small. Significant hypothermia was evident across laboratory means at the TOPE and at 24 hours (Figure 22). Body weight group means clearly decreased during the week after dosing, and the dose-by-time interaction factor was significant.

No laboratory obtained the precise profile which was presented in the laboratory-averaged data. Only three laboratories obtained significant Neuromuscular or Sensorimotor domain changes, and only two recorded effects in the Excitability and Activity domains. Thus the overall analysis did not predict well the individual laboratory profiles.

Table 11 presents the results from each individual laboratory. The data from the high-dose group in laboratory B are included at the TOPE and 24 hours, at which time no rats had died. Since 60% of that group died before the one-week test, however, these data were not included in the repeated-measures analyses.

Motor activity depression was the most consistent functional change recorded, as shown in Figure 23. Five of seven laboratories obtained decreases at both the TOPE and 24 hours. In addition, these effects were significant at one week in three laboratories. The magnitude of hypoactivity was greatest at the TOPE, and in four laboratories all dose groups were significantly lower than control at that time.

General excitability was depressed in three of the five laboratories using 200 mg/kg as the TD. These laboratories reported decreased arousal in the open field, and two laboratories reported lowered reactivity to being removed from the cage and/or handled; these effects were most prominent at one week. Interestingly, increases in arousal and removal reactivity were reported in two laboratories, but only at the lower dose levels (16.7-66.7 mg/kg).

Neuromuscular function was not altered in any pronounced or consistent manner. Mild to moderate gait

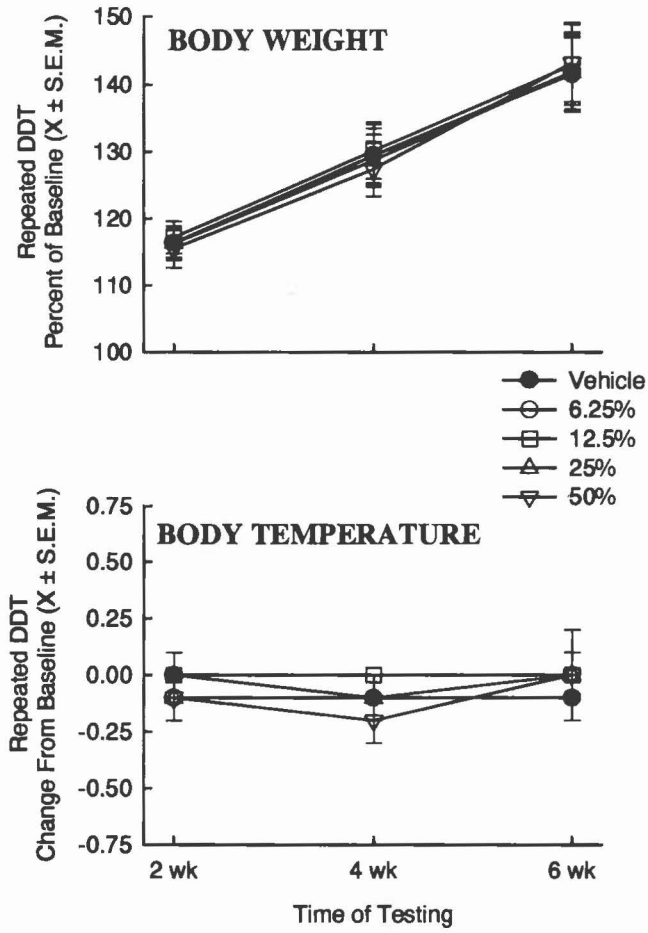


FIG. 20. Effects of repeated administration of DDT on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed no significant overall effects.

changes were reported in four laboratories, described as tip-toe gait with hunched posture in three laboratories, and as ataxia in the fourth laboratory. These significant gait effects were obtained mostly in the 100%-TD dose group, except for one laboratory (G) in which effects were only seen at the two lower doses. One laboratory reported moderate decreases in forelimb grip strength, another laboratory reported decreased hindlimb grip strength, and two laboratories obtained smaller foot splay values; no pattern of effects was obvious.

In the remaining domains, the data were even less consistent; the effects may have been random differences

TABLE 11. Lead Acetate: Effects[a,b] of a Single Dose in Each Laboratory, Presented in Descending Order of Top Dose and TOPE.

Lab	TD	TOPE	Time of Testing		
			TOPE	24 hr	1 wk
Activity					
D	200	4	↓ MA	↓ MA	↓ ma
A	200	3	↓ ma	↓ ma	↓ ma
H	200	3	↓ ma, ↓ rear	↓ ma, ↓ rear	↓ ma, ↓ rear
B	200	2	↓ ma	-, [↓ MA]	-
F	200	1.5	-	-	-
E	133.3	4	↓ MA	↓ MA	-
G	133.3	1	-	-*	-
Autonomic					
D	200	4	-	↓ def	-
A	200	3	-	-	-
H	200	3	-	-	-
B	200	2	-	PUPIL	lacrim
F	200	1.5	-	-	-
E	133.3	4	-	-	-
G	133.3	1	-	↓ def	-
Convulsive					
D	200	4	-	-	-
H	200	3	myoc[1], smack[1]	smack	-
B	200	2	-, [trem]	trem[1]	-
F	200	1.5	-	-	-
E	133.3	4	-	-	-
G	133.3	1	-	-	-
Excitability					
D	200	4	↓ ar	↓ AR	↓ AR, ↓ REM, ↓ HAND
A	200	3	↓ ar, ↓ hand	↓ HAND	↓ hand
H	200	3	↓ ar	↓ ar	↓ ar
B	200	2	-	-	-
F	200	1.5	-	-	-
E	133.3	4	↑ rem[1]	-	-
G	133.3	1	↑ ar[1]	↑ ar[1]	↑ ar[1]
Neuromuscular					
D	200	4	-	-	-
A	200	3	-*	↓ splay, ↓ fgrip[1]	↓ HGRIP
H	200	3	GAIT	GAIT	gait
B	200	2	-, [gait]	gait	-
F	200	1.5	↓ FGRIP	↓ FGRIP, RIGHT	-*
E	133.3	4	gait	gait	-
G	133.3	1	gait[1]	gait[1], ↓ splay	-
Sensorimotor					
D	200	4	↓ TP	↓ TP, ↓ APPR	↓ TOUCH, ↓ APPR
A	200	3	↓ TP	-*	↓ CLICK, ↑ APPR
H	200	3	↓ appr	↓ appr[1]	↓ click, ↓ appr[1]
B	200	2	-	-	-
F	200	1.5	-	↓ touch	-
E	133.3	4	-	↓ appr[1]	↓ appr[1]
G	133.3	1	↑ touch	↓ CLICK	↑ touch[1]
Physiological Measures					
D	200	4	↓ temp	↓ temp	↓ wt
A	200	3	↓ temp, pilo	↓ wt[1], pilo	↓ wt, ↓ temp, pilo
H	200	3	↓ temp, pilo	↓ wt, ↓ temp, pilo	↓ wt, ↓ temp, pilo
B	200	2	↓ temp, [pilo]	↓ wt, pilo, [↓ temp]	↓ wt
F	200	1.5	↓ temp	-	↓ wt
E	133.3	4	↓ temp	↓ wt	↓ wt, ↓ temp[1]
G	133.3	1	↓ temp	↓ wt	↓ wt

[a] See Table 5 for key

[b] Effects listed in brackets are those produced only by the doses which were excluded from analyses due to high lethality (>50%; laboratory B only).

[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose

* Domain significant but no individual measures affected

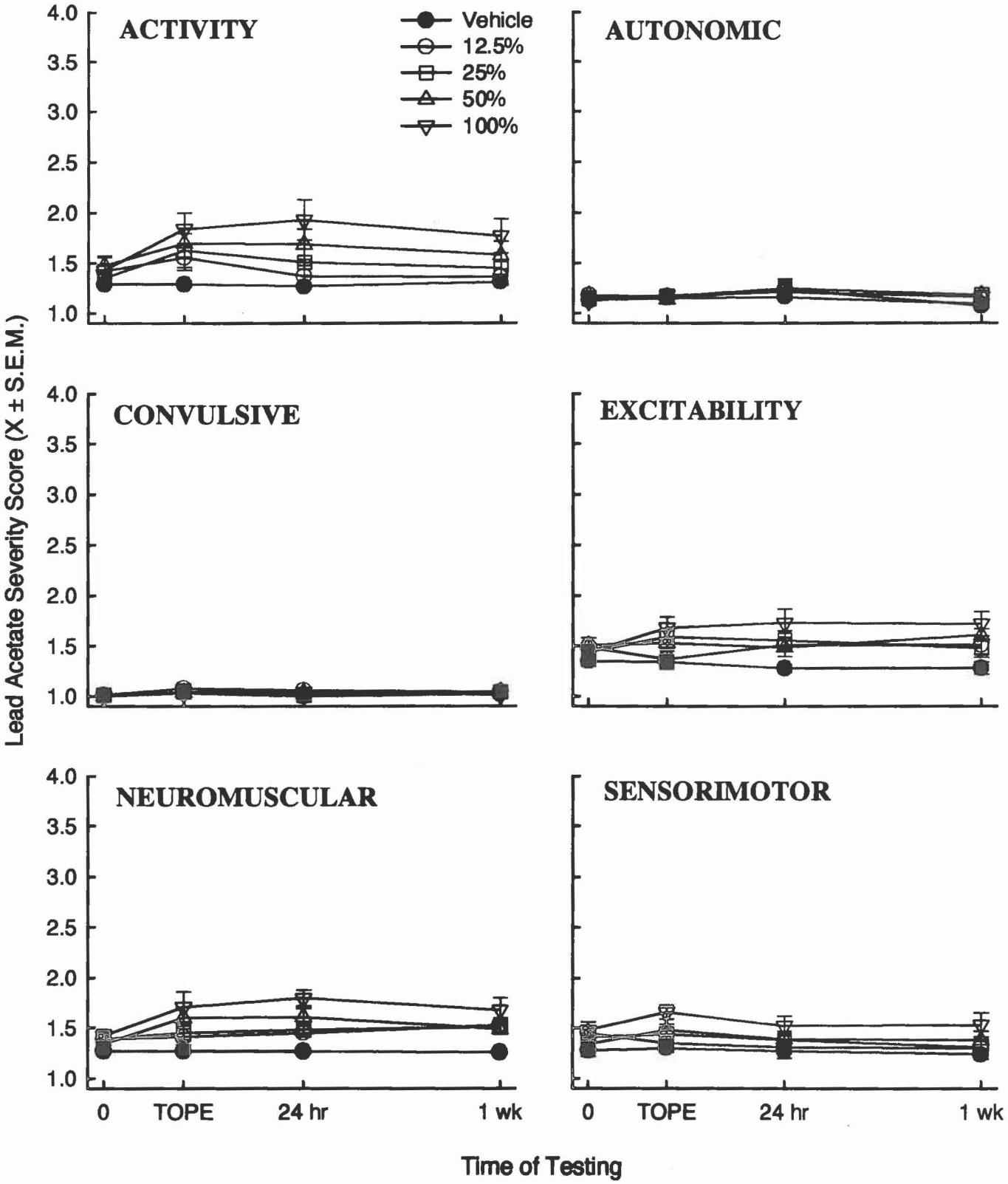


FIG. 21. Effects of acute lead acetate on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects in the Activity, Excitability, Neuromuscular, and Sensorimotor Domains.

rather than treatment-related changes. Variable changes in the Sensorimotor responses were obtained across laboratories; however, no clear pattern of effects on specific endpoints emerged, nor did any particular response appear to be more sensitive. Almost no autonomic measures were altered by acute exposure to lead acetate. Two laboratories reported decreased defecation at 24 hours, which could have been a result of decreased food intake; these laboratories also reported the greatest weight loss at that time point. Involuntary abnormal movements were reported in a few laboratories. One laboratory reported a low incidence (20-30%) of mild tremors which were scattered across the lower dose groups at 24 hours. Additionally, one laboratory reported a low incidence (30-40%) of myoclonus and smacking at the TOPE, but only at the lowest dose, and about 40% of rats in the high-dose group showed smacking at 24 hours.

Only the physiological measures were consistently affected in all laboratories. Pronounced hypothermia (1.1-3.0°C decrease) on the day of dosing was a clear sign of toxicity, mostly occurring only at the higher doses. Likewise, effects on body weight were reported in all laboratories at one week; five of the laboratories also reported changes at 24 hours. Weight loss was evident at higher doses, and by one week the mean weights of the highest dose groups ranged from 81-98% of control values. At lower dose groups, depressed weight gain over the one-week period was evident. Despite the compromised health of the rats, only three laboratories reported the observation of piloerection in 20-80% of rats (mostly in the high-dose group) at most test times.

Repeated Lead Acetate Exposures. The most pronounced effects of repeated administration of lead acetate was high lethality (40-100%) that occurred at the 25%- and 50%-TD dose levels in all laboratories (see Table 4). Furthermore, the rats in the two higher dose groups that did survive were emaciated and moribund. After two weeks of dosing, up to 30% lethality had occurred at the high dose in six laboratories, with 75% lethality at that dose in the seventh laboratory. By the end of the study, however, there was 90-100% lethality at the 50%-TD dose, 40-100% lethality at 25%-TD, and 12.5-100% lethality at 12.5%-TD. Only one laboratory reported deaths (20%) at the lowest dose (6.25% TD).

The data for the functional domains, body weight and temperature, averaged across laboratories, are presented in Figures 24 and 25. These figures include the severity scores of rats who subsequently died; most of the test measures as well as the domains were adversely affected in these higher dose groups. All except the Convulsive domain were altered at two and/or four weeks, before the high lethality occurred. The most prominent and consistent effects were in the Excitability domain, consisting of decreased arousal, and lower removal and handling reactivities, and Neuromuscular effects, mostly gait changes, and decreased grip strength and landing foot splay. A few laboratories reported autonomic changes of inhibited pupil response and altered urination and defecation, and depression of some of the Sensorimotor reactions. Piloerection was recorded in some laboratories, as well as reports of rough coat and poor appearance.

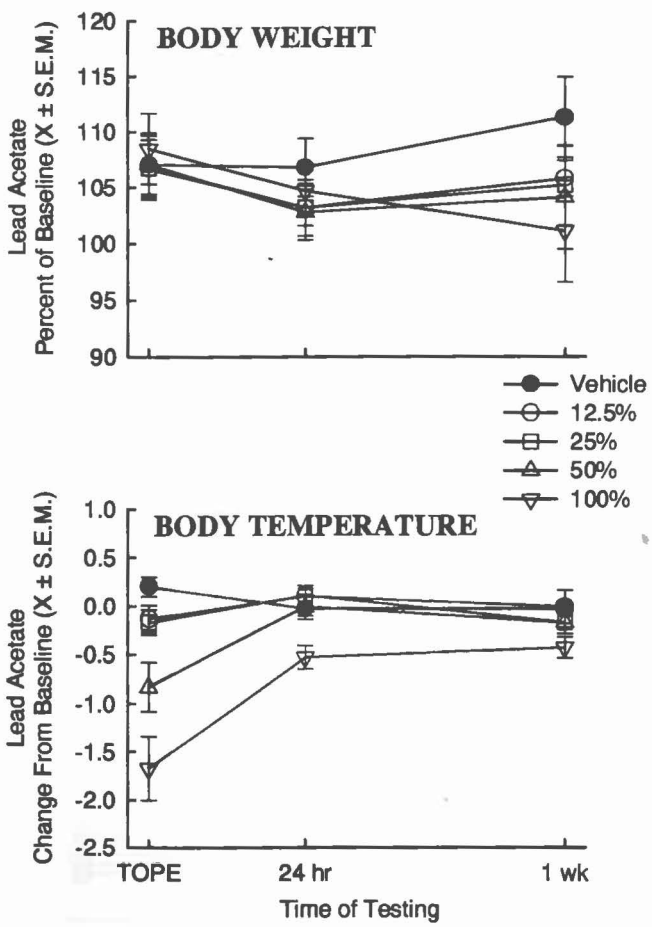


FIG. 22. Effects of acute lead acetate on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed a significant overall effect on both measures.

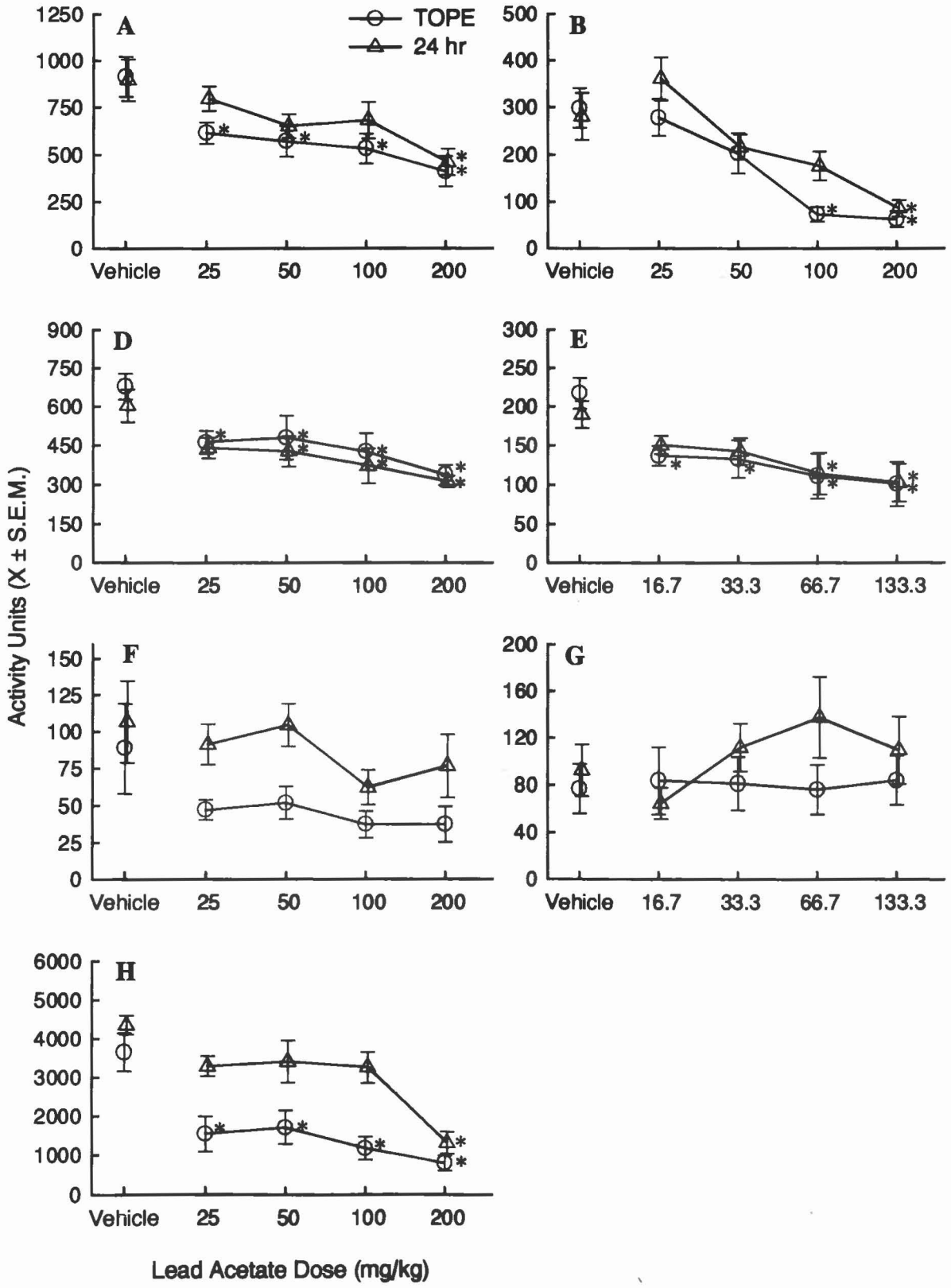


FIG. 23. Effects of acute lead acetate on motor activity (mean total activity units during the session ± S.E.M.) in individual laboratories, at the TOPE (circles) and at 24 hours (triangles). Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

TABLE 12. Lead Acetate: Effects[a,b] of Repeated Doses in Each Laboratory, Presented in Descending Order of Top Dose.

Lab	TD	Time of Testing		
		2 wk	4 wk	6 wk
Activity				
A	100	-, [↓ MA, ↓ REAR]	↓ ma	-
B	100	-, [↓ MA]	↓ ma	-
D	100	↓ ma	↓ ma	↓ MA
F	100	-	-	↓ rear
H	100	↓ ma	↓ ma	-
E	66.7	-, [↓ ma]	-, [↓ ma]	-
G	66.7	-	-	-
Autonomic				
A	100	-	-, [pupil]	-
B	100	lacrim	lacrim, [PUPIL]	-
D	100	-, [↑ DEF, ↑ URIN, PUPIL]	-	-
F	100	-	-	-
H	100	-, [↓ DEF[1], PTOSIS]	-	-
E	66.7	-, [↓ def]	↓ urin	-
G	66.7	-, [↑ urin]	-	↑ def
Convulsive				
B	100	[trem]	[TREM]	-
D	100	-	-	-
F	100	-	[trem]	-
H	100	MYOC, [SMACK, TREM, OPISTH]	myoc, opisth	-
E	66.7	-	-	-
G	66.7	-	-	-
Excitability				
A	100	↓ ar, [↓ REM, ↓ HAND]	↓ AR, ↓ REM, ↓ HAND	↓ AR, ↓ HAND
B	100	-	-, [↓ ar, ↓ hand]	-
D	100	-, [↓ AR, ↓ REM, ↓ HAND]	-, [↓ ar]	-
F	100	↓ AR, [↓ REM]	↓ ar	↓ ar
H	100	-, [↓ AR, ↓ REM, ↓ HAND]	↓ ar, ↓ hand	-
E	66.7	-	↓ AR	-
G	66.7	-, [↓ ar]	↓ ar[1], [↓ HAND]	-
Neuromuscular				
A	100	-, [GAIT, ↓ FGRIP, ↓ HGRIP, RIGHT]	GAIT, ↓ HGRIP, [↓ FGRIP, RIGHT]	GAIT, ↓ HGRIP
B	100	-, [gait, ↓ hgrip]	-, [gait]	-
D	100	-, [GAIT, ↓ FGRIP, ↓ HGRIP, RIGHT]	-, [GAIT, ↓ FGRIP, ↓ HGRIP, ↓ SPLAY, RIGHT]	GAIT, RIGHT, ↓ FGRIP
F	100	-, [GAIT, ↓ FGRIP, ↓ HGRIP]	right, [↓ hgrip]	-
H	100	gait, [↓ FGRIP, ↓ HGRIP]	gait, ↓ splay	gait, ↓ splay
E	66.7	-, [GAIT, ↓ FGRIP, ↓ HGRIP, ↓ SPLAY]	-, [gait]	-
G	66.7	-, [GAIT]	-, [GAIT, ↓ FGRIP, ↓ HGRIP]	-
Sensorimotor				
A	100	↓ tp	↓ tp, [↓ CLICK, ↓ TOUCH]	-
B	100	-	-	↑ tp
D	100	-	-, [↓ appr]	-
F	100	-	-	↓ touch[1]
H	100	↓ appr, [↓ TP, ↓ CLICK[1], ↓ TOUCH]	-	-
E	66.7	-	-	-
G	66.7	-	-	-
Physiological Measures				
A	100	↓ wt, ↓ temp, pilo	↓ wt, ↓ temp, pilo	↓ wt, ↓ temp, pilo
B	100	↓ wt, pilo, [↓ temp]	↓ wt, ↓ temp, pilo	↓ wt
D	100	↓ wt, [↓ temp]	↓ wt	↓ wt
F	100	↓ wt	↓ wt, [↓ temp]	↓ wt
H	100	↓ wt, ↓ temp, pilo	↓ wt, ↓ temp, pilo	↓ wt
E	66.7	↓ wt	↓ wt, [↓ temp]	↓ wt
G	66.7	↓ wt	↓ wt, [↓ temp]	↓ wt

[a] See Table 5 for key

[b] Effects listed in brackets are those only produced only by the doses which were excluded from analyses due to high lethality (>50%); this occurred in the 50%- and 25%-TD dose groups, and also in the 12.5%-TD dose group in laboratories D and H.

[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose

* Domain significant at time point but no individual measure was significant

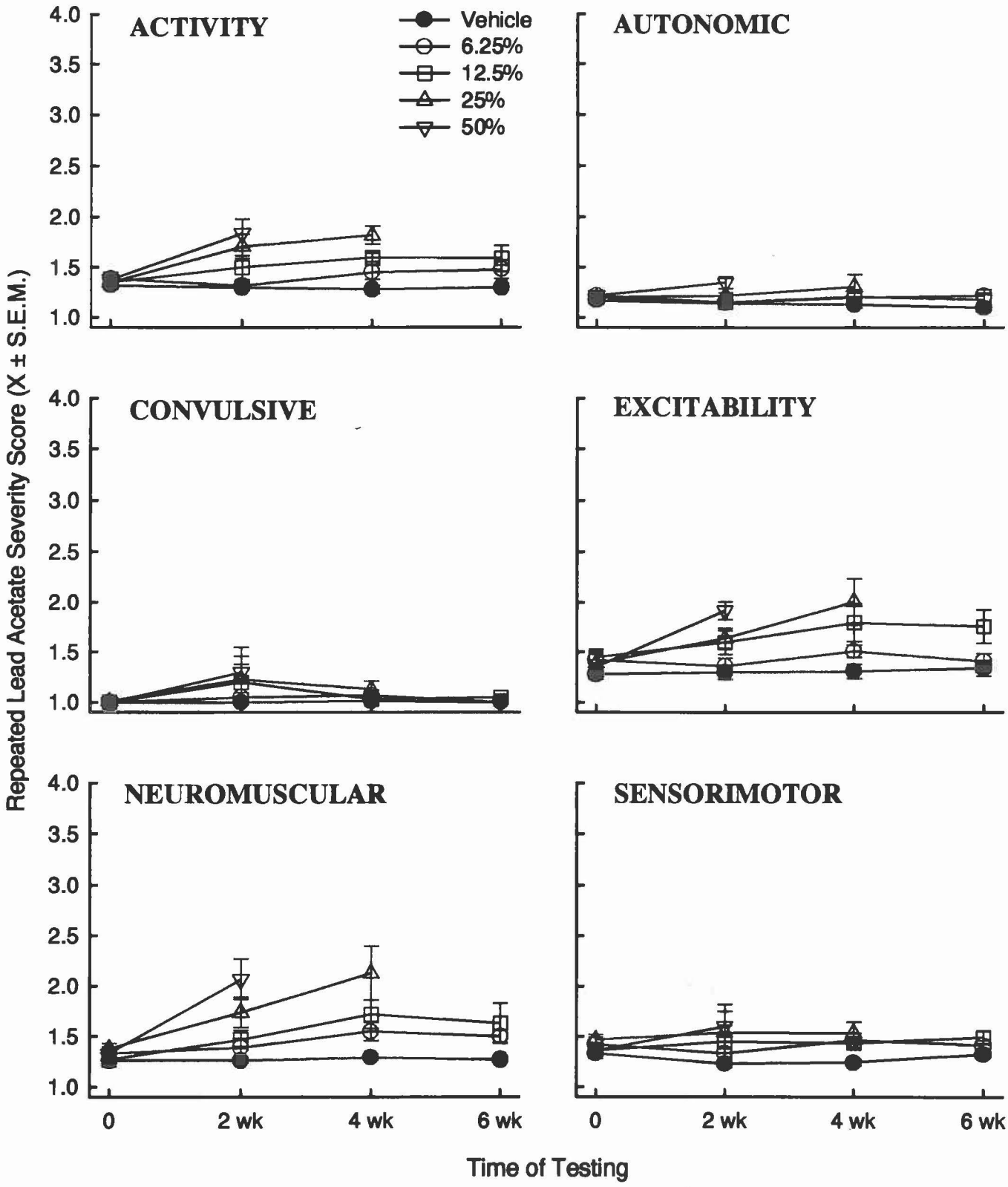


FIG. 24. Effects of repeated administration of lead acetate on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Data for the 50%-TD treatment are shown to two weeks only, and the 25%-TD group at two and four weeks only, due to lethality in those dose groups; all domains except for Convulsive were altered by those doses. Two-way ANOVAs of the lower dose groups revealed significant overall effects in the Activity, Excitability, Neuromuscular, and Sensorimotor Domains.

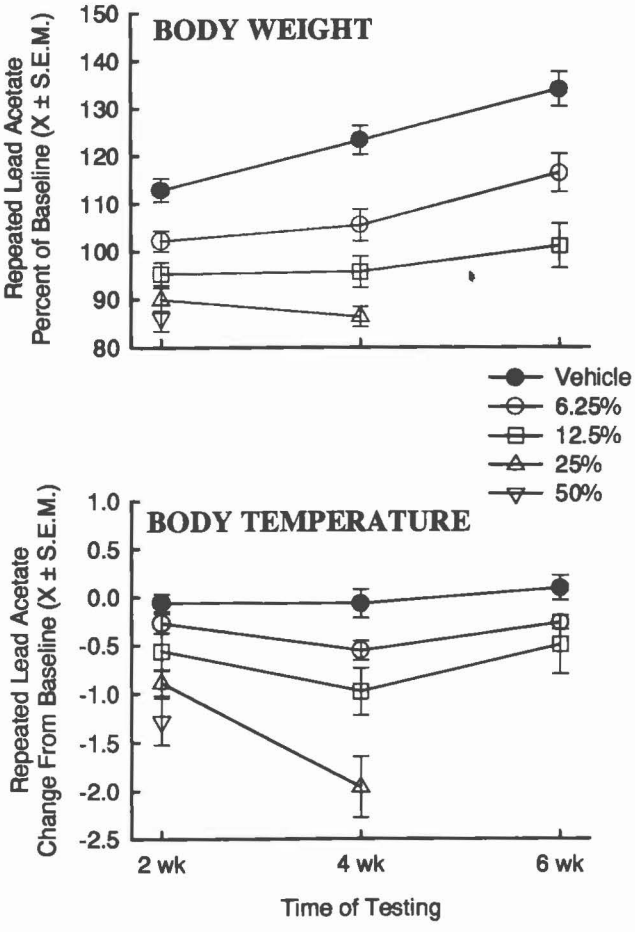


FIG. 25. Effects of repeated administration of lead acetate on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Data for the 50%-TD treatment are shown to two weeks only, and the 25%-TD group at two and four weeks only, due to lethality in those dose groups. Two-way ANOVAs revealed significant overall effects on both measures.

Hypothermia was recorded in all laboratories, with decreases ranging from 1° to 4°C (see Figure 25). Weight loss was severe, such that at the end of dosing body-weight group means were 50-80% of control. The only exception was one laboratory (G), whose few remaining high-dose rats actually gained about 100 grams between two and four weeks (observations of enlarged livers and ascites were also recorded for those rats), even though weight loss was evident in lower dose groups in that laboratory. Due to the profound toxicity expressed in the two higher dose groups (and in the three higher dose groups in two laboratories, D and H), the data from those rats were excluded from the repeated-measures analyses.

The repeated-measures analyses therefore included only controls and either the two lower doses, or the lowest dose only (in laboratories D and H). At the end of dosing (four-week time point), significant effects were obtained in the Activity, Excitability, Neuromuscular, and Sensorimotor domains (see Figures 24 and 25). Only the Activity changes were still significant at six weeks. These were the same domains affected in the single-dose study at this level of analysis. In addition, the overall analysis revealed significant body weight deficits even in the lowest dose group, as well as hypothermia.

The profile shown by the across-laboratory analysis was not indicative of the individual laboratories. Only three laboratories showed significant changes in the Excitability domain at either two or four weeks, and two produced Neuromuscular domain changes at four and/or six weeks. The remaining domains were either not altered (Autonomic, Sensorimotor) or affected in only one laboratory (Activity, Convulsive). There were few individual measures which were consistently affected in the low dose groups, and the changes, when seen, were generally small. There were even fewer effects in the laboratories using the lower dose range (laboratories E and G). The data for each laboratory are presented in Table 12.

Within the Excitability domain, the most consistent effect was that of decreased arousal in five of the seven laboratories at the end of dosing. A few instances of lower reactivity to being handled or removed from the cage were also obtained. Activity changes were mostly comprised of decreased motor activity, observed in four laboratories at four weeks and in two laboratories at two weeks. The magnitude of hypoactivity was 43-56% of control values at four weeks.

There was little consistency within the Neuromuscular and Sensorimotor domains in terms of any measure being affected by more than three laboratories, and usually not even as many as two. One laboratory reported a significant proportion of rats showing myoclonus and opisthotonos at two and four weeks. Since this was also the laboratory that obtained 100% lethality in the 12.5%-TD group, it is possible that this strain of rat was extremely sensitive to the effects of lead acetate.

In contrast to the variable behavioral measures, there was complete agreement on the effects on body weight; the data are presented in Figure 26. Generally the 12.5%-TD dose group showed weight loss, and the 6.25%-TD group did not gain weight during the dosing period. At

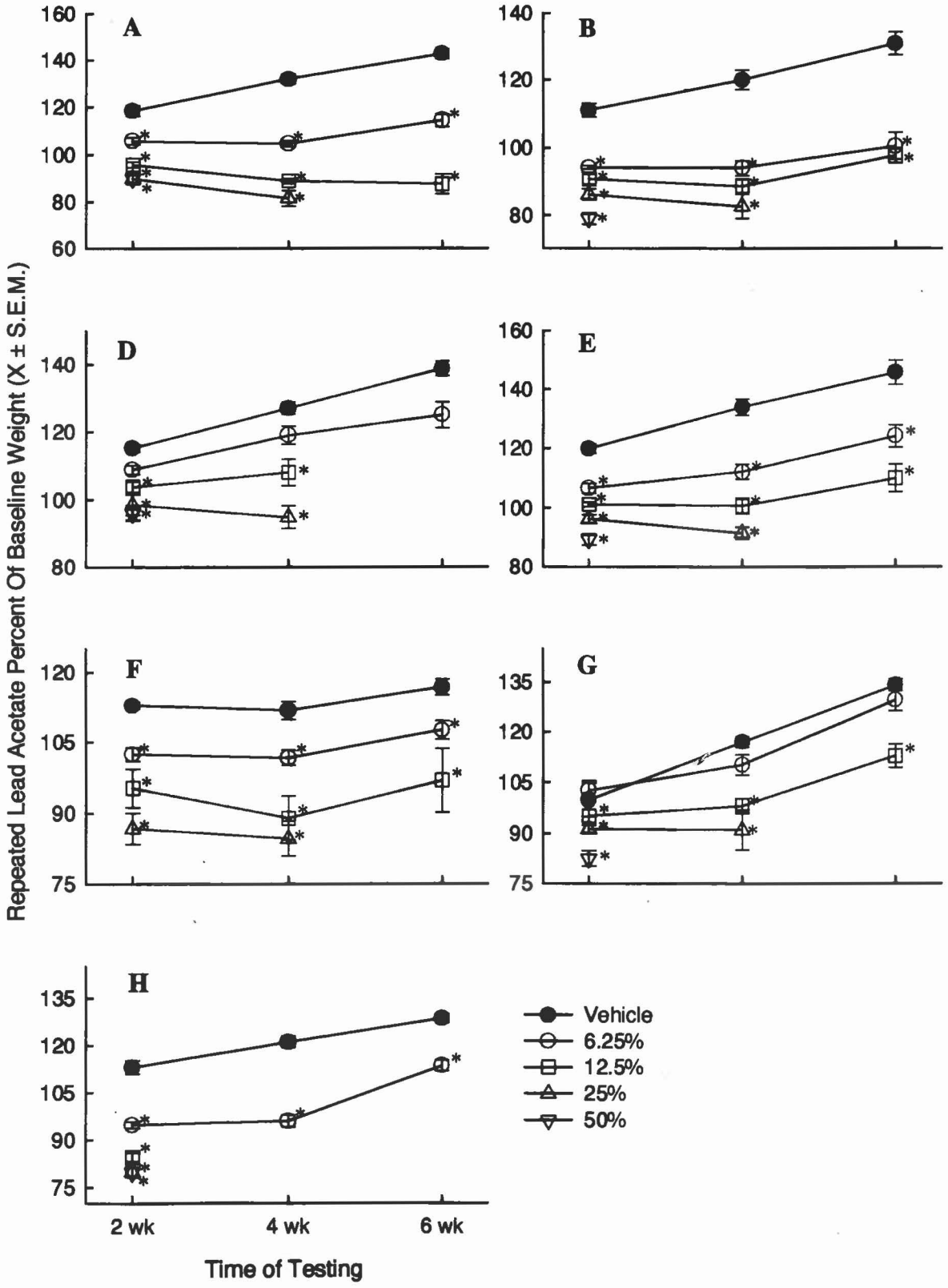


FIG. 26. Effects of repeated administration of lead acetate on body weight, expressed as mean percent of baseline body weight (± S.E.M.), in individual laboratories at two, four, and six weeks. Data for the 50%-TD treatment are shown to two weeks only, and the 25%-TD group at two and four weeks only, due to lethality in those dose groups. Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

the end of the study, all treated rats showed some weight gain but at six weeks they had not recovered. At four weeks, the group means were 77-95% and 68-85% of control values for the 6.25%- and 12.5%-TD groups, respectively. In addition, three laboratories reported hypothermia (0.6-1.5°C lower than controls) and piloerection. Thus, the effects of lead acetate on general measures of toxicity were more profound than the observed behavioral effects.

Parathion

There was a threefold range in the doses determined as the Top Dose, from 3.0 mg/kg to 10.13 mg/kg (listed in Table 3). In general, Wistar and Sprague-Dawley rats appeared the most sensitive, and the inbred rats were the least sensitive (highest TD). The distribution of individual TDs was symmetrical around the calculated median top dose of 5.62 mg/kg. The determinations of the time of peak effect (TOPE) based on changes of gait and/or arousal are given in Table 2. Almost all laboratories chose either two or three hours as the TOPE, and one laboratory chose 1.5 hours.

Acute Parathion Dosing. As shown in Table 4, lethality at the TD was at or below 20% in seven out of eight laboratories; the exception was one laboratory that obtained 50% lethality at a TD of 6.75 mg/kg.

Two-way ANOVAs indicated that a single dose of parathion induced statistically significant effects in all of the functional domains at the TOPE, with significant, albeit smaller changes observed at 24 hours post-injection for the Activity, Convulsive, and the Neuromuscular domains. These data are shown in Figure 27. Almost all effects were obtained at the high dose (100%-TD), with considerably fewer effects at 50% of the TD. Significant hypothermia was also observed at the TOPE (see Figure 28). A non-significant trend for body weight loss was evident at 24 hours (dose-by-time interaction $p<0.07$).

In general there were many similarities across laboratories in the profile of domain effects. The Neuromuscular and Convulsive domains were altered by parathion in all laboratories, and the remaining domains were significantly affected in at least five laboratories each. The effects on each domain and individual measure are listed in Table 13.

Autonomic cholinergic signs were present at the TOPE for most laboratories, and only at the TD. The Autonomic domain was significant for five laboratories, wherein the most consistent observation was salivation. The dose-response curves for the ranking of salivation are presented in Figure 29. Four laboratories also reported a lack of the pupil response, and there was a trend (dose $p<0.09$) in a fifth laboratory: in most cases miosis was recorded. Interestingly, only one laboratory reported lacrimation. In the two laboratories where defecation was altered it was decreased, whereas in several instances diarrhea in the home cage was reported. Only one laboratory showed an increase in urination.

Five out of the eight laboratories obtained significant effects in the Activity domain at TOPE. Motor activity decreases, in some cases quite pronounced (7-27% of control levels), were obtained in four laboratories and showed a trend in the fifth (dose factor $p<0.06$). In three laboratories, all of which used the higher top doses, rearing in the open field was decreased at the TOPE and motor activity was still depressed at 24 hours. Flattened posture and lack of activity in the home cage was reported in six laboratories.

The Convulsive domain was significant at the TOPE in all laboratories for which data were available. There was complete agreement in the reporting of tremors, and half of the laboratories also reported chewing, or mouth smacking. In two instances myoclonus or opisthotonos were recorded as well.

The Excitability domain showed significant effects in five out of the eight laboratories at the TOPE. Arousal was most consistently reduced, in the four laboratories with the highest TD's. There was less consistency in reports of decreased resistance to handling (four laboratories) or removal of the rat from the home cage (three laboratories).

All laboratories obtained significant effects on the Neuromuscular domain at the TOPE. Individual measures most consistently affected in all eight laboratories included gait alterations (primarily described as ataxia) and righting deficits. Gait changes were still observed at 24 hours in half of the laboratories, whereas almost all other neuromuscular changes had recovered at that time. Weakness, manifest as decreased forelimb and/or hindlimb grip strength, was evident at the high dose in seven laboratories. These measures did not show pronounced changes, nor were there differential effects on either forelimbs or hindlimbs (forelimb grip strength values were 62-83% of control, hindlimb grip strengths were 63-87% of control). Furthermore, landing foot splay was increased (124-188% of control values) in half of the laboratories, and there was a trend in a fifth laboratory (dose-by-time interaction $p<0.07$).

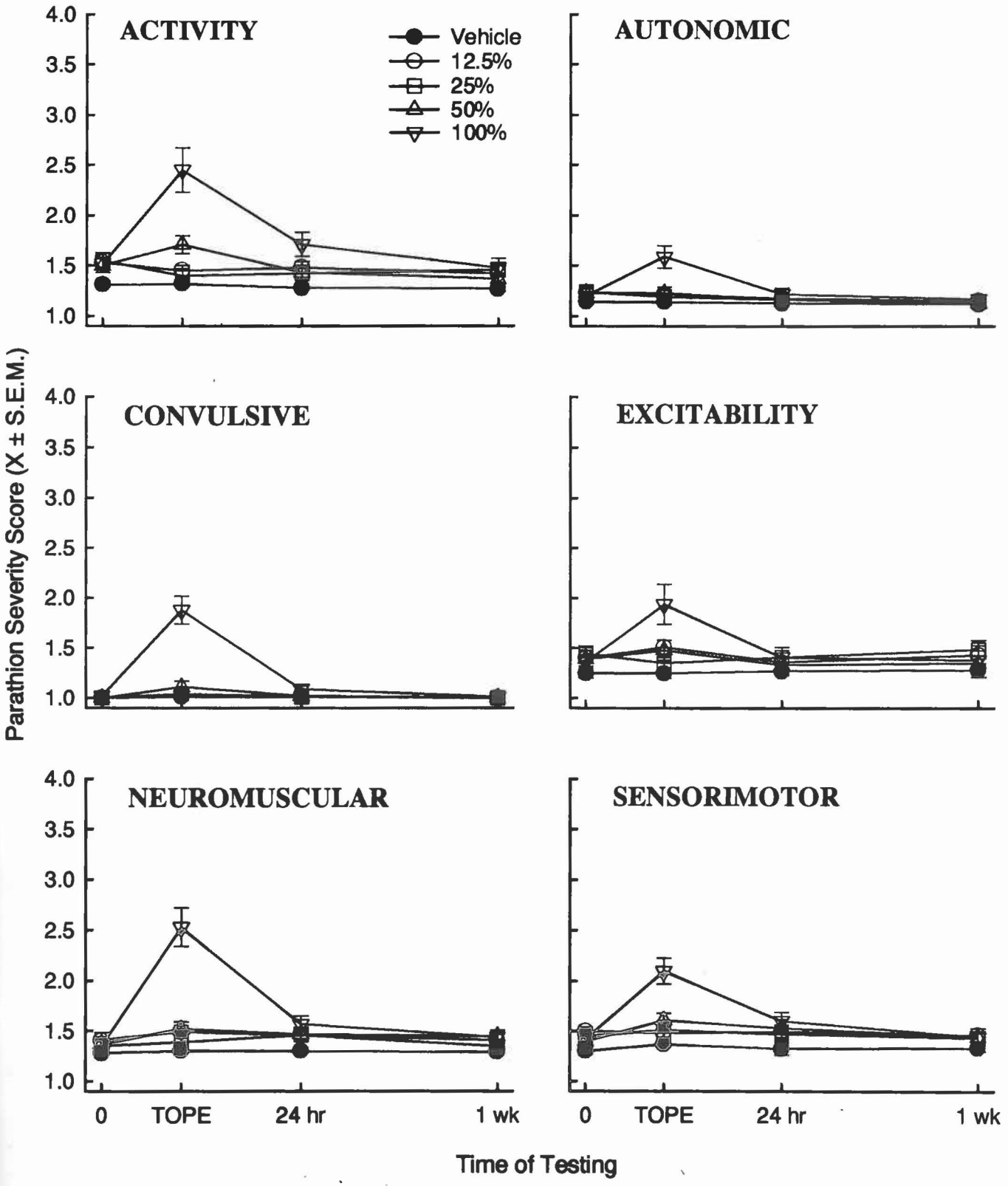


FIG. 27. Effects of acute parathion on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects in all of the Domains.

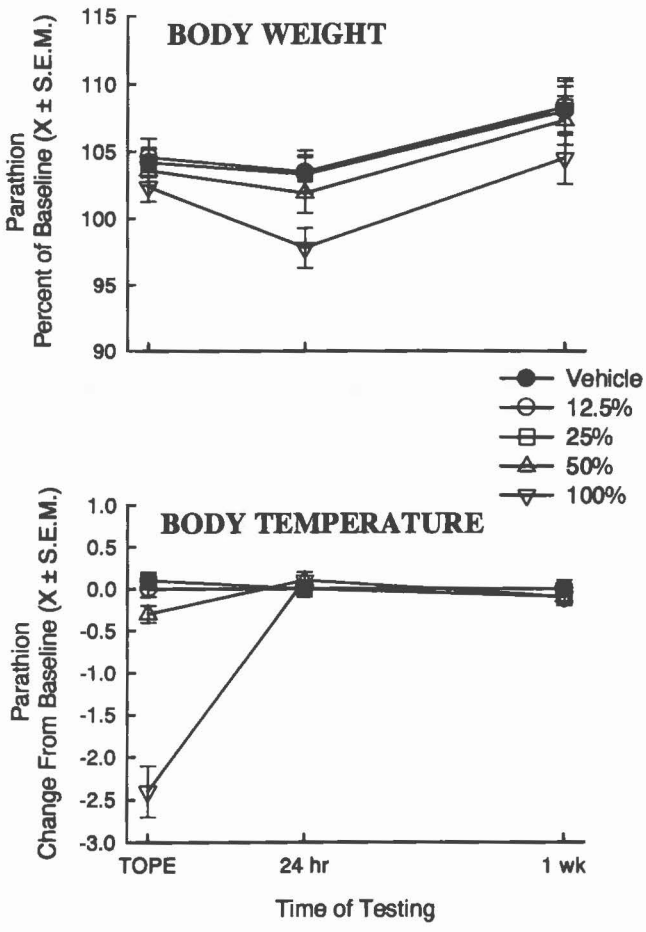


FIG. 28. Effects of acute parathion on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed a significant overall effect on body temperature.

Six laboratories reported significant Sensorimotor domain effects at the TOPE. The tail-pinch response was decreased in six laboratories, but one laboratory showed a non-dose-related increased response. These data are shown in Figure 30. When this measure was significant, it was quite pronounced: 57-100% of rats in the high-dose groups showed no response whatsoever to the tail pinch. In the two laboratories in which this effect was not statistically significant, the control levels of reactivity were quite low: in fact, these were among the laboratories which had the lowest baselines on this measure (see Moser *et al.*, 1997c). The click and touch responses were decreased in half of the laboratories, and there was essentially no effect on approach response (one laboratory reported increased response).

All laboratories reported a marked decrease in body temperature, ranging from 1.3° to 3.8°C less than controls. Almost all of the laboratories showed weight loss, which occurred primarily 24 hours after administration of the high dose; three laboratories still reported differences in mean weights at one week.

Repeated Parathion Exposures. With repeated dosing, significant lethality (70%, 100%) was obtained in two laboratories (D and G) at the high dose (50% of the TD), and in a third laboratory (A) who sacrificed all the rats in the high-dose group following the death of three rats within the first week of dosing. Of the remaining four laboratories, the high dose produced 30% and 40% lethality in the laboratories using higher doses, and 0% and 12.5% in laboratories with lower TD's (see Table 4). Generally, lethality occurred throughout dosing, i.e., at any time during the dosing regimen. Since repeated-measures analyses were used, the rats who died during testing were eliminated from the statistical analysis. In the instances where more than 50% of the dose group died, that entire dose group was eliminated from analysis.

Overall, parathion had very few behavioral effects with repeated dosing. Analysis of the severity scores across all laboratories indicated significant Activity domain effects throughout testing (two through six weeks), and Neuromuscular domain effects during dosing (two and four weeks). As can be seen in Figure 31, these changes were trivial, especially those representing the Neuromuscular domain. Body temperature and weight data across laboratories indicated no significant alterations (Figure 32).

Domain analysis for the individual laboratories showed only sporadic effects, with no clear pattern of toxicity. Changes in all measures and domains are indicated in Table 14. Unlike the profile of domains across laboratories, no laboratory obtained effects on both the Activity and Neuromuscular domains. One laboratory obtained a significant effect on the Neuromuscular domain at four weeks, but there were no significant measures within that domain; the same situation occurred for the one laboratory regarding significant Activity effects at four weeks. Three laboratories produced significant changes in the Excitability domain, but again there was either no corresponding significant measure, or else decreased arousal was recorded. Most affected measures were sporadic: in fact, there were only four instances in which two labora-

TABLE 13. Parathion: Effects[a] of a Single Dose in Each Laboratory, Presented in Descending Order of Top Dose and TOPE.

Lab	TD	TOPE	Time of Testing		
			TOPE	24 hr	1 wk
Activity					
H	10.13	2	↓ MA, ↓ REAR, POST	↓ MA	-
E	6.75	2	↓ MA, ↓ REAR, POST	↓ ma	-
D	6.75	2	↓ MA, ↓ REAR, POST	↓ ma	-
G	6.75	1.5	post	-	-
B	4.5	3	↓ MA, POST	-	-
A	4.5	3	POST	↓ ma	-
C	4.5	2	-	-	-
F	3.0	3	-	-	-
Autonomic					
H	10.13	2	pupil	-	-
E	6.75	2	SALIV, LACRIM, PUPIL, ↓ DEF	-	↑ def
D	6.75	2	SALIV, PUPIL	pupil	-
G	6.75	1.5	SALIV, ↓ DEF, PUPIL	↓ def[1]	-
B	4.5	3	-	pupil	-
A	4.5	3	SALIV, ↑ URIN	-	-
C	4.5	2	SALIV	↑ def[1]	-
F	3.0	3	-	-	-
Convulsive					
H	10.13	2	TREM, SMACK, MYOC	myoc	-
E	6.75	2	TREM, SMACK, OPISTH	-	-
D	6.75	2	TREM, SMACK	-	-
G	6.75	1.5	TREM	-	-
B	4.5	3	TREM	-	-
C	4.5	2	TREM, SMACK	-	-
F	3.0	3	TREM	-	-
Excitability					
H	10.13	2	↓ AR, ↓ HAND[1], ↑ REM	-	-
E	6.75	2	↓ AR, ↓ HAND, ↓ REM	↓ hand, ↓ rem	↓ rem[1]
D	6.75	2	↓ AR	-	-
G	6.75	1.5	↓ AR, ↓ HAND, ↓ REM	-	-
B	4.5	3	↓ hand	-	-
A	4.5	3	↓ REM	-	-
C	4.5	2	-	-	↑ rem
F	3.0	3	-	↑ hand[1]	-
Neuromuscular					
H	10.13	2	GAIT, RIGHT, ↓ FGRIP, ↓ HGRIP, ↑ SPLAY	-	-
E	6.75	2	GAIT, RIGHT, ↓ FGRIP	gait	-
D	6.75	2	GAIT, RIGHT, ↓ FGRIP, ↑ SPLAY	gait	-
G	6.75	1.5	GAIT, RIGHT, ↓ HGRIP, ↑ SPLAY	↓ splay[1]	-
B	4.5	3	GAIT, RIGHT	-	-*
A	4.5	3	GAIT, RIGHT, ↓ FGRIP, ↓ HGRIP, ↑ SPLAY	gait, right	-
C	4.5	2	GAIT, RIGHT, ↓ HGRIP	GAIT, RIGHT	gait
F	3.0	3	GAIT, RIGHT, ↓ HGRIP	-	-
Sensorimotor					
H	10.13	2	↓ TP	-	-
E	6.75	2	↓ TP, ↓ TOUCH	-	-
D	6.75	2	↓ TP, ↓ TOUCH, ↓ CLICK	↓ appr	-
G	6.75	1.5	↓ tp, ↓ touch, ↓ click	-	↑ tp[1], ↑ appr
B	4.5	3	↓ CLICK	-	-
A	4.5	3	↓ TP, ↓ TOUCH, ↓ CLICK, ↑ APPR	↓ TP	-
C	4.5	2	↓ TP	-	-
F	3.0	3	↑ tp[1]	↑ click[1]	-
Physiological Measures					
H	10.13	2	↓ temp	↓ wt	-
E	6.75	2	↓ temp, ↓ wt	↓ wt	↓ wt
D	6.75	2	↓ temp	↓ wt	wt
G	6.75	1.5	↓ temp	↓ wt, pilo	-
B	4.5	3	↓ temp	↓ wt	-
A	4.5	3	↓ temp, ↓ wt	↓ wt	↓ wt
C	4.5	2	↓ temp, pilo	pilo	-
F	3.0	3	↓ temp	-	-

[a] See Table 5 for key
[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose
* Domain significant at time point but no individual measure was significant

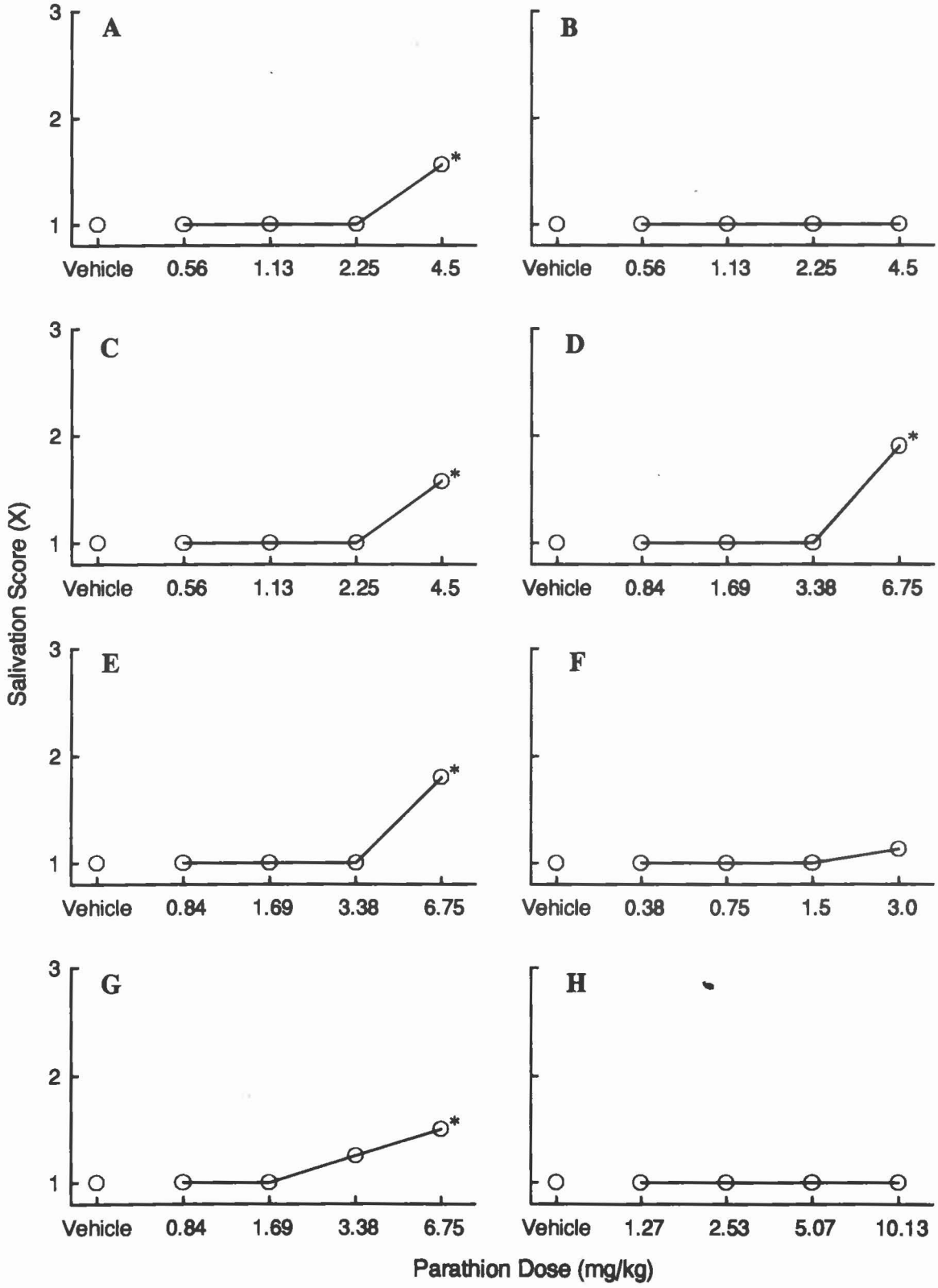


FIG. 29. Effects of acute parathion on salivation in individual laboratories, at the TOPE. Salivation is scored on a scale as '1' (no salivation), '2' (slight), and '3' (severe salivation). Data are presented as mean score for each dose group. Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control (Kruskal-Wallis analyses of the TOPE data; see Methods).

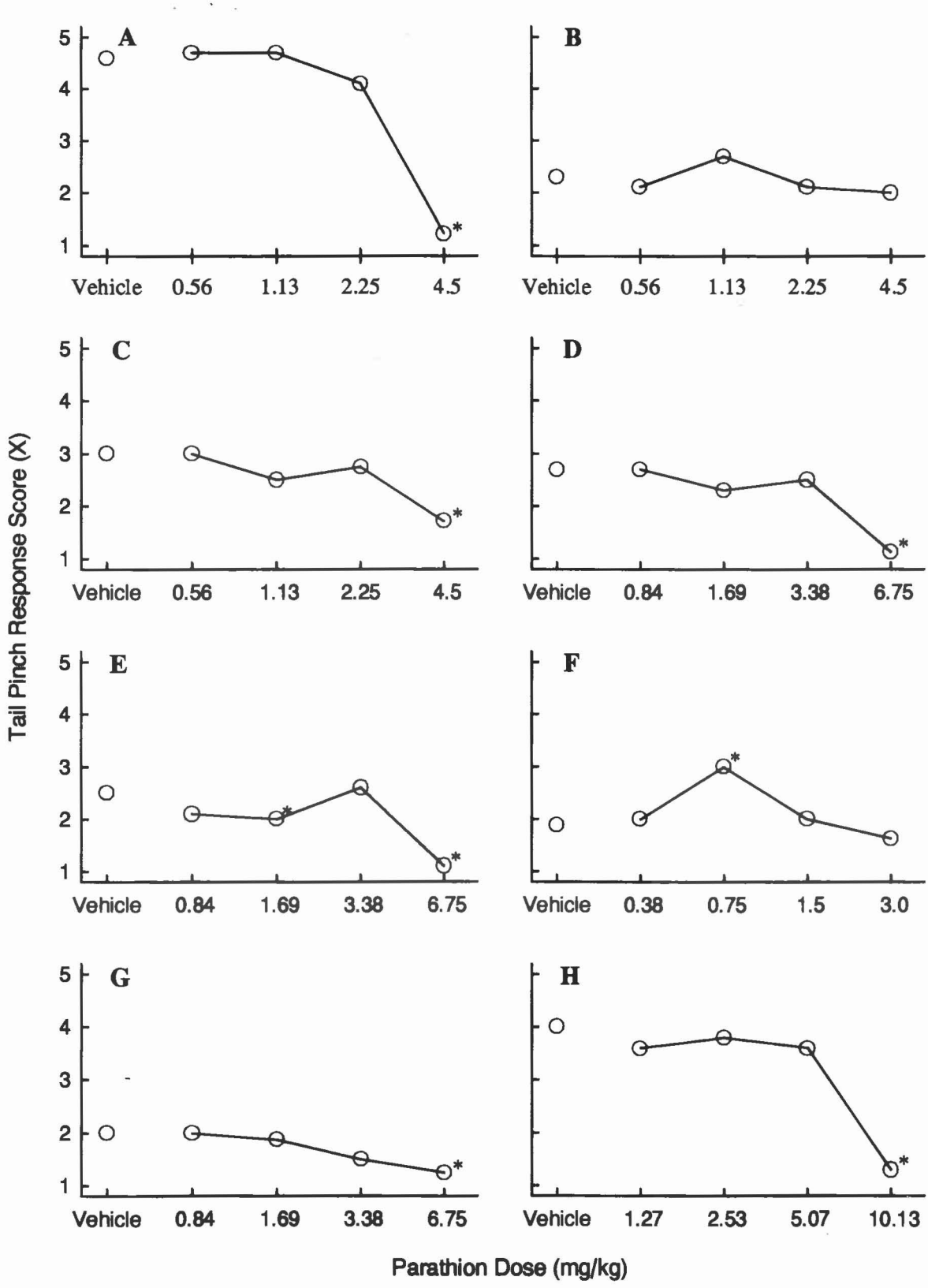


FIG. 30. Effects of acute parathion on the tail-pinch response of rats in individual laboratories, at the TOPE. The tail-pinch response is ranked on a scale ranging from '1' (no reaction) to '5' (exaggerated reaction). Data are presented as mean score for each dose group. Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

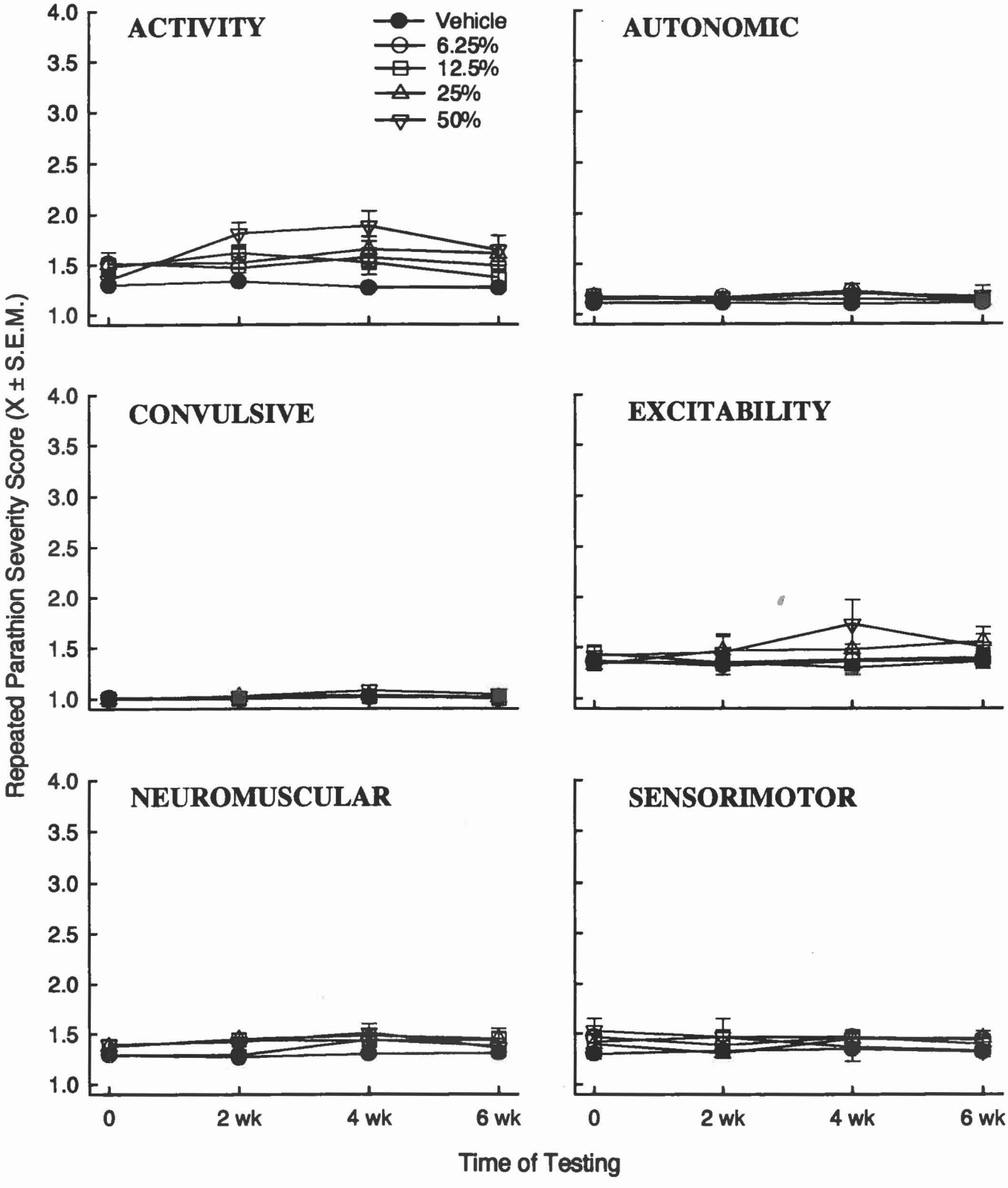


FIG. 31. Effects of repeated administration of parathion on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects only in the Activity and Neuromuscular Domains.

TABLE 14. Parathion: Effects[a,b] of Repeated Doses in Each Laboratory, Presented in Descending Order of Top Dose.

Lab	TD	Time of Testing		
		2 wk	4 wk	6 wk
Activity				
H	5.07	↓ ma	↓ ma	-
E	3.38	-	-	-
D	3.38	-	↓ rear	↓ rear, ↑ ma
G	3.38	-	-	-
B	2.25	-	-	-
A	2.25	-	-	-
F	1.5	-	-*	-
Autonomic				
H	5.07	-	↑ urin[1]	-
E	3.38	-	-*	-
D	3.38	-	-	-
G	3.38	-	-	-
B	2.25	-	-	-
A	2.25	↓ def	-	-
F	1.5	-	-	-
Convulsive				
H	5.07	-	-	-
E	3.38	-	-	-
D	3.38	-	-	-
G	3.38	-	-	-
B	2.25	trem[1]	trem	trem
F	1.5	-	-	-
Excitability				
H	5.07	-	-*	↓ AR
E	3.38	↑,↓ hand	↓ hand	-
D	3.38	↓ ar	↓ AR	↑ ar[1]
G	3.38	-	↓ rem	-
B	2.25	-	↓ hand	↓ ar
A	2.25	-*	-	-
F	1.5	↓ rem[1]	-	-
Neuromuscular				
H	5.07	-	-	-
E	3.38	-	-	-
D	3.38	-	-	-
G	3.38	-	-*	-
B	2.25	-	-	-
A	2.25	-	-	-
F	1.5	-	-	-
Sensorimotor				
H	5.07	-	-	↑ touch[1]
E	3.38	-	↑ touch	-
D	3.38	↓ touch, ↓ appr	↓ touch	↓ tp, ↓ touch
G	3.38	-*	-	-
B	2.25	-	↑ touch	-
A	2.25	-	-	-
F	1.5	-	-	↓ tp[1]
Physiological Domain				
H	5.07	↓ wt	↓ wt	-
E	3.38	-	↓ wt	-
D	3.38	↓ wt	↓ wt	-
G	3.38	-	-	-
B	2.25	↓ wt[1]	↓ wt[1]	-
A	2.25	-	-	-
F	1.5	-	-	-

[a] See Table 5 for key.
[b] Effects excluded those of the high dose in laboratories when that dose group had 70%-100% lethality (laboratories A, D, and G).
[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose.
* Domain significant at time point but no individual measure was significant.

tories reported the same effect at the same time point (decreased handling reactivity at four weeks, decreased arousal at six weeks, increased touch response at four weeks, and decreased tail-pinch response at six weeks).

Most effects were detected only at the high dose (50% of the TD). In six of the seven laboratories, the 25%-TD dose produced either no effects, or only one significant change on any measure. The next-lower dose, 12.5% TD, was a clear no-effect level in all laboratories.

Repeated administration of parathion produced only moderate effects on body weight. Four laboratories reported depressed weight gain, the remaining laboratories showed no effects on body weight. There were no other signs of compromised general health (body temperature, piloerection).

Toluene

Six of the seven laboratories included in this analysis used the limit dose, 2000 mg/kg, as the top dose; the seventh laboratory obtained 1333 mg/kg. This illustrates that orally-delivered toluene in a corn oil vehicle is not potent.

There was, however, considerable range of times selected for the TOPE, from one to four hours with a median value of three hours. Interestingly, the shortest time point was used by the laboratory which had the lowest TD; this laboratory was also the only one which found significant acute effects of toluene on both arousal and gait score (the measures used to determine TOPE). Several participants commented that toluene appeared to show a biphasic time-course, with the arousal measure showing changes earlier than gait score. The differences in peak times probably reflected the differences in how individual laboratories chose their time point.

Acute Toluene Dosing. No lethality occurred during the single-dose toluene study in any laboratory. At the overall level of analysis, only the Neuromuscular domain was significantly altered at the TOPE. This was not a large effect, as illustrated in the domain data in Figure 33. A slight loss of body weight was apparent at 24 hours, but the overall analysis did not reveal a statistically reliable effect (see Figure 34).

Table 15 provides the results of the severity scoring analysis as well as for each test measure for each individual laboratory. Although the Neuromuscular domain was significant overall, only one laboratory (H) showed effects in that domain. Laboratory H also had statistically significant changes in other domains, which were not

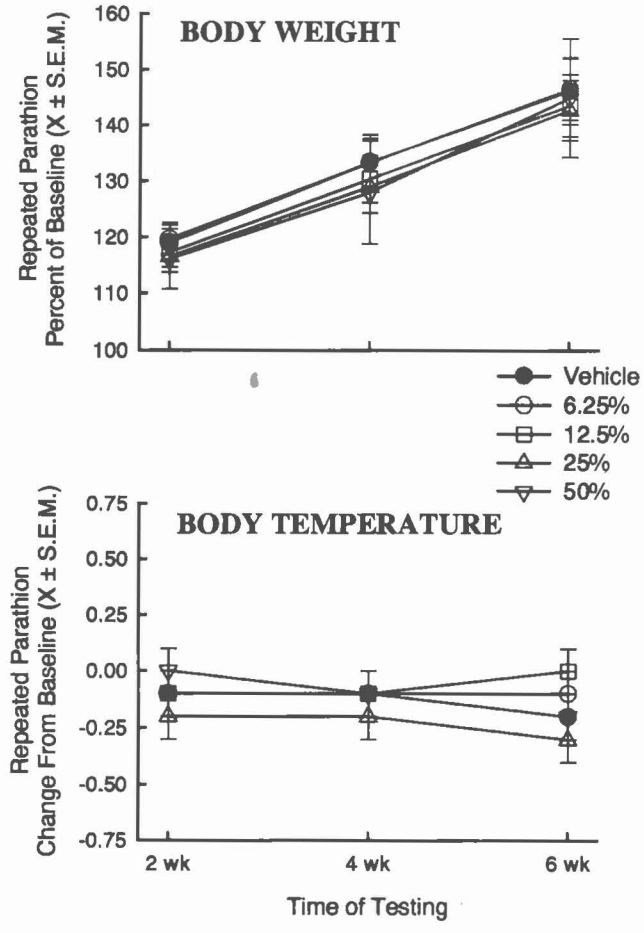


FIG. 32. Effects of repeated administration of parathion on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed no significant overall effects.

affected in the across-laboratory analysis (specifically, the Autonomic, Activity, and Sensorimotor domains). There was little effect of toluene in the other laboratories, with four laboratories showing no significant domain effects, and the remaining two showed changes in only one domain each. Thus, the data from laboratory H did not appear similar to those from the other laboratories.

Significant gait disorder, decreased forelimb and hindlimb grip strength, increased landing foot splay, and righting impairment all contributed to the significant Neuromuscular domain effect in laboratory H. Although two other laboratories also reported gait abnormalities

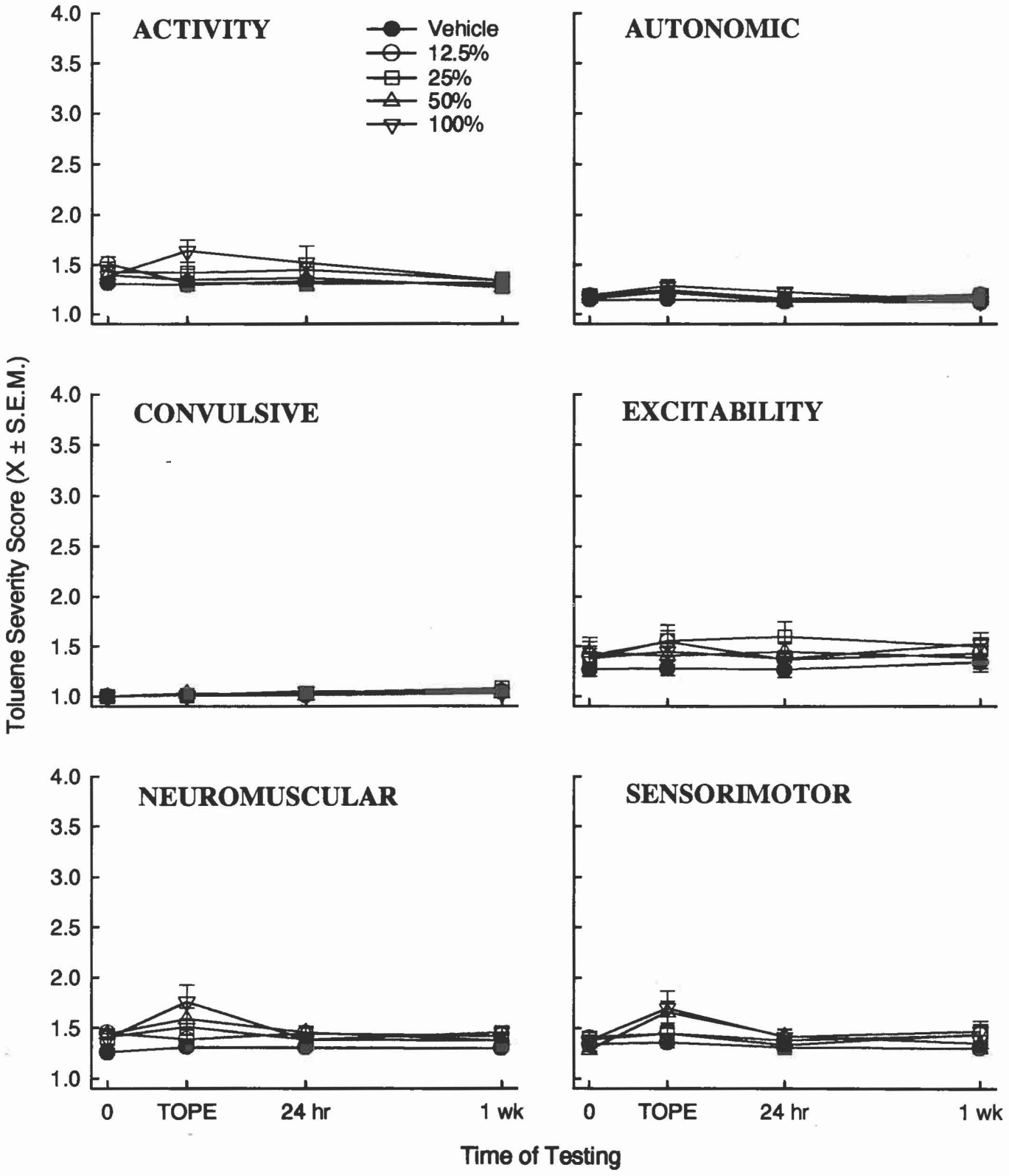


FIG. 33. Effects of acute toluene on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects only in the Neuromuscular Domain.

and decreased forelimb grip strength, the magnitude of the changes was considerably less than that recorded in laboratory H. For instance, the data for gait score are presented in Figure 35: in two laboratories (A and G), the changes were mild and described as tip-toe walking, but in laboratory H the effects were mostly ranked as severe, consisting of uncoordinated limb placement and tip-toe gait, and in addition splayed hindlegs at higher doses. The magnitude of forelimb grip strength decrease ranged from 72-90% of control levels in two laboratories, whereas laboratory H recorded a 50% decrease on this measure.

Activity changes following toluene were recorded as increased motor activity levels in two laboratories at the TOPE, but also as decreased activity at 24 hours in three laboratories. Similar biphasic tendencies were observed in the frequency of rearing for laboratory H. Three laboratories observed significant increases in arousal level at the TOPE, and a few increases were noted at 24 hours and one week. Reactivity to removal and handling were also mostly increased.

In the Sensorimotor domain, five laboratories demonstrated significantly weaker reactions to tail pinch at the TOPE. Thus this measure showed the most concordance of effect, although the magnitude of change was not great. Significant effects on the other sensory endpoints were sporadic, with no clear pattern of time-course or direction of change. For the sensorimotor and excitability measures in several laboratories, *post-hoc* contrasts revealed that intermediate, but not the highest, dose groups were different from control. This indicates a biphasic function, which was U-shaped for some measures (e.g., tail pinch response), or inverted U-shaped (e.g., arousal).

There were mostly no autonomic alterations recorded, except for a few changes in excretions. One laboratory recorded a small but significant incidence (37.5% of the high dose group) of lacrimation at the TOPE. Likewise, there were no convulsive abnormalities.

Body weight declined significantly at 24 hours after dosing in two laboratories, and this represented only a 2-4% loss. Only one laboratory reported hypothermia (0.6°C less than controls) at 24 hours.

Repeated Toluene Exposures. Lethality (30% of the dose group) occurred at the high dose level in only one laboratory during repeated dosing with toluene. There were also cases of lethality at lower doses in three laboratories, but these were dispersed across dose groups ranging from 0 to 250 mg/kg and were never greater than 10% of the group. In some of these instances, the lethality could have been due to aspiration of oil during gavage.

Figure 36 shows graphs for each domain, and Figure 37 shows body weight and temperature, using the data averaged across laboratories. In the overall laboratory analysis, no domain showed significant effects related to treatment. There was also no effect on weight gain or thermoregulation.

Within the individual laboratories, there were a few significant domain effects (Neuromuscular, Excitability, and Sensorimotor) but in these cases there were no corresponding effects on the measures within that domain. Only one laboratory (H) obtained significant domain

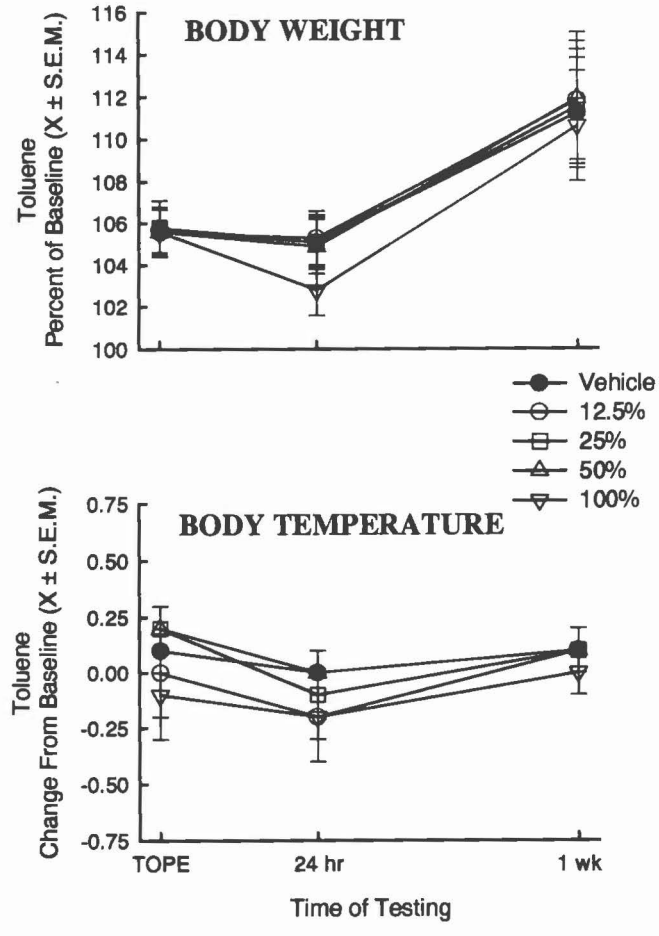


FIG. 34. Effects of acute toluene on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed no significant overall effects.

TABLE 15. Toluene: Effects[a] of a Single Dose in Each Laboratory, Presented in Descending Order of Top Dose and TOPE.

Lab	TD	TOPE	Time of Testing		
			TOPE	24 hr	1 wk
Activity					
D	2000	4	↓ rear	-	-
H	2000	4	↑ MA, ↑ REAR	↓ MA, ↓ REAR	-
A	2000	3	↑ ma	-	-
E	2000	3	-	↓ ma	-
F	2000	2	-	↓ ma	-
B	2000	1.5	-	-	-
G	1333	1	-	-	-
Autonomic					
D	2000	4	↑ def[1]	-	-
H	2000	4	↓ URIN	-*	-
A	2000	3	-	-	↑ urin[1]
E	2000	3	-	-	-
F	2000	2	-	-	↑ urin[1]
B	2000	1.5	↑ def	-	↓ urin
G	1333	1	lacrim	-	-
Convulsive					
D	2000	4	-	-	-
H	2000	4	-	-	-
E	2000	3	-	-	-
F	2000	2	-	-	-
B	2000	1.5	-	-	-
G	1333	1	-	-	-
Excitability					
D	2000	4	-	-	-
H	2000	4	↑ rem	-	-
A	2000	3	-	↑ ar	-
E	2000	3	↑ AR, ↓ REM	-	↑ rem
F	2000	2	-	-	-
B	2000	1.5	↑ ar[1]	↑ AR[1], ↑ REM[1], ↑ HAND[1]	-
G	1333	1	↑ ar	-	↑ ar
Neuromuscular					
D	2000	4	-	-	-
H	2000	4	GAIT, RIGHT, ↓ FGRIP, ↓ HGRIP, ↑ SPLAY	-	-
A	2000	3	gait	-	-
E	2000	3	-	-	-
F	2000	2	↓ fgrip	-	-
B	2000	1.5	-	-	-
G	1333	1	gait, ↓ fgrip	-	-
Sensorimotor					
D	2000	4	↓ tp	-	-
H	2000	4	↓ TP	-	↓ click[1]
A	2000	3	↓ tp	↓ tp[1]	-
E	2000	3	↓ tp[1]	↑ touch	-
F	2000	2	-	-	-
B	2000	1.5	-	↑ appr[1]	-
G	1333	1	↓ tp[1]	↑ click	-
Physiological Measures					
D	2000	4	-	-	-
H	2000	4	-	↓ wt, ↓ temp	-
A	2000	3	-	-	-
E	2000	3	-	↓ wt	-
F	2000	2	↓ temp	-	-
B	2000	1.5	-	-	-
G	1333	1	-	-	-

[a] See Table 5 for key.
[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose.
* Domain significant at time point but no individual measure was significant.

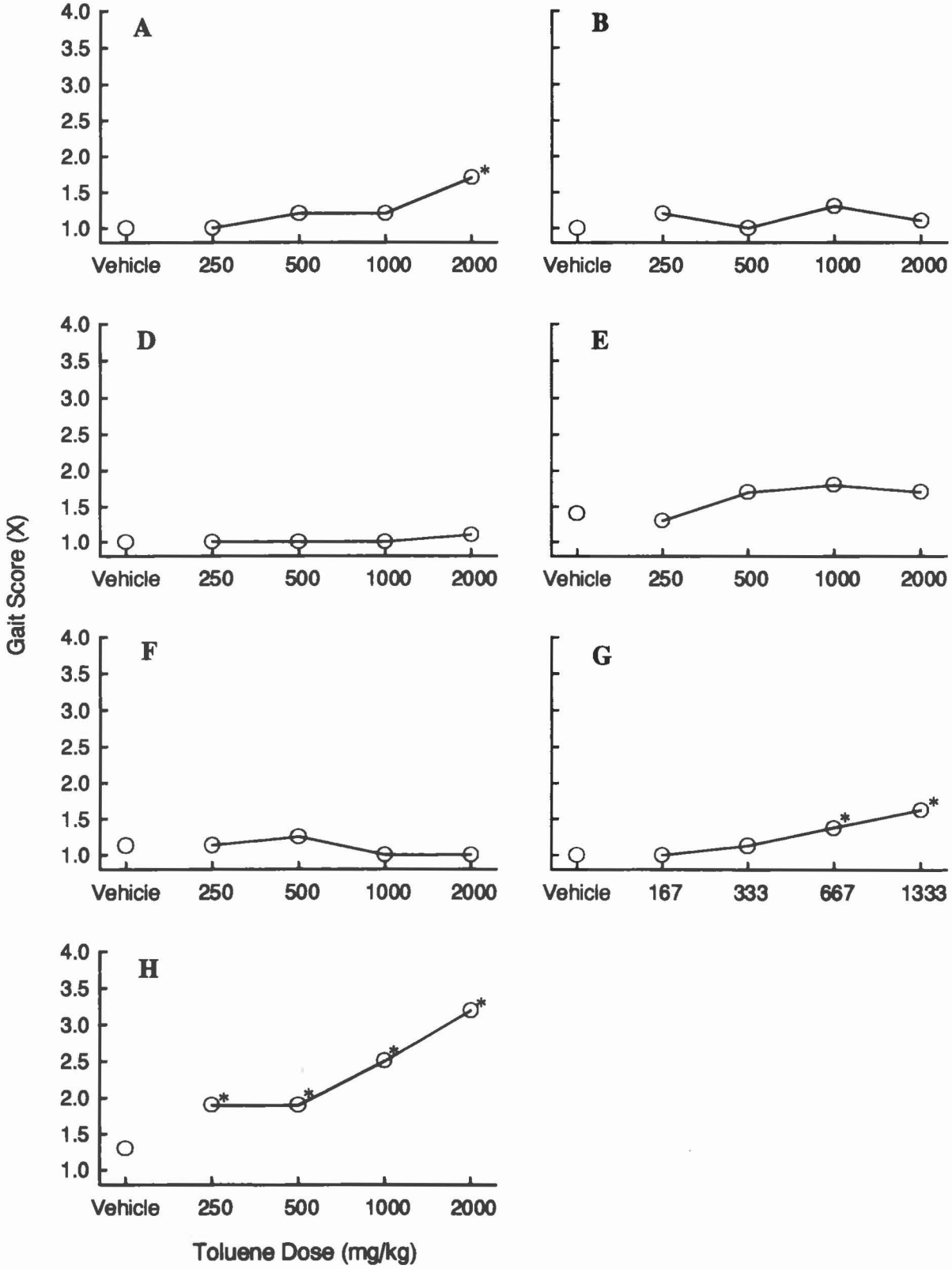


FIG. 35. Effects of acute toluene on the ranking of gait abnormalities in individual laboratories, at the TOPE. Gait is scored as '1' (normal), '2' (slightly abnormal), '3' (moderately abnormal), or '4' (severely abnormal). Data are presented as mean score for each dose group. Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

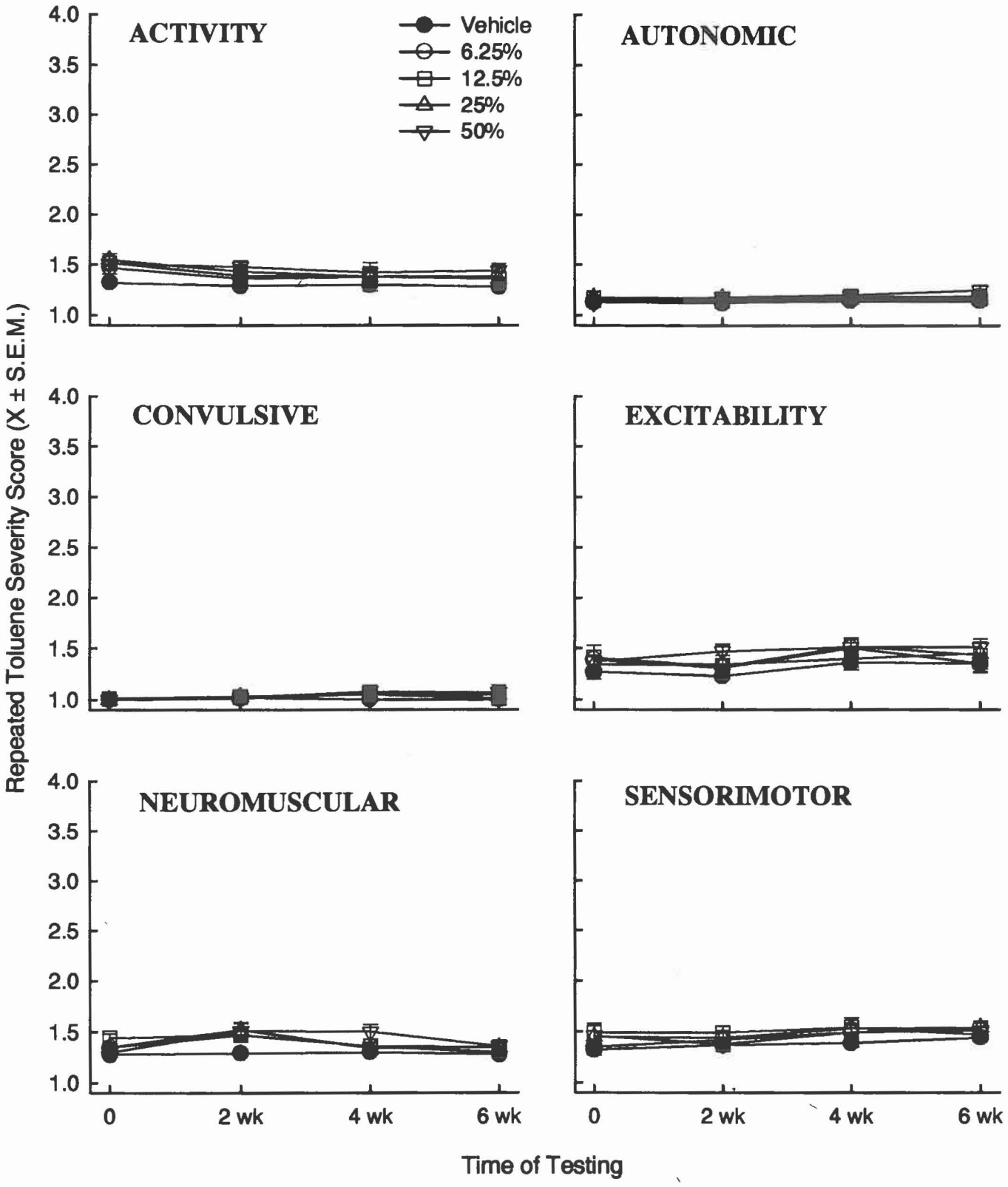


FIG. 36. Effects of repeated administration of toluene on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed no significant overall effects.

TABLE 16. Toluene: Effects[a] of Repeated Doses in Each Laboratory, Presented in Descending Order of Top Dose.

Lab	TD	Time of Testing		
		2 wk	4 wk	6 wk
Activity				
D	1000	-	-	-
H	1000	-	-	-
A	1000	-	-	-
E	1000	-	↓ ma[1]	-
F	1000	-	-	-
B	1000	↓ ma[1]	-	-
G	667	↑ rear[1]	-	-
Autonomic				
D	1000	-	-	-
H	1000	-	-	-
A	1000	↓ def[1]	-	-
E	1000	-	-	-
F	1000	-	-	↓ def[1]
B	1000	-	-	-
G	667	-	-	-
Convulsive				
D	1000	-	-	-
H	1000	-	SMACK, TREM	smack, trem
E	1000	-	-	-
F	1000	-	trem[1]	-
B	1000	-	-	-
G	667	-	-	-
Excitability				
D	1000	-	-	-
H	1000	-	-	-
A	1000	↓ rem[1]	↑ ar[1], ↑ rem[1]	-
E	1000	-	↑ hand[1]	↑ hand
F	1000	-	-	-
B	1000	-	-	-
G	667	↑ hand	-	-*
Neuromuscular				
D	1000	-*	-	-
H	1000	-	-	-
A	1000	-	-	-
E	1000	gait	gait	-
F	1000	-*	-	-
B	1000	-	-*	-
G	667	↓ splay	-	-
Sensorimotor				
D	1000	-	-	↑ click[1]
H	1000	-	-*	-
A	1000	↓ tp[1], ↑ touch[1]	-	-
E	1000	-	-	-
F	1000	-	-	-
B	1000	-	↓ appr[1]	-
G	667	-	-	-
Physiological Measures				
D	1000	-	-	-
H	1000	-	-	↓ temp
A	1000	-	-	-
E	1000	-	-	-
F	1000	↓ wt	↓ wt	↓ wt
B	1000	↓ wt	↓ wt	-
G	667	-	-	-

[a] See Table 5 for key.
[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose.
* Domain significant at time point but no individual measure was significant.

changes which corresponded to the observations of mouth-smacking and mild tremors. The Activity and Autonomic domains were consistently not affected. Table 16, which presents the results from the individual measure analyses, shows how few tests were significantly altered in these studies. In fact, there was almost never more than one laboratory detecting a significant change on any one endpoint at any given time. The only exception was the occurrence of mild tremors at four weeks, which was observed in two laboratories (30-50% incidence).

Weight gain was significantly depressed during dosing in only two laboratories; at the end of dosing, the high dose groups were 89% and 94% of the respective control groups. One laboratory recorded a slight but significant decrease in body temperature at the six-week time point (0.2-0.4°C less than controls).

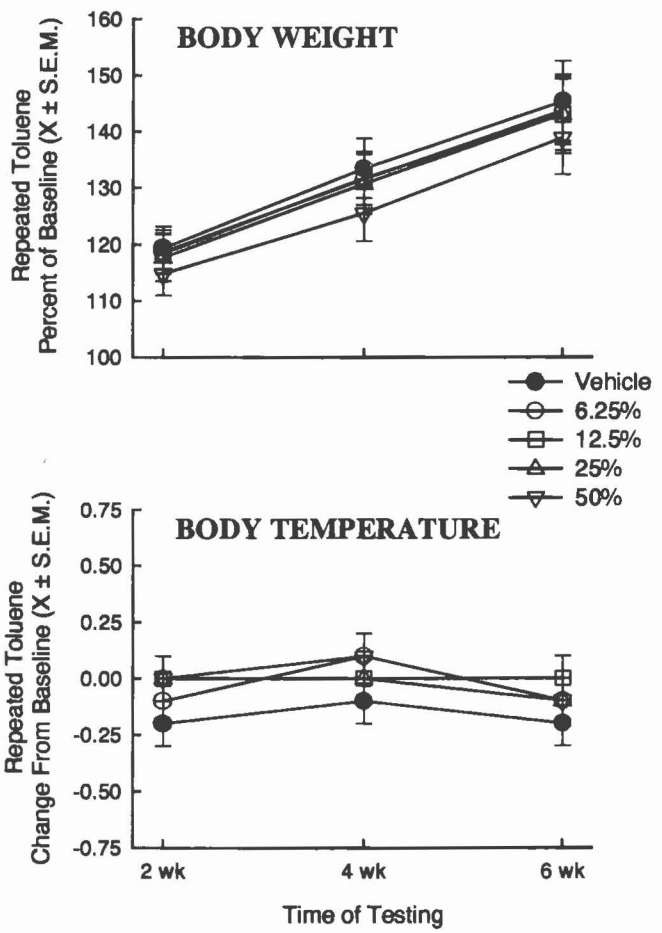


FIG. 37. Effects of repeated administration of toluene on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed no significant overall effects.

Triethyl Tin

The Top Doses for triethyl tin (TET) determined by seven laboratories ranged from 4 mg/kg to 9 mg/kg (presented in Table 3), with the median value 6 mg/kg. The Wistar rat appeared somewhat more sensitive, since both laboratories using that rat strain chose the lowest TD (4 mg/kg). All laboratories agreed that the time of peak effect was generally short: one to two hours in six laboratories, and three hours in the seventh laboratory (Table 2).

Acute TET Dosing. The lower TDs, 4 and 6 mg/kg, produced little lethality in the single-dose study; only one laboratory reported 10% lethality at the high dose (see Table 4). In the two laboratories using 9 mg/kg as the TD, however, lethality occurred in 70 and 90% of the rats. Therefore, the data from the high-dose group in those laboratories were excluded from the across-laboratory analyses. All deaths occurred between one day and one week after dosing.

Analysis of the severity scores across domains, using each laboratory mean as an observation, revealed significant effects in the Activity, Neuromuscular, and Excitability domains. These data are presented in Figure 38. The Activity and Neuromuscular domains showed large alterations which lasted throughout the study, whereas the Excitability changes were somewhat more transient. Body temperature was decreased across laboratories at the TOPE and at 24 hours, as shown in Figure 39. Whereas weight loss at one week was apparent in the group means, and there was a statistically significant dose-by-time interaction; however, that time point did not reveal a significant dose factor.

The profile of predominantly neuromuscular and activity effects, with some changes in excitability, was mirrored in almost all laboratories. Significant Neuromuscular domain effects were obtained in all laboratories at the TOPE, in six laboratories at 24 hours, and three laboratories continued to show significant effects at one week. Likewise, the Activity domain was affected in six of the seven laboratories at the TOPE. The Excitability domain was also altered in six laboratories, mostly only at the TOPE but at 24 hours or one week in a few laboratories. The Sensorimotor domain was affected in five laboratories, but there was less consistency in the effective

times and no more than three laboratories showed effects at any single time point. There were significant changes in the Autonomic and Convulsive domains in only a few laboratories, and these effects were very small.

Table 17 presents the effects on the test measures for each laboratory. The Activity and Neuromuscular domains clearly showed the most effects, with most endpoints within those domains significantly altered. Motor activity was greatly decreased in all but one laboratory (G). Group mean activity levels for the high-dose groups were ≤28% of control values in most laboratories, and in laboratory A the decrease reached 49% of control. Recovery was evident in most laboratories by one week, but in a few cases motor depression remained significant. While the primary activity change was decreased locomotion, a few laboratories also reported fewer rears in the open field, and some reported that rats (20-40%) in the high-dose group were lying with a flattened, prostrate posture in their home cages. One laboratory (E) reported increases in rearing at 24 hours and one week; examination of the data, however, revealed that while all rats displayed fewer rears during the course of the study, the control group showed a much larger decrease than did the other groups, leading to an apparent increase in treated rats at the later time points. This control group appeared atypical compared to the historical controls for that laboratory.

All laboratories obtained pronounced neuromuscular changes. Pronounced gait abnormalities, described as ataxia and uncoordinated hindlimb placement, and impaired righting reflex were reported by all laboratories. The data for gait score are presented in Figure 40, and data for the righting reflex score at the TOPE are shown in Figure 44. These effects peaked at the TOPE with little recovery at 24 hours, and gait was still significantly altered in most laboratories one week after dosing. In the laboratories using the higher dose levels (TD's of 6 or 9 mg/kg), both forelimb and hindlimb grip strength were decreased. The decrements in forelimb and hindlimb grip strength were approximately equivalent; maximum effects obtained by these laboratories were 54-73% and 52-82% for forelimb and hindlimb grip strength, respectively. Landing foot splay was also transiently increased in three laboratories. In two of the three laboratories using the lowest TD (4 mg/kg), a persistent and selective decrease (57-64% of control values) in hindlimb grip strength was noted; landing foot splay and forelimb grip strength were not altered.

TET effects on excitability measures were evident mostly at the TOPE and at 24 hours. Reactivity to being removed from the cage (ease of removal) and being handled (handling reactivity) were the measures most affected, with significantly lowered reactivity in six laboratories at the TOPE, and in five laboratories at 24 hours. The data for ease of removal at the TOPE are shown in Figure 41. Arousal was affected less often, being significantly lowered in four laboratories, but often these data did not show clear dose-response or time-course characteristics. In laboratory E, arousal showed significant increases in many of the dose groups, but as was the case with rearing, this was due more to abnormally low arousal scores in the control group than to treatment effects.

The sensorimotor changes were more erratic, with few measures showing consistent changes. Six laboratories showed decreases in the tail-pinch and/or the click response at one or more time points. The magnitude of hypo-responsiveness was considerable, and most rats (>50%) in the high-dose groups showed no response whatsoever. Decreased reactions to the approach and touch stimulus were also recorded, but with even less consistency of pattern. In several instances, the response was significantly increased, but these changes were seen only at one week or occurred at low or middle doses, but not the high dose (i.e., no monotonic dose-response).

Almost no convulsive or involuntary movements were reported following TET administration. Three laboratories reported a low incidence of mild tremors (20-40%), and one reported that 30% of the rats showed myoclonus.

Decreases in defecation and urination, inhibited pupil response (with accompanying notes of miosis), and ptosis were the most often reported autonomic changes. In one laboratory these effects were seen only in the rats who subsequently died. These changes did not show a clear dose-response and were not of large magnitude.

TET produced hypothermia at the TOPE in six laboratories, and average temperature of the high-dose groups ranged from 1.4° to 3.7°C lower than controls. The laboratories using the higher dose levels showed the greatest change. The data from the seventh laboratory showed a 0.7° drop, yet the overall dose effect failed to attain significance ($p<0.06$). Piloerection was recorded in three laboratories, usually in the high-dose group only, and mostly at the TOPE and 24 hours. The greatest effects on body weight were recorded one week after dosing in all laboratories. At that time the surviving high-dose groups (TD's of 6 or 4 mg/kg) showed either weight loss of up to 15%, or a suppression of weight gain.

Repeated TET Exposures. Daily dosing with TET produced considerable lethality in all laboratories (see Table 4). Death occurred before the two-week time point

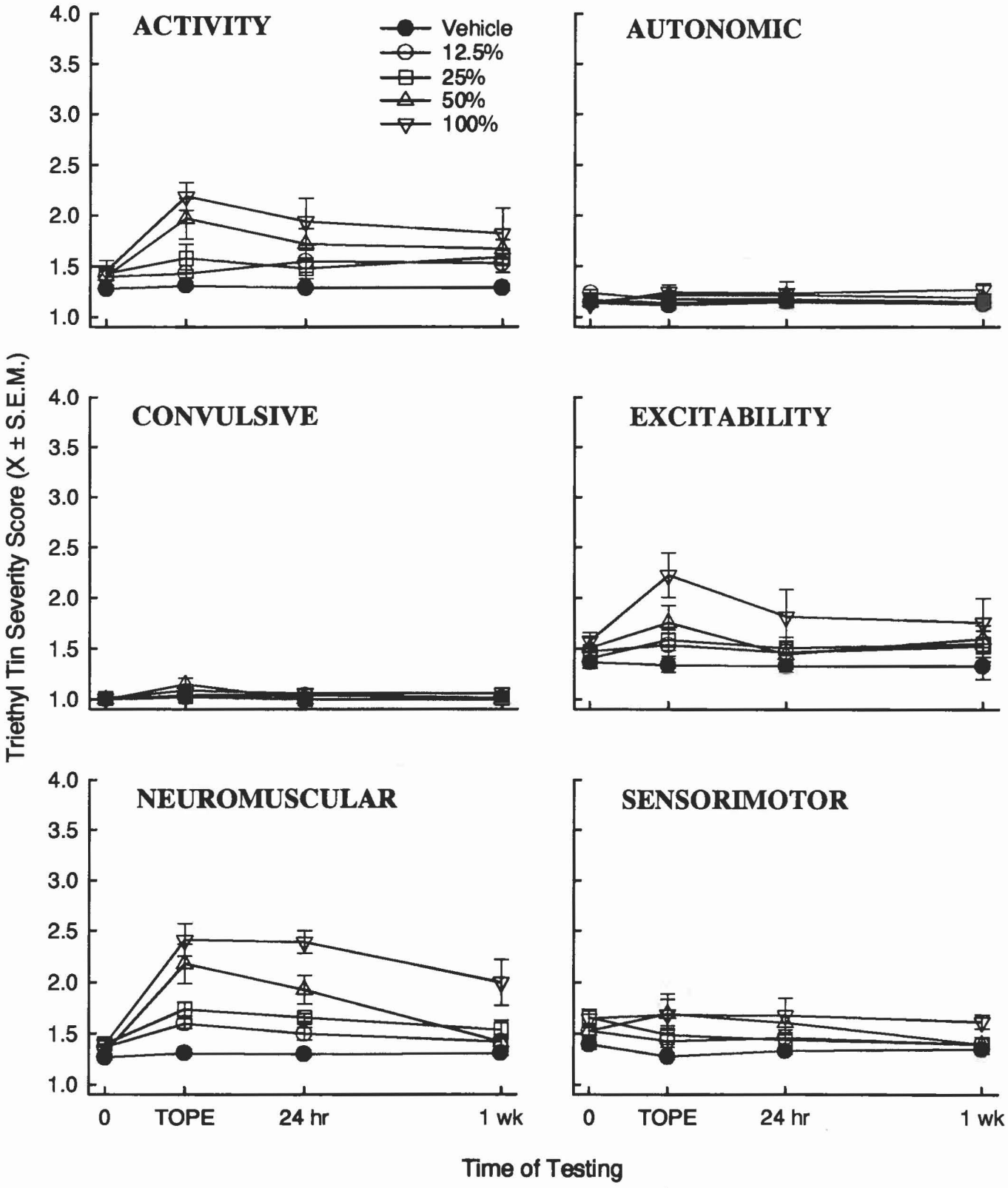


FIG. 38. Effects of acute triethyl tin on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed significant overall effects in the Activity, Excitability, and Neuromuscular Domains.

TABLE 17. Triethyl Tin: Effects[a,b] of a Single Dose in Each Laboratory, Presented in Descending Order of Top Dose and TOPE.

Lab	TD	TOPE	Time of Testing		
		TOPE	24 hr	1 wk
Activity				
D	9	3	↓MA	-, [↓ MA]	-
H	9	2	↓MA, ↓REAR, POST	↓ MA, ↓REAR	↓ ma
E	6	2	↓MA, POST	↓ MA, ↑REAR	↓ MA, ↑REAR
G	6	1	-	-	-
A	4	2	↓ MA, POST	-*	-
F	4	1.5	↓ MA, ↓REAR	↓ ma	-*
B	4	1	↓ MA	↓ MA	↓ MA
Autonomic					
D	9	3	-, [↓ def]	-, [↓ def, pupil, ptosis]	↓ urin[1]
H	9	2	pupil, ptosis	pupil, [ptosis]	-
E	6	2	↓ def[1], ↑ urin[1], ptosis	↓ def	-*
G	6	1	↓ DEF	↓ def, ↓urin[1]	↓ DEF, ↓URIN[1]
A	4	2	-	-	-
F	4	1.5	-	-	-
B	4	1	pupil, ↓ urin[1]	PUPIL	-
Convulsive					
D	9	3	-	-	-
H	9	2	myoc, [smack]	-	-
E	6	2	trem	-	-
G	6	1	-	-	-
F	4	1.5	TREM[1]	-	-
B	4	1	trem	-	-
Excitability					
D	9	3	-	↑ ar[1]	-
H	9	2	↓ AR, ↓REM, [↓HAND]	↓ ar, ↓rem, [↓HAND]	-
E	6	2	↓ REM, ↓HAND	↓ REM, ↓HAND, ↑ AR	↓ REM, ↓HAND, ↑ AR
G	6	1	↓ REM, ↓HAND	↓ rem, ↓hand	↓ REM
A	4	2	↓ AR, ↓HAND	↓ rem	-
F	4	1.5	↓ REM, ↓HAND	↓ hand	↓ ar[1]
B	4	1	↓ ar, ↓rem, ↓hand[1]	↑ REM[1]	↓ HAND, ↓AR[1]
Neuromuscular					
D	9	3	GAIT, ↑ SPLAY, [↓FGRIP, ↓HGRIP, RIGHT]	gait, [↓ FGRIP, ↓HGRIP, RIGHT]	-
H	9	2	GAIT, RIGHT, ↓HGRIP, [↓FGRIP, ↑ SPLAY]	GAIT, RIGHT, ↓HGRIP, [↓FGRIP, ↑ SPLAY]	↑ splay[1], right[1]
E	6	2	GAIT, RIGHT, ↓FGRIP, ↓HGRIP, ↑ SPLAY	GAIT, RIGHT, ↓FGRIP, ↓ HGRIP	GAIT, RIGHT, ↓ FGRIP, ↓ HGRIP
G	6	1	GAIT, RIGHT, ↓FGRIP	GAIT, ↓FGRIP	GAIT, ↓FGRIP, ↓HGRIP
A	4	2	GAIT, RIGHT	GAIT, RIGHT	-
F	4	1.5	GAIT, RIGHT, ↓HGRIP	GAIT, RIGHT, ↓HGRIP	GAIT, RIGHT, ↓HGRIP
B	4	1	GAIT, RIGHT, ↓HGRIP	GAIT, RIGHT, ↓HGRIP	gait, ↓hgrip
Sensorimotor					
D	9	3	↓ tp, ↓click, ↓appr	↓ tp, [↓CLICK]	↓ click
H	9	2	↓ TP, ↓CLICK, ↓APPR, ↓TOUCH	↓ TP, ↓CLICK, ↓APPR, ↓TOUCH	↑ appr
E	6	2	↓ TP, ↓CLICK	↓ tp, ↓click, ↓appr[1]	↓ tp, ↑ appr
G	6	1	↓ TP, ↓TOUCH	↓ CLICK	↓ click
A	4	2	↓ click, ↓touch	↓ TOUCH	↓
F	4	1.5	↑ click[1]	↓ click, ↓tp[1]	tp, ↓touch, ↓click[1]
B	4	1	↑ touch[1]	↓ tp, ↓click, ↑ touch[1]	↓ TP
Physiological Measures					
D	9	3	↓ temp	-, [↓temp, pilo]	↓ wt
H	9	2	↓ temp, pilo	↓ wt, pilo, [↓ temp]	↓ wt
E	6	2	↓ temp	↓ temp, ↓wt	↓ wt
G	6	1	↓ temp	↓ temp	↓ wt
A	4	2	↓ temp, pilo	-	↓ wt
F	4	1.5	↓ temp	↓ temp	↓ wt
B	4	1	pilo	pilo	↓ wt

[a] See Table 5 for key.

[b] Effects listed in brackets are those only produced only by the doses which were excluded from analyses due to high lethality (>50%); this occurred in the 100%-TD dose groups in laboratories D and H.

[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose.

* Domain significant at time point but no individual measure was significant.

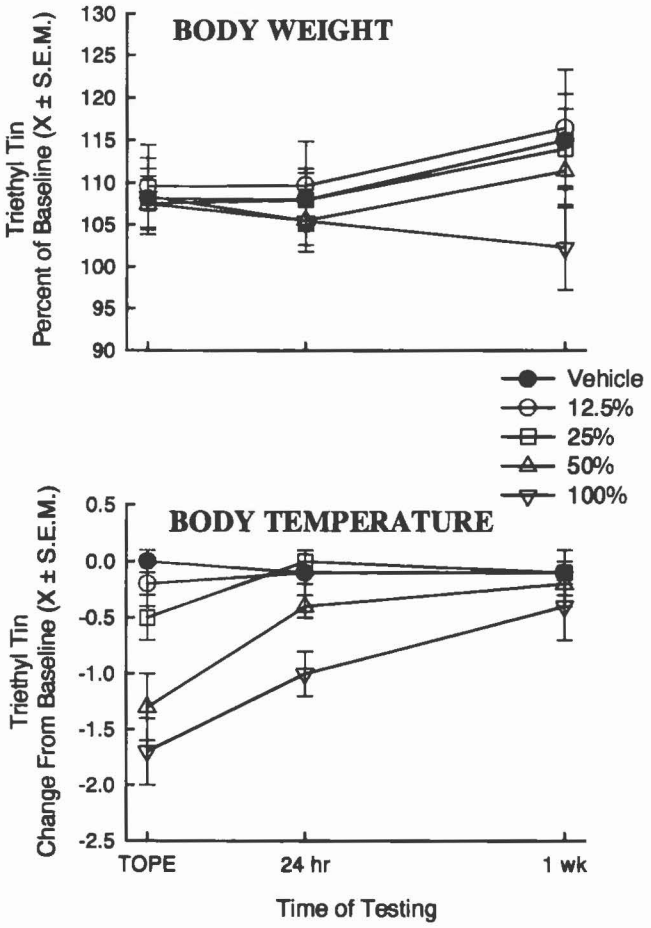


FIG. 39. Effects of acute triethyl tin on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Two-way ANOVAs revealed a significant overall effect on both measures.

in the high-dose group (50% of the TD). In the next-highest dose group (25%-TD), almost all of the lethality had occurred by four weeks. In other words, the time to death was twice as long when dosing occurred at half the dose level. All laboratories reported 100% lethality in the 50%-TD dose group, and all but one had complete lethality in the 25%-TD dose group (the exception was 70% lethality). The next-lower dose (12.5% of the TD), however, produced no deaths in five laboratories, and 10% and 40% lethality in the remaining two laboratories. For statistical analyses, the data for the high-dose were eliminated completely (since all were eliminated before the two-week test), and the data for the 25%-TD dose group were excluded from the repeated-measures analyses. Therefore, for the overall analyses the dose-response information was based on control and two doses. The two-week data, which included the 25%-TD dose group, were also analyzed separately.

Figure 42 presents the effects of daily administration of TET on the composite severity scores, and Figure 43 presents the body weight and temperature data. Since the overall analyses included the 6.25%- and 12.5%-TD dose groups only, significant domain effects were only observed for Neuromuscular measures throughout the study, and for the Sensorimotor responses during dosing (two and four weeks). No effects were obtained in the laboratory means on body weight or temperature. At two weeks, the 25%-TD dose group produced significant effects in all domains except Convulsive and Excitability. Marked weight loss was also noted in this dose group, as well as pronounced hypothermia. This is indicative of the pronounced toxicity observed in those rats at that time point. While the 12.5%-TD dose groups showed more effects at four weeks, the magnitude of effect at that time point was not as great as the 25%-TD dose at two weeks.

Data for all laboratories are presented in Table 18. While Table 18 includes the data for the 25%-TD dose group at two weeks, the two laboratories which used the highest doses (laboratories D and H) did not have enough surviving rats (one or two) at that time to obtain meaningful data; i.e., those data are not included for that dose. Most effects were observed at the 25%-TD dose: four laboratories showed Neuromuscular effects, and there were a few effects in the other domains as well. When only the two lower doses were included in the analyses, however, repeated dosing with TET produced even fewer effects than might be predicted by the overall analysis. Three laboratories obtained significant Neuromuscular effects; these were the laboratories using higher dose levels (12.5% of the TD was 1.1 or 0.8 mg/kg/day). Sensorimotor effects were documented in only two laboratories. Three laboratories showed no alterations at the domain level, and no laboratories obtained significant changes in the Autonomic, Activity, Excitability, and Convulsive domains.

There was a striking difference between the wide range of effects obtained at 25% of the TD and the very few effects produced by 12.5% of the TD. These effects at 25%-TD included compromised neuromuscular ability, lowered reactivity to specific stimuli as well as to general

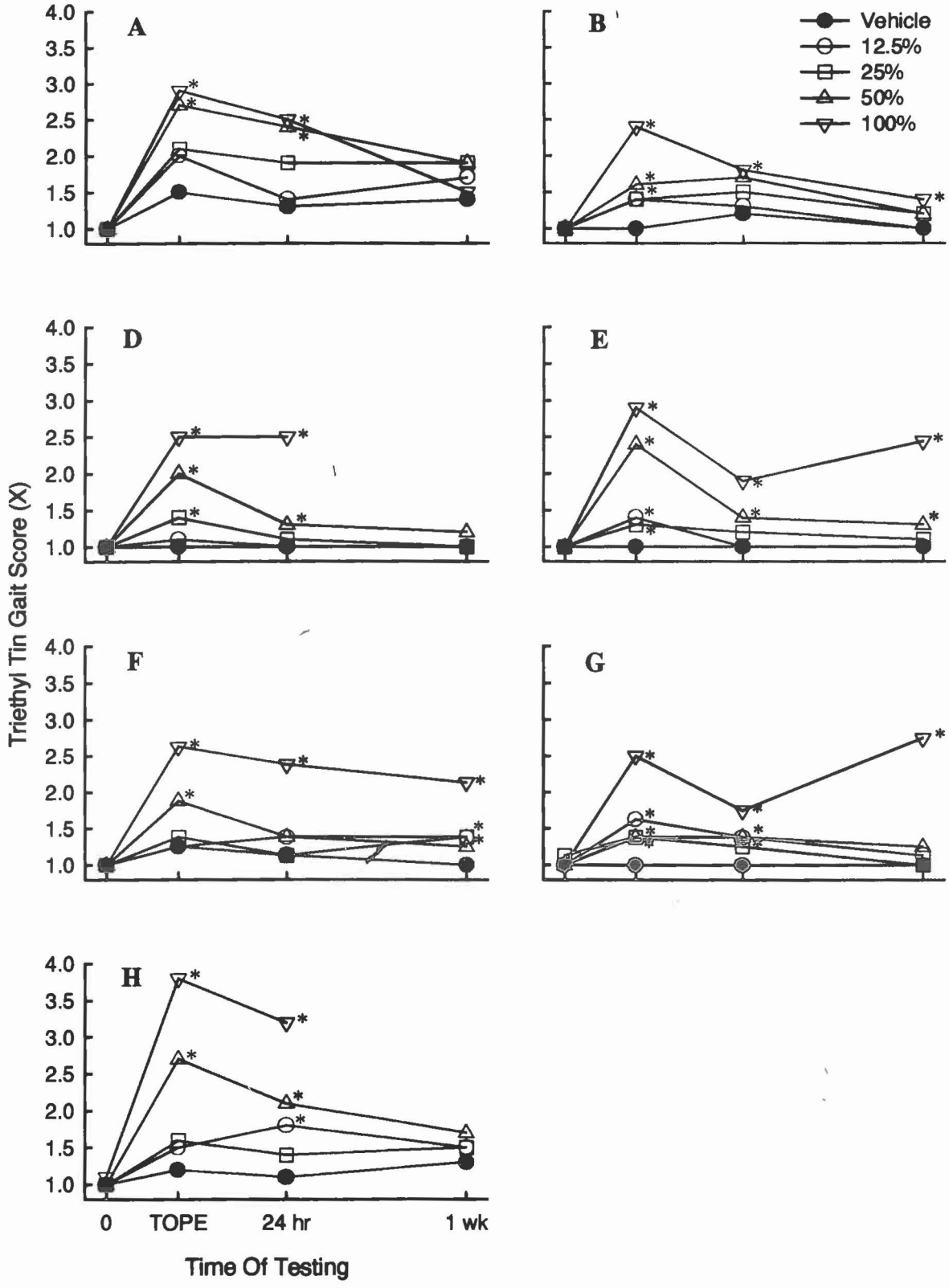


FIG. 40. Effects of acute triethyl tin on the ranking of gait abnormalities in individual laboratories, at the TOPE. Gait is scored as '1' (normal), '2' (slightly abnormal), '3' (moderately abnormal), or '4' (severely abnormal). Data are presented as the group means for each dose group at each time point. Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

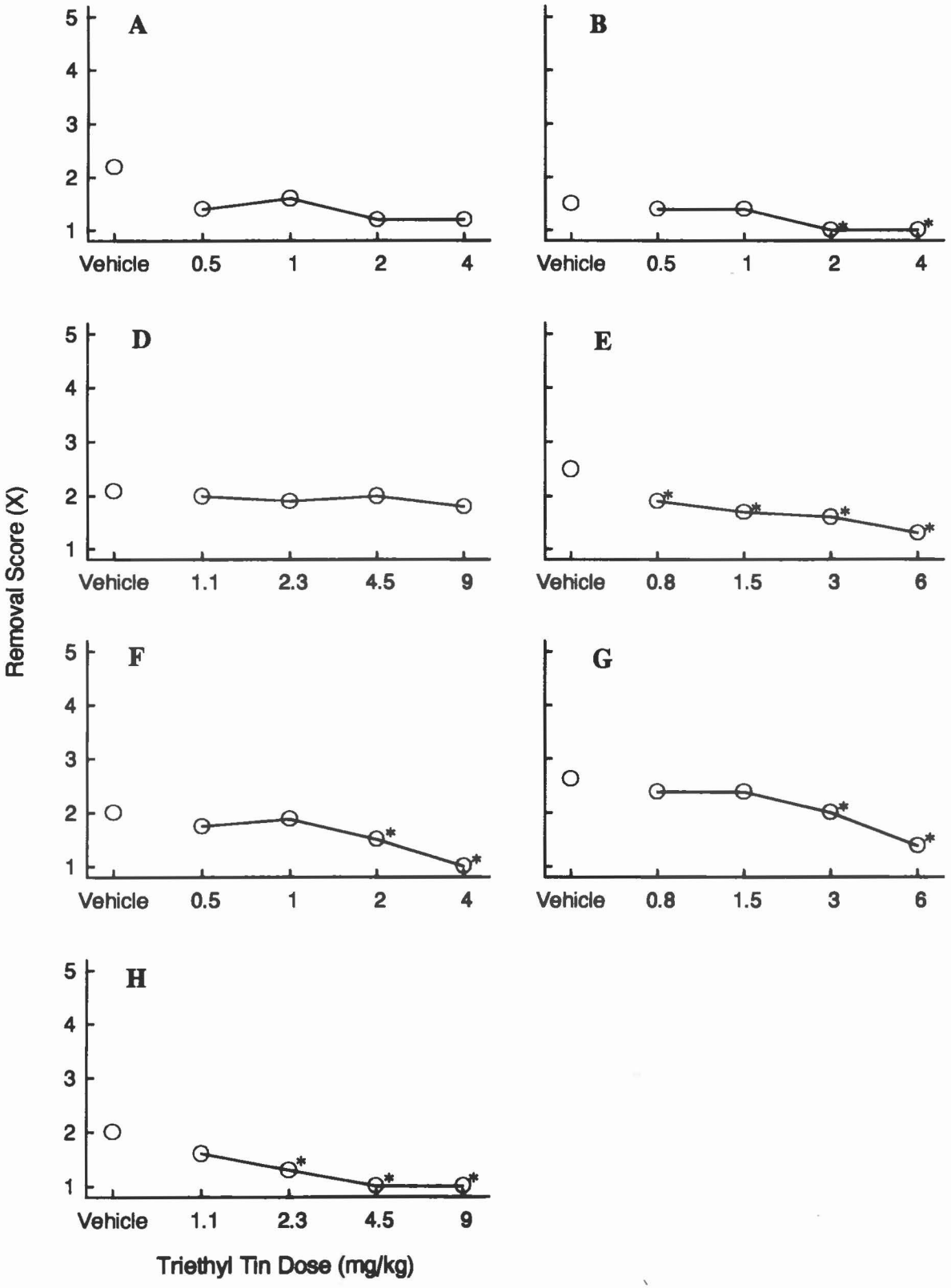


FIG. 41. Effects of acute triethyl tin on the ranking of ease of removal in individual laboratories, at the TOPE. The ease of removal is ranked on a scale ranging from '1' (no reaction) to '5' (exaggerated reaction). Data are presented as mean score for each dose group. Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

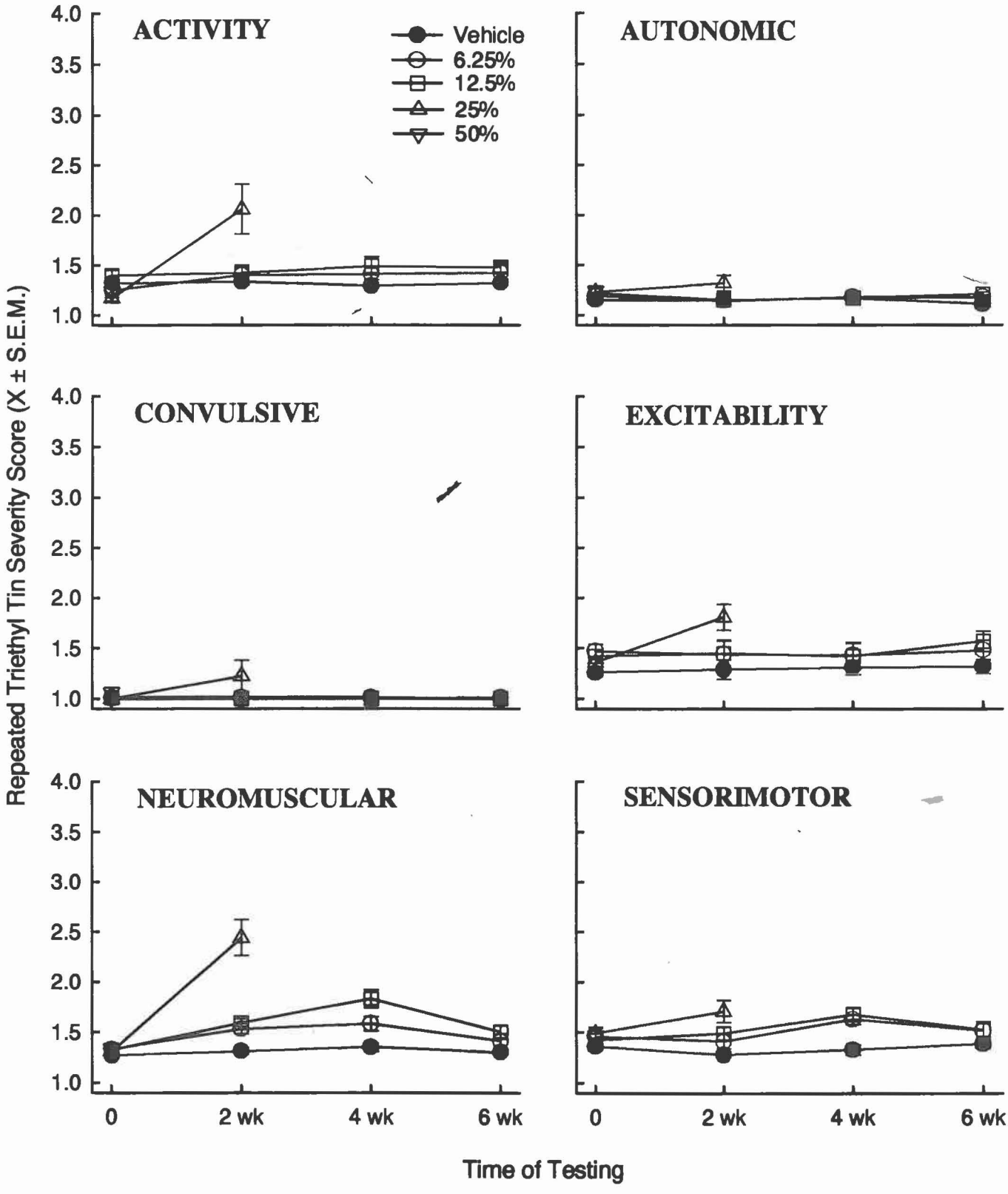


FIG. 42. Effects of repeated administration of triethyl tin on the neurological functional domains, across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Data for the 25%-TD treatment are shown at two weeks only, due to lethality in that dose group; Autonomic, Activity, Neuromuscular, and Sensorimotor domains were altered by that dose. Two-way ANOVAs of the lower dose groups revealed significant overall effects in the Neuromuscular and Sensorimotor Domains.

TABLE 18. Triethyl Tin: Effects[a,b] of Repeated Doses in Each Laboratory, Presented in Descending Order of Top Dose.

Lab	TD	Time of Testing		
		2 wk	4 wk	6 wk
Activity				
D	2.3	-	↓ ma	-
H	2.3	-	-	-
E	1.5	-	-	-
G	1.5	-	-	-
A	1	[↓ ma]	-	-
B	1	-, [↓ MA, ↓ REAR, POST]	↑ rear	-
F	1	↓ ma, [post]	↓ ma	-
Autonomic				
D	2.3	-	-	-
H	2.3	-	-	-
E	1.5	-	-	-
G	1.5	-	-	-
A	1	↓ def	-	-
B	1	-, [LACRIM, PUPIL]	-	-
F	1	-	-	-
Convulsive				
D	2.3	-	-	-
H	2.3	-	-	-
E	1.5	-, [trem]	-	-
G	1.5	-	-	-
B	1	-, [TREM]	-	-
F	1	-	-	-
Excitability				
D	2.3	-	-	↑ ar
H	2.3	-	-	-
E	1.5	-, [↓ rem]	-	↑ rem
G	1.5	-	-	-
A	1	-, [↓ hand]	↓ hand[1]	-
B	1	-, [↓ REM, ↓ HAND, ↓ AR]	-	↑ ar
F	1	-, [↓ rem]	-	↑ ar[1]
Neuromuscular				
D	2.3	-	GAIT, RIGHT, ↓ FGRIP, ↓ SPLAY	-
H	2.3	gait, right	RIGHT, ↑ FGRIP, ↓ SPLAY	↑ fgrip
E	1.5	gait, right, [↓ FGRIP, ↑ SPLAY]	gait, right	gait
G	1.5	↓ SPLAY, [GAIT, RIGHT, ↓ FGRIP, ↓ HGRIP]	gait	-
A	1	-, [right]	-	-
B	1	gait, right, ↓ hgrip, [↓ FGRIP, ↓ SPLAY]	↓ hgrip	-
F	1	-, [GAIT, RIGHT, ↓ FGRIP, ↓ HGRIP]	right	-
Sensorimotor				
D	2.3	-	↓ tp	-
H	2.3	↓ tp	↓ tp	↑ APPR[1]
E	1.5	-, [↓ tp, ↓ click]	↓ click	-
G	1.5	-, [↓ click]	↓ CLICK	-
A	1	↓ CLICK, ↓ TP[1]	↓ TP, ↓ CLICK[1]	↓ touch[1]
B	1	-, [↓ tp, ↓ touch]	↓ tp	-
F	1	-, [↓ tp]	↓ tp	-
Physiological Measures				
D	2.3	-	↓ wt	-
H	2.3	-	-	-
E	1.5	-, [↓ wt]	-	-
G	1.5	-, [↓ wt]	-	-
A	1	-	-	-
B	1	-, [↓ wt, ↓ temp, pilo]	↓ wt[1]	↓ wt
F	1	-, [↓ wt]	-	-

[a] See Table 5 for key.
[b] Effects listed in brackets occurred only at higher doses (with 100% lethality) at two weeks.
[1] data did not show monotonic dose-response, i.e., low dose(s) were significant but not the highest dose.

stimulation such as handling, weight loss, and a few reports of lowered activity and mild tremors (30% of the dose groups). Motor activity was lowered in three laboratories: interestingly, this occurred only in the laboratories using the lowest dose range. Reactivity to being removed from the cage was affected somewhat more so than handling reactivity, and the response to the tail pinch was lowered more so than the other stimuli. Grip strength values, primarily forelimb, were generally lowered. This profile resembled the acute effects of higher doses of TET.

On the other hand, most behavioral changes observed at two weeks of dosing with the lower doses were in the Neuromuscular domain. At that time, three laboratories showed abnormalities in the righting reflex, as well as alterations in gait, described mostly as ataxia, uncoordinated hindlimbs, and tip-toe walking. The data for righting reflex are shown in Figure 44, which illustrates how the dose-response curves for repeated exposures were similar to, but shifted to the left of, those obtained exposure to a single dose. At the completion of four weeks of dosing, these effects were generally more pronounced, although only one or two dose groups had survived at that time. Landing foot splay was decreased in the two laboratories using the higher doses. Grip strength, however, did not show a clear pattern of effect. For instance, forelimb grip strength was actually increased in one laboratory at both four and six weeks, but this appeared to be due only to an atypical decrease with repeated testing in the grip strength values of the control group. Decreased responses to the tail pinch and/or click stimuli were recorded in all laboratories. Indeed, the most consistently affected measure at four weeks was the tail- pinch response, which was lowered in five laboratories (and also showed a non-significant trend in a sixth).

Weight gain was depressed at these lower doses in only two laboratories. Very few, if any, residual effects were observed at six weeks in all laboratories.

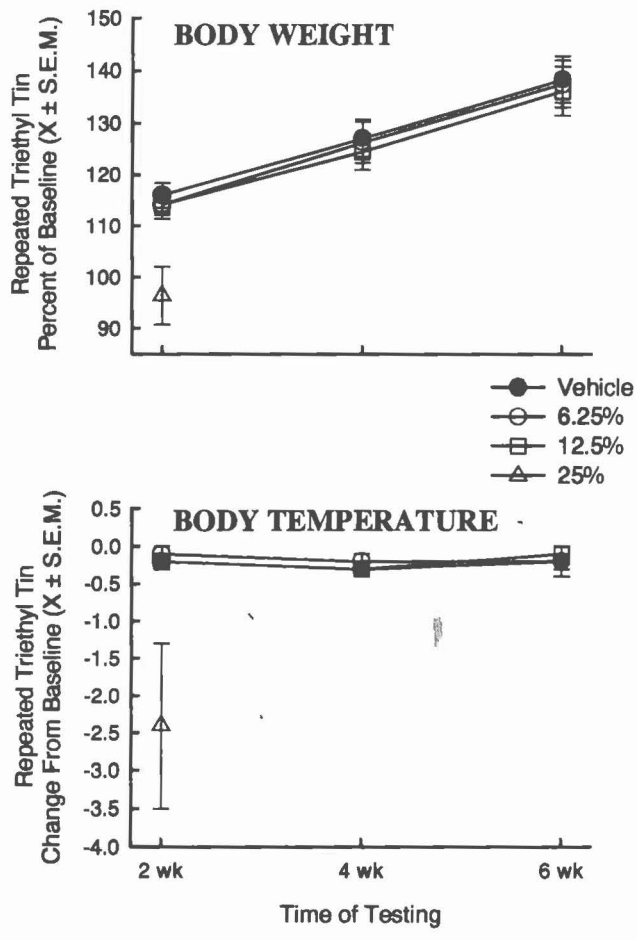


FIG. 43. Effects of repeated administration of triethyl tin on body weight and temperature (°C), across all laboratories. Data are presented as the mean of the group means from each laboratory (± S.E.M.), i.e., each laboratory mean represents one observation. Dose groups are either control (vehicle) or percentages of the top dose. Data for the 25%-TD treatment are shown at two weeks only, due to lethality in that dose group; both measures were significantly altered. Two-way ANOVAs of the lower dose groups, however, revealed no significant overall effects.

DISCUSSION

During the conduct of this study many problems and questions arose in the data which were submitted. Some of these problems were resolved following discussions with the project directors. Others were carefully evaluated and determined to be acceptable, even though in some cases the practices were not endorsed. Only a few problems were considered too serious for inclusion in the data analysis. In addition, three test measures were considered useless for interpretation. The amount of data excluded was not large enough to seriously compromise the inter-laboratory comparisons in this study.

Chemical Discussion: Acrylamide

Overall, the profile of effect produced by a single dose of acrylamide did not show very specific effects,

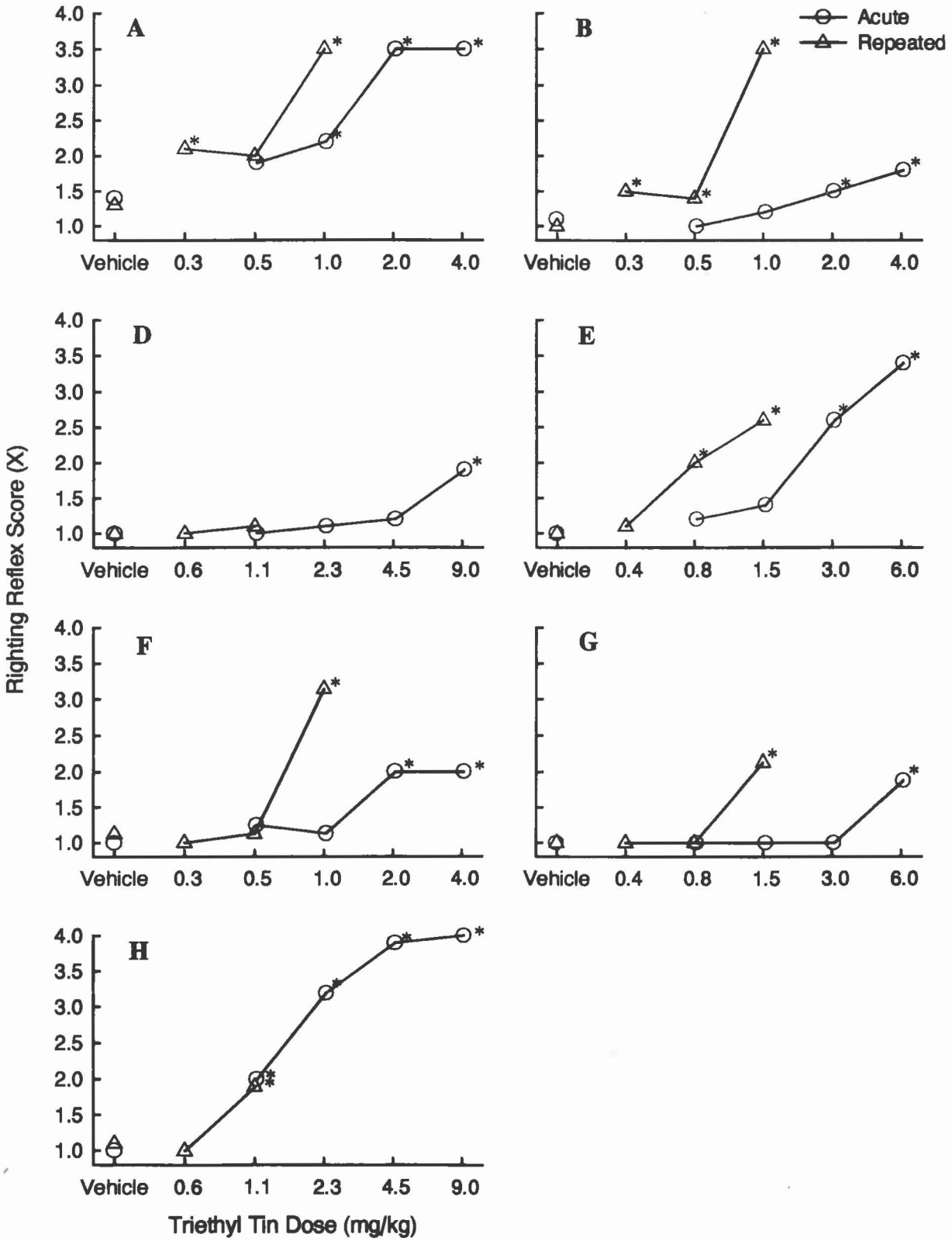


FIG. 44. Effects of triethyl tin on the righting reflex of rats in individual laboratories, at the TOPE (acute study; circles) or at two weeks (repeated exposures; triangles). Righting reflex is ranked on a scale ranging from '1' (normal response) to '4' (severely impaired reaction). Data are presented as mean score for each dose group. Laboratories are indicated by letter. Asterisks indicate dose groups which were different from control in the laboratories for which the initial two-way ANOVAs were significant.

with most laboratories detecting only mild to moderate signs of neurotoxicity. Changes reported by the majority of laboratories were decreased motor activity, mild gait abnormalities, hypothermia, and weight loss. The laboratories agreed more on the lack of dose-related effects on most of the remaining endpoints. Thus, the concordance was relatively good in determining that acrylamide had few serious neurological effects following single-dose exposure. General toxicity, i.e., weight loss, hypothermia, may have contributed to the signs that were reported most consistently across laboratories such as lowered activity, but probably would be unrelated to increased excitability or tremors. In most cases, effects were only detected at the high dose (100% TD), but some laboratories (E, C) reported significant effects on some endpoints at lower doses.

The literature contains many reports on the acute effects of acrylamide with which to compare the findings of these studies (reviewed in Kulig, 1994; Tilson, 1981). Generally, single doses of 50-150 mg/kg produced tremors, ataxia, incoordination, and decreased rearing (Crofton *et al.*, 1994; Gipon *et al.*, 1977; Kulig *et al.*, 1985; Sterman and Sheppard, 1983). Several laboratories obtained this spectrum of effects. Higher doses (≥200 mg/kg) also produced decreased hindlimb grip strength, and affected performance on an inclined screen (Tilson and Cabe, 1979); however, no laboratory in this study used doses that high. Although rearing was decreased in less than half of the laboratories, motor activity was depressed in most of them. While these changes are not thought to be due to the peripheral neuropathy which results from repeated acrylamide exposures, some acute neuronal changes have been demonstrated following a single dose, e.g., decreased axonal transport and nerve terminal degeneration (DeGrandchamp *et al.*, 1990; Sickles, 1991).

If acrylamide had been tested as an unknown compound under these conditions, the general toxicity observed following single-dose administration would have been overshadowed by its cumulative neurotoxicity seen during repeated dosing. Neuromuscular deficits dominated the pattern of toxicity. All laboratories detected these neurotoxic effects, and the differentiation between single- and repeated-dosing effects (Yoshimura *et al.*, 1992) was evident in all laboratories. The agreement across laboratories was fairly good, although there were two obvious outliers: the laboratory which used the lower dose range (B), and the one which obtained 70% lethality in the 25%-TD dose group (E). In the former instance, the major difference was the lack of effect on hindlimb grip strength, although many of acrylamide's other effects were detected (e.g., increased foot splay, altered gait). In the latter laboratory, the findings of decreased grip strength, gait abnormalities, and lowered motor activity were present at four weeks, but only in the 25%-TD dose group which had seven of 10 rats alive for that evaluation (only 30% survived through six weeks).

There is a vast literature regarding the nature of acrylamide toxicity in animals and humans. Despite decades of research, however, the mechanism of action has not yet been determined (reviewed in Kulig, 1994; Tilson, 1981). The progressive pattern of ataxia, incoordination, mild tremors, proprioceptive deficits, muscular weakness (preferentially in the hindlimbs), increased landing foot splay, and weight loss are hallmarks of the central-peripheral distal axonopathy produced by acrylamide (Kulig, 1994). Urinary retention and distended bladder is also commonly reported; one laboratory that performed general necropsy on their rats verified this finding as well.

The reliability of these neurological signs have made acrylamide a prime choice for validating neurotoxicological procedures (e.g., Moser *et al.*, 1992; Shell *et al.*, 1992). Indeed, acrylamide originally served to validate the landing foot splay and grip strength measures used in this test battery (Edwards and Parker, 1977; Jolicoeur *et al.*, 1979; Tilson and Cabe, 1979; Tilson *et al.*, 1979). While most sensory function is unaffected by acrylamide (Edwards *et al.*, 1991), the striking exception is proprioceptive function. It is this deficit in limb positioning which is evaluated using the landing foot splay measure. In contrast, the responses to other sensory stimuli showed no clear pattern of change.

The emergence of neuromuscular effects in the 25%-TD group at four weeks, which were similar to those found at 50% of the TD at two weeks, clearly indicates cumulative toxicity. This finding is reminiscent of the hypothesis that acrylamide's effects depend on the total accumulated dose, rather than dose level. At the end of two weeks, total administered dose for seven laboratories was 567 mg/kg, which is very close to the theoretical threshold of 600 mg/kg proposed by others to produce overt neurotoxicity (e.g., Gipon *et al.*, 1977; Rafales *et al.*, 1982; Spencer and Schaumberg, 1974). Gait changes and increased splay were the only alterations clearly evident at two weeks in the 25%-TD group, which occurred at a total accumulated dose of approximately 283 mg/kg. While recent studies have shown that dose rate as well as total dose are important factors in predicting neurotoxic-

ity, this concept of a threshold level is still generally applicable (Crofton *et al.*, 1994).

Thus, in this study all laboratories detected non-specific signs of toxicity following a single dose of acrylamide, along with progressive neuromuscular toxicity during repeated dosing. This compound would have been detected in a screening study, and the resultant axonopathy would be confirmed with appropriate neuropathological evaluation. The test measures which are specifically altered by acrylamide proved to be sensitive and reliable across all laboratories.

Bis-Acrylamide

Overall, the signs of toxicity produced by exposure to bis-acrylamide resembled very closely those of single-dose acrylamide exposure, and there appeared to be good agreement across laboratories. The predominant effects similar to both compounds were decreased motor activity, mild gait abnormalities, hypothermia, and weight loss; in addition decreased defecation and piloerection were also reported in most laboratories. As with acrylamide, general toxicity could account for some of the signs that were reported most consistently across laboratories. Unlike acrylamide, interestingly, there was no hint of increased levels of reactivity, and almost no reports of tremorigenic activity. In a screening situation, this compound would raise concern primarily for the pronounced general toxicity produced both by a single dose and by repeated exposure. This toxicity was especially notable due to the relatively long time-course and magnitude of body weight deficits.

As was observed with a single exposure to bis-acrylamide, the general toxic effects of repeated dosing (weight loss, hypothermia) were much more prominent than the behavioral changes. It is likely that the dramatic weight loss, prolonged hypothermia, and general toxicity contributed to some of the effects reported herein, since almost no effects were obtained in rats who did not show such large weight loss. Further examination of individual laboratories showed that the magnitude of effects were directly proportional to the weight loss obtained in the high-dose groups. Reports of various changes in these test measures following food restriction paradigms have generally shown that effects are reliably seen only with severe deprivation, e.g., 50% baseline food intake (Albee *et al.*, 1987; Gerber and O'Shaughnessy, 1986; Serrone *et al.*, 1989). Different profiles have been reported in those studies, doubtless due to procedural differences, but in general signs similar to those described here, e.g., decreased grip strength and decreased foot splay, are common findings.

Bis-acrylamide is sometimes used, as it was in this collaborative study, as a negative control in studies of acrylamide neurotoxicity. The available literature indicates that bis-acrylamide produces weight deficits and poor general health with repeated exposures (Edwards, 1975). Some effects such as testicular atrophy, minor peripheral nerve changes, and decreased retrograde axonal transport, are observed with both bis-acrylamide and acrylamide; bis-acrylamide, however, has been repeatedly shown to not produce weakness, ataxia, or impair rotorod performance, and does not produce central-peripheral distal axonopathy, as does acrylamide (Edwards, 1975; Hashimoto *et al.*, 1981; Miller and Spencer, 1985; O'Donoghue, 1985; Schotman *et al.*, 1978).

All laboratories in this study distinguished differences in the toxicity of acrylamide and bis-acrylamide. The neuromuscular effects obtained with repeated dosing of bis-acrylamide were quite unlike those of repeated exposure to acrylamide, which increased foot splay and decreased grip strength, preferentially hindlimb, in a time- and dose-related progressive manner. The effects of bis-acrylamide included decreased foot splay, non-specifically decreased grip strength, and the effects did not show a clear progression as dosing continued. Thus these laboratories showed that, while bis-acrylamide is a toxic compound, it did not produce neurotoxicity that resembled that of its analogue, acrylamide.

p,p'-DDT

The times chosen by most laboratories for acute testing were fairly long (≥ three hours). Indeed, DDT is slowly absorbed from the gastrointestinal tract and shows a slow onset of effects following oral dosing, with signs first appearing at two to five hours and reaching a peak at 12 hours (Hayes, 1982a; Henderson and Woolley, 1970; Hudson *et al.*, 1985). One laboratory, however, chose one hour as the testing time, which was probably too short for DDT effects to become evident. In fact, that laboratory detected almost no effects of DDT, and it was the only one that did not observe tremors, myoclonus, or hyperthermia. A possible explanation for their choice may be that effects were not observed when conducting the TOPE test, since this laboratory used a higher dosing volume than specified. It appears that increasing the dosing volume for DDT, thereby decreasing its concentration, markedly decreases its potency (McDaniel and Moser, 1997).

DDT's apparent mechanism of action involves an alteration of the neuronal sodium current, which leads to repetitive discharges following a single stimulus (Narahashi, 1982; Narahashi and Yamasaki, 1960). Studies have also reported changes in neurotransmitter levels following DDT exposure (Hrdina *et al.*, 1973; Hudson *et al.*, 1985). Thus DDT affects the entire nervous system and produces multiple neurological signs. If this compound had been an unknown, almost all laboratories would have identified it as neurotoxic using these behavioral test methods. The profile would have been described as hyperthermic, tremorigenic progressing to myoclonus and convulsions at higher doses, and producing increased reactivity to specific stimuli. In fact, these are precisely the descriptions used by others (Hayes, 1982a; Henderson and Woolley, 1970; Hrdina *et al.*, 1973; Hudson *et al.*, 1985; Woolley, 1982). Only one laboratory would not have detected these effects, due to the short TOPE; this finding underscores the critical importance of choosing an appropriate TOPE in conducting studies, especially for screening.

Along with tremors and myoclonus, another known effect of DDT is increased responsiveness to tactile and auditory stimuli, which generally has been subjectively scored (as in this study), but also objectively measured as increased amplitude of the startle response (Crofton and Reiter, 1988; Herr *et al.*, 1987; Hrdina *et al.*, 1972). The findings in this study of increased responses to the click and touch stimuli correlate well with these previous reports. The lack of the click response effect in two laboratories is more puzzling. The laboratory with the shortest TOPE obtained a decreased click response, but this effect was not large, nor was it dose-related (see Figure 18). The other laboratory (H) showed no effects at all on the click response. This laboratory did detect increased click response in the proficiency study, but a higher dose was used (75 mg/kg in the proficiency study, and 58 mg/kg used in the formal study). Another possible explanation is the higher baseline responses in this laboratory (see Figure 18), which may have precluded further increases; however, the scores were not maximal.

Other effects of DDT that are sometimes reported include incoordination and weakness, but these are not common findings (Hayes, 1982a). The gait changes observed in this study, which were fairly consistent, could correspond to incoordination and ataxia reported by others (Hayes, 1982a). Indeed, the descriptions of these gait alterations included mostly ataxia and uncoordinated placement of limbs. Furthermore, the four laboratories which recorded the largest gait changes were also the ones with the longest TOPE, indicating perhaps that this effect occurs later. Weakness can be assessed by grip strength, which was decreased in only a few laboratories in this study. These decreases were not large, being at the most 67% of control values, and were not specific for either forelimbs or hindlimbs. Thus, these motor changes may be those that can only be detected under certain circumstances (i.e., optimal dose, time of testing).

Few studies have assessed motor activity using an automated device, but Crofton and Reiter (1988) did show decreased activity following DDT. In the present study, only two laboratories obtained decreased activity, and interestingly, these were the two laboratories that used figure-8 maze devices as were those used in Crofton and Reiter (1988). Thus, this effect may be device-specific, possibly due to the spacing of the photobeams or the criteria used to determine one activity unit. One laboratory reported increased activity which may have been due to the detection of tremors, since their activity chambers had closely-spaced photobeams.

The results of the repeated-exposure scenario agree with the findings of others. That is, since DDT accumulates in the body (mostly fat), levels of DDT can become much greater than those obtained with one dose but with no side effects (Hayes, 1982a; Mitjavila *et al.*, 1981a, 1981b). DDT also stimulates its own metabolism (Hayes, 1982a). In the acute study, DDT's effects showed almost complete recovery at 24 hours after dosing; the tests in the repeated-dosing study were conducted before the day's dose, i.e., at least 23 hours after the previous day's dose. Thus repeated dosing with these lower doses (50% TD and less) would not be expected to produce progressive or lasting signs of toxicity.

The concordance with which the laboratories detected the effects of DDT was quite good, and these laboratories agreed on the absence of signs of toxicity during repeated dosing. The cardinal signs of toxicity (tremors and myoclonus, hyperthermia, and stimulus-specific hyperreactivity) were identified in all laboratories which used a TOPE of three hours or longer (with the exception of the click response in laboratory H). Thus, this battery of tests was sufficient to detect potential neurotoxic effects of DDT when the appropriate time of testing was used. The effects of DDT during and after repeated dosing differed greatly from its acute effects, and all laboratories could distinguish these differences in outcome.

Lead Acetate

Overall, single-dose administration of lead acetate produced a variety of changes in the different laboratories, but few measures emerged as being consistently altered. Decreased motor activity was the most consistent functional change, whereas hypothermia and weight loss were more pronounced. Had this been an unknown compound, interpretation of these effects would have been somewhat difficult due to the lack of a clear pattern of effect. Hypothermia and weight changes verify that the compound was biologically active. Motor activity decreases could be an indicator of either general or neurotoxicity. Without concomitant neuromotor weakness or generalized depression, tentative conclusions could be drawn that support more generalized toxicity rather than neurotoxicity. In addition, the participating laboratories agreed fairly well on the lack of effects from acute exposure to lead acetate. On almost all measures the majority of laboratories agreed that there was no effect: the exceptions were arousal, motor activity, and body weight.

The overall conclusion based on the repeated-dosing data would be that lead acetate produces considerable general toxicity, with weight loss and lethality exceeding that which is acceptable for standard toxicity studies. The behavioral effects which were observed could well be secondary to this extreme toxicity. For instance, motor activity showed decreases in both the single- and repeated-dosing studies, but there were no corroborating decreases in rearing or compromised motor function which would suggest neuronal effects. Most of the gait changes were described as hunched posture and tip-toe gait, which could be secondary to the intraperitoneal irritation produced by injection of lead acetate. Indeed, several laboratories reported that general necropsy revealed peritonitis and ascites in treated rats. A similar profile was obtained during a subchronic study of 2-hydroxyethyl acrylate, which was also administered intraperitoneally and also produced local irritation (Moser *et al.*, 1992). Finally, interpretation of these data is confounded by the pronounced weight loss observed, since no laboratory had any dose group which showed less than a 5% decrease in weight gain, and most had considerably larger effects.

Few studies have addressed the neurotoxicity of lead acetate in adult rats; the vast majority of studies focus on the known cognitive deficits produced by lead exposure in developing/immature subjects (for review, see Jason and Kellogg, 1980). Lead exposure clearly compromises general health, and the kidney appears to be the primary target organ (Choie and Richter, 1980). Human studies have revealed neurological effects as well, but this has not been well correlated with effects obtained in animal studies. High doses of lead produce neurological effects, such as tremors, ataxia, and muscular weakness. These signs were observed in both the single- and repeated-dosing studies, but only in rats who subsequently died or were moribund. In studies using lower doses in adult animals, only equivocal changes were reported on learning and memory paradigms, and results were still confounded by general toxicity and motor dysfunction (e.g., Brown *et al.*, 1971; Ogilvie, 1977).

Using several similar behavioral measures (grip strength, motor activity, thermal sensitivity, body temperature), Pryor and co-workers (1983) examined lead acetate along with several other chemicals in adult rats. As did the participating laboratories in this study, Pryor *et al.* (1983) reported body weight loss and motor activity decreases during subchronic exposures, with no effects on other measures. They also determined LD50 values for single-dose exposures as well as during subacute (28-day) and subchronic (15-week) dosing. With repeated dosing of lead acetate, they reported that lethality occurred at doses which were very low proportions of the acute LD50. The subacute LD50 (24 mg/kg/day) was only 8% of the acute LD50. In the present study, the next-to-lowest dose, 12.5% of the TD (17-25 mg/kg/day) produced 10-100% lethality. Thus lethality produced by lead acetate was the predominant feature of its toxicity.

Parathion

Following administration of parathion, only the high dose or next-highest dose (100% and 50% of the TD) were significant; i.e., almost all changes were clustered at the high end of the dose range. The two lower doses generally had no effects. This illustrates the steep dose-response characteristics of parathion. The steep nature of this dose-response also makes the determination of the TD more difficult, since the protocol specified only one rat to be used at each dose. Furthermore, the spacing of doses used in this study may have been too broad for a compound such as parathion.

General descriptions of the effects of acetylcholinesterase (AChE) inhibitors using these test methods include tremors, miosis, salivation, gait changes, hypothermia, muscle weakness, and depressed motor activity (Lochry *et al.*, 1990; Moser, 1995; Moser *et al.*, 1988;

Newton *et al.*, 1992; Weiler *et al.*, 1994). All of the participating laboratories identified the majority, or all, of these effects. At this overall level of analysis, therefore, there was good agreement. Differences emerge, however, when the data are more closely compared. It is important to note that in one laboratory, 50% of the high-dose group had died by the time of testing (only four rats remaining), thereby decreasing the statistical power of the data from that group.

In a previous study, systematic comparisons of seven organophosphates and carbamates revealed that the Autonomic, Activity, Convulsive, and Neuromuscular domains were consistently altered by treatment, the Excitability domain showed significant effects more often than not, and the Sensorimotor domain was affected in about half of the studies (Moser, 1995). In the present study, only the Convulsive and Neuromuscular domains were identified by all laboratories, and remaining domains were affected in five or six of the eight laboratories. Thus the profiles of effect on the functional domains were somewhat similar to the earlier study (Moser, 1995). Of the three laboratories which did not show an overall effect on the Autonomic domain, two used a lower dose range (TD=4.5 mg/kg or 3.0 mg/kg) and a longer TOPE (three hours). The third laboratory (H) commented that when conducting the TOPE study, it became evident that the motoric changes appeared somewhat later than cholinergic signs. They expressed concern that the TOPE, which was determined based on arousal and gait changes, did not adequately reflect the onset of cholinergic signs. Therefore, the lack of effects on Autonomic function in that laboratory could be due to the time of testing. The subjective assessment of this type of time course for other AChE inhibitors has recently been described (Lammers and Kulig, 1997; Moser *et al.*, 1995b).

A cluster of specific measures in the neurobehavioral screening battery have been identified which are consistently altered by AChE inhibitors (Moser, 1995). In that study, all AChE inhibitors produced autonomic signs of cholinergic over-stimulation (salivation, lacrimation, and miosis which prohibits the pupil response), hypothermia, mild tremors and mouth-smacking (chewing motions), lowered motor activity, decreased tail-pinch response, and altered neuromuscular function (gait changes and increased foot splay). The measures which were generally the most sensitive were body temperature, motor activity, gait, and the presence of mouth-smacking and fine tremors. In this study, only gait changes, tremors, and hypothermia were observed in all laboratories. While the majority of laboratories reported salivation, decreased tail-pinch response and smacking, half of them or less identified increased splay, lowered motor activity, or miosis as toxic signs of exposure. Of interest to note was the relative lack of lacrimation (seen only in one laboratory).

Possible explanations for the differences in laboratory results include strain differences, since chronic feeding studies have shown marked differences in sensitivity of rat strains (Hayes, 1982b). In this study the inbred rats were relatively resistant to parathion effects and required a higher dose range for effects: similar findings have been reported comparing the effects of DFP in Fischer-344 rats (inbred) to Long-Evans or Sprague-Dawley rats (outbreds) (Gordon and MacPhail, 1993). The TD determined in Wistar rats was also on the low end of the range. The laboratories which used the higher TDs (≥6.75 mg/kg) reported significant changes in 70-90% of the cardinal signs, whereas those laboratories using the lower doses (≤4.5 mg/kg) reported only 30-60% of these signs. Thus, a possible scenario is that rat strain influences sensitivity to the lethal effects of parathion, but the functional changes are more dependent on absolute dose.

The nature of the dose-response could also contribute to these differences. Such a steep function makes the dose selection, preparation, and administration critical. Other possibilities include inherent, as yet unexplainable, differences; lacrimation is a case in point. In studies with Long-Evans rats, lacrimation is predictably observed at high doses of AChE inhibitors (Moser, 1995; Moser *et al.*, 1988; Padilla *et al.*, 1992). Other studies using Sprague-Dawley rats, however, do not report lacrimation (Lochry *et al.*, 1990; Newton *et al.*, 1992; Weiler *et al.*, 1994). This may be an example of qualitative strain-related differences in the effects of parathion.

The acute signs of toxicity, identified here and in other studies, can be attributed to cholinergic over-stimulation due to inhibition of AChE and subsequent elevation of acetylcholine levels. Effects such as salivation, miosis which precludes the pupil response, and lacrimation are modulated by the muscarinic system, whereas nicotinic effects are generally those produced by stimulation of the neuromuscular junction (Taylor, 1985). The neuromuscular signs included tremors, weakness, and loss of muscle tone. Other effects also appear to be cholinergically-mediated, including decreased tail-pinch response (indicative of antinociception; Koehn and Karczmar, 1978; Iwamoto and Marion, 1993; Pedigo *et al.*, 1975), mouth-smacking, or chewing (Kelley *et al.*, 1989; Rupniak *et al.*, 1990; Salamone *et al.*, 1986, 1990), and hypothermia (Gordon, 1994).

An inhibited pupil response produced by AChE inhibitors is usually due to miosis, i.e., the pupils are so small that further constriction due to the light is not readily apparent. Different AChE inhibitors differ in their efficacy on this measure, but clear effects were obtained with parathion (Moser, 1995). Although four laboratories detected this effect, these laboratories were those using the highest TD; therefore this particular effect may depend more on the absolute dose.

Although mouth-smacking, or chewing, following AChE inhibition has been described by many (Kelley *et al.*, 1989; Rupniak *et al.*, 1990; Salamone *et al.*, 1986, 1990), in this study only half of the laboratories reported it. It is possible that there was a lack of understanding, or an inadequate description, of this particular effect. For training purposes, visual examples of this particular movement would be better than verbal explanation. It is possible, however, that this observation was simply not seen in some laboratories.

A transient increase in landing foot splay has been a consistent finding with AChE inhibitors but with some compounds the magnitude of the effect is not large: in the study reported by Moser (1995), parathion (7 mg/kg) produced only a 30% increase over control. In this study, the statistically significant findings were also relatively small, ranging from 24% to 88% over control. Thus this effect of AChE inhibitors may not be as robust as others, which could explain why only half of the laboratories detected it.

For most test measures, recovery was evident by 24 hours for all laboratories. Even though reactivation of the AChE enzyme is much slower than this time-course indicates, it has been reported by others as well that behavioral deficits recover long before AChE inhibition returns to normal (e.g., Carr and Chambers, 1991; Reiter *et al.*, 1973).

The profile produced by repeated exposure to parathion was quite different from the acute effects, due to both the time of testing (approximately 23 hours after a dose) and the development of tolerance. The agreement on essentially no effects at the end of dosing was evident. As noted in the single-dose study, very few of parathion's effects were evident at the 24-hour test time. Furthermore, tolerance to repeated exposure to AChE inhibitors has been shown to be due to receptor down-regulation (Costa *et al.*, 1982; Hoskins and Ho, 1992).

Had this been an unknown compound, the pattern of effects revealed with repeated dosing, which contrasted so markedly to the acute effects, would have served to denote a compound with acute toxicity only. However, considering the lethality (>50%) obtained in three laboratories, conclusions may be drawn that this compound produces clear acute toxicity, wherein rats either succumb or else develop tolerance to the effects with repeated dosing. This is a pattern of effects that is well-accepted for AChE inhibitors in general.

Toluene

Administration of toluene produced only mild and somewhat inconsistent effects in six of the seven laboratories. The seventh laboratory, however, showed pronounced effects of toluene. These included changes in the Neuromuscular (gait and righting changes, decreased grip strength, and increased foot splay, on the day of dosing), Activity (increased motor activity and rearing at the TOPE, decreases at 24 hours), and Sensorimotor domains (decreased tail-pinch response on the day of dosing). These findings describe increases in activity followed by depression, and compromised motoric function. This profile is similar to that of CNS depressants, which cause general relaxation (neuromuscular effects) and stimulation of central activity (depression of inhibitory influences) followed by general depression. Indeed, many have reported these types of effects for toluene (for reviews, see Benignus, 1981; Evans and Balster, 1991).

The agreement across laboratories in terms of no effect of toluene was generally good. One might conclude this to be a nonpotent chemical which, given by this route of administration, produced little behavioral effects and may not pose a health risk. In actual practice, organic solvents such as toluene might be tested either during or after inhalation exposure. Under those conditions, these test methods should and do detect neurological effects of toluene (Tegeris and Balster, 1994).

A possible explanation for the lack of effect in six of the seven laboratories is the dose of toluene administered. Solvents in general require higher doses when given orally to produce effects. It is conceivable that these laboratories were on the ascending portion of the dose-response curve, but not quite at the threshold level. The pronounced effects observed in laboratory H may be due simply to higher sensitivity to toluene evident in their strain of rat. Few behavioral studies employ the oral route, so it is difficult to compare these results with other toluene studies. However, a study using these neurobehavioral methods to characterize other organic solvents (Moser *et al.*, 1995a) reported significant effects at doses of

5000 mg/kg and only marginal effects at 1500 mg/kg, a dose which was closest to the limit dose used in this Collaborative Study. Other explanations include prolonged absorption from the gastrointestinal tract due to the presence of food, since rats were not fasted before dosing.

Studies of blood values after oral exposure have shown that this route of administration can produce high levels, but that the time-course of distribution and elimination is protracted compared to that following inhalation (Gospe and Al-Bayati, 1994; Pyykko *et al.*, 1977; Sullivan and Conolly, 1988). Using the equation derived in a recent study (Gospe and Al-Bayati, 1994), a single oral dose of 2000 mg/kg should produce blood levels equivalent to that following a three-hour inhalation exposure to about 13,585 ppm; based on the literature, one could expect neurobehavioral changes at that concentration. Maximal blood toluene levels obtained two to six hours after oral dosing are strongly dependent on the dose (increasing dose level slows absorption). The long period of time over which asymptotic levels are reached may make it difficult to observe a peak effect using a single test time. Thus this route may be more appropriate for long-term dosing to study chronic changes, not immediate acute effects.

Repeated administration of toluene also produced almost no effects in all laboratories. This lack of effect might be expected from the essentially negative effects observed at 24 hours in the single-dose study. There was no evidence of cumulative toxicity with toluene, and several studies have shown that blood levels are at or below minimal detectable quantities at 24 hours after an oral dose (Gospe and Al-Bayati, 1994; Sullivan and Conolly, 1988). These negative results are similar to previous experience in this laboratory with 14-day dosing of other organic solvents (Moser *et al.*, 1995a).

There are, however, known neurotoxic consequences of repeated exposure to toluene (mostly by inhalation). Ototoxicity is produced by toluene exposure, and it has been reported that oral administration is sufficient to produce this effect (Sullivan *et al.*, 1988). In that study, toluene was given daily at dose levels (867 mg/kg/day) which were similar to those used in these studies, but for 42-49 days instead of 20 days as in this study. Auditory dysfunction, presumably detectable by the click response, was not reported in any participating laboratory. This was not necessarily expected, however, since the clickers used to elicit the response have a broadband frequency, and ototoxicity produced by solvents is a selective mid-frequency hearing loss. Other studies have verified that the click response cannot detect the hearing loss of rats exposed to trichloroethylene, which produces a similar ototoxicity (Crofton and Moser, unpublished).

Other neurobehavioral effects of long-term toluene exposure, such as motor and cognitive deficits, also require considerably longer exposure durations. For example, daily inhalation exposure for 23 weeks was required to produce a syndrome of ataxia, shortened and widened gait, and increased landing foot splay (Pryor, 1991). Thus the lack of effects with this screening battery may be due more to the insufficient exposure dose levels and/or duration, as well as the specific nature of the neurotoxicity produced by toluene, rather than a lack of sensitivity of the test measures themselves.

Triethyl Tin

All laboratories identified TET to be an acutely toxic compound, producing severe neuromotor dysfunction. These deficits presented as lowered locomotor activity, impairment of postural function and possibly vestibular reflexes, generalized weakness manifest as lowered grip strength and decreased responsiveness to various stimuli, as well as decreased reactivity to manipulations. This overall pattern of effects was seen in all seven laboratories, although specific endpoints did not show complete agreement. All laboratories would have recognized this compound as one having considerable neurotoxic potential.

TET effects were fairly rapid, with all laboratories using one to three hours as the TOPE. Only slight recovery was evident at 24 hours for most measures, and the neuromuscular changes in particular were still prominent even at one week. This indicates a fast uptake and distribution, but long elimination characteristics. Kinetic studies have indeed shown that TET is rapidly distributed to tissues, reaching asymptote by about one hour with levels staying fairly constant throughout 24 hours (Cook *et al.*, 1984a, 1984b; Rose and Aldridge, 1968). The half-live of TET in brain tissue has been reported as 4.6-8.5 days (Long-Evans rats appear to have a faster elimination than Sprague-Dawley rats; Cook *et al.*, 1984a, 1984b). Studies have also reported similar time-course characteristics for the behavioral changes following a single dose of TET (Dyer and Howell, 1982; Eto *et al.*, 1971; Stoner *et al.*, 1955).

Repeated dosing with high doses of TET for two weeks or less produced severe toxicity, characterized by

weakness, motor incoordination, and lowered reactivity to specific and general stimuli, progressing to death. Four weeks of dosing with lower doses caused alterations in righting ability and gait characteristics, and depressed reactivity to some responses, specifically the tail pinch but in some cases also the click stimulus. Body weight and general health was generally not affected by these lower doses. There was very good agreement across laboratories in the effects of these lower doses at four weeks.

Early studies of TET described severe toxicity and generalized weakness resulting from administration of high TET doses, which eventually caused death (Barnes and Stoner, 1958; Stoner *et al.*, 1955; Magee *et al.*, 1957). The behavioral effects of sublethal doses of TET were subsequently examined and have been reviewed (McMillan and Wenger, 1985; Reiter and Ruppert, 1984); these effects are very similar to those reported here. The neuromotor dysfunction produced by TET is consistent with the findings of central/peripheral myelin sheath splitting and vacuolation, and cerebral edema. The toxicity progresses with continued exposure, but when dosing is discontinued recovery occurs within weeks. Using measures similar to those in this neurobehavioral screening battery, others have reported decreased motor activity (Gerren *et al.*, 1976; Reiter *et al.*, 1980), decreased grip strength (Squibb *et al.*, 1980), lowered responsiveness to painful stimuli (Squibb *et al.*, 1980), and decreased foot splay (Reiter *et al.*, 1980). These findings (except for the changes in splay) were evident in almost all laboratories following a single dose. During the repeated-dosing study these effects were also noted, but mostly in the 25%-TD dose group which subsequently died. At the end of dosing, the tail-pinch response was altered in five laboratories but the other changes (motor activity, grip strength, and splay) were only affected in, at most, two laboratories. It is possible that these effects would have become more evident if dosing had continued, or if slightly higher non-lethal doses had been used.

The screening battery therefore was able to detect and describe the acute neurotoxicity - generalized weakness and depression - of TET. Repeated dosing, using the pre-established fractions of the TD, resulted in similar effects in rats who thereafter died. Significant effects were also evident in the surviving dose groups, but the severity of response was not as great at the end of dosing as was seen in the groups which died. These data would have revealed this compound to be a potential neurotoxicant, both acutely and with repeated exposure.

GENERAL DISCUSSION

The time of peak effect (TOPE) determination was required so that acute testing would take place at a time when maximal effects were evident. The time-course of effects could differ due to rat strain, dosing conditions, etc. The protocol only specified that gait and arousal be evaluated for this determination, which may have presented problems in some laboratories if chemicals did not show pronounced effects on these measures, or did not show a clear time-course. The participants felt that while some laboratories used only those data to determine the TOPE, others may have included additional signs as well. At the least, autonomic signs and clonic abnormalities should be routinely assessed as criteria for the TOPE. A more complex issue is raised when there is a clear time dissociation of the various criteria, and using two TOPEs might be a better solution than choosing just one (although this increases the difficulty of test scheduling). Another option is to give preference to a longer TOPE value, since the latency of effects at lower doses is likely to be longer than that of a high dose.

The top dose (TD) was intended to be similar to the maximally-tolerated dose, in that it would produce either clear behavioral or generally-toxic effects without producing significant lethality which would compromise data interpretation. The approach used to determine the TD was similar to the up-down method of Bruce (1985, 1987), except that all seven rats were dosed at one time and evaluated. No additional iterations were conducted, except in cases where lethality occurred in the TOPE determination. The approach used in this protocol for using a TD as the high dose for single-dose studies worked reasonably well, but the algorithm was not appropriate for setting dose levels for repeated-dosing studies. This illustrates the necessity of determining a repeated-dosing TD, i.e., conducting more range-finding studies. These conclusions have also been reached by others (MacPhail *et al.*, 1995; Pryor *et al.*, 1983). One option suggested by the study participants was to use the rats from the TOPE determination to pretest for possible cumulative toxicity.

As stated at the beginning of this paper, the objectives of this collaborative study were: 1) to evaluate the reliability, sensitivity and specificity of a standardized set of neurobehavioral screening tests designed to detect potential human neurotoxicants; 2) to develop a data-base of neurotoxic effects with a known set of chemical agents and to compare those effects with compounds having little or no reported

neurotoxicity; and 3) to analyze data from participating laboratories to determine the dose- and time-dependent effects of chemical exposure and to determine the influence of laboratory on such treatment effects (Moser *et al.*, 1997a).

The original hypotheses concerning treatment-related effects were generally met, and the anticipated behavioral signs were reported in most to all laboratories. Acrylamide was expected to produce a syndrome of neuromotor dysfunction characteristic of a dying-back axonopathy, with a more pronounced response seen following repeated exposures than with a single dose. Bis-acrylamide, on the other hand, was not expected to produce the same profile of neuromuscular effects. Indeed, all laboratories reported increased landing foot splay, decreased grip strength, and altered gait with acrylamide. These changes were cumulative, and generally occurred at doses which showed no greater than a 10% difference in weight. Four-week exposure to bis-acrylamide produced only slightly altered gait, decreased landing foot splay in most laboratories, and affected grip strength only at dose levels where body weight was 60-80% of controls. Although there were differences in the effects of repeated acrylamide and bis-acrylamide, single-dose exposure to both compounds acutely decreased motor activity. Unlike bis-acrylamide, however, a single dose of acrylamide caused increased reactivity and tremors in some laboratories. Thus while the neurotoxicity of acrylamide appeared to be progressive neuromotor dysfunction, the effects of bis-acrylamide did not show the same profile, and the effects occurred along with more general toxicity.

Administration of TET also produced neuromuscular signs, somewhat like acrylamide, but unlike acrylamide it produced generally decreased reactivity and sensory responding. These effects of TET were cumulative with repeated exposure. The most sensitive and pronounced effects of TET, with both single- and repeated-dosing regimens, were gait alterations and impaired righting reflex. Thus TET effects could be differentiated from those of acrylamide.

The acute DDT neurotoxic syndrome was observed in all laboratories, except the one which used the short TOPE. Likewise, a single dose of parathion produced acute cholinergic signs in all laboratories, except in the laboratory which felt that they had used an inappropriate TOPE. The cardinal signs of exposure with these compounds were relatively unique, and should be considered seriously if observed in neurobehavioral testing: these included tremors, myoclonus, hyperthermia, and increased click response for DDT, and tremors, lacrimation, miosis, hypothermia, and smacking for parathion. For both of these compounds there were marked differences between the single-dose profiles and the lack of effects with repeated dosing.

Lead acetate generally lowered activity and reactivity levels in a few laboratories, but the biggest effects were on weight and temperature. This was also the case with repeated exposure, wherein almost all signs of depressed activity, reactivity, and motor dysfunction were in rats who died. Surviving rats showed very few effects in all laboratories, in spite of great weight deficits and hypothermia. While lead acetate can not be considered an inactive compound, its effects were quite unlike those of others known to produce neurotoxicity.

The selection of dose levels and route of administration of toluene was unfortunate. Only one laboratory obtained an acute syndrome of motor incoordination and changes in activity/reactivity, while the profiles obtained in all the other laboratories could be interpreted as negative data. Repeated exposure to toluene was not expected to produce pronounced effects, which was verified by the data.

The individual measures were also evaluated for reliability and sensitivity, and compared across laboratories. Decreased rearing and activity levels were obtained during single-dose studies with most of these chemicals. Indeed, the automated measure of motor activity often detected alterations even when there was no change in the frequency of rearing, or else at doses lower than those that affected rearing. Motor activity was consistently decreased following a single dose of all chemicals except DDT and toluene in all but one laboratory. The exception to this was laboratory G which never obtained significant motor activity effects, possibly due to their highly variable data (see Figures 3, 23, and Moser *et al.*, 1997b). The most consistent chemical effects on rearing were decreases produced by administration of parathion and acrylamide. Home-cage posture was also affected during single-dose studies, but was not as sensitive a measure since almost all effects were obtained only at the highest doses.

The autonomic endpoints (lacrimation, salivation, pupil response, palpebral closure) were generally not affected by chemical treatment, except acutely by parathion; thus, the agreement across laboratories on the lack of effects was good. The exception was laboratory B, which reported lacrimation and/or altered pupil response in more than half of the studies. The count of excretions (urination, defecation) was much less reliable, with significant changes (increases and decreases) appearing sometimes inconsistently at various time points across studies. Control data for these measures were also quite variable (Moser *et al.*, 1997b). There was no study where laboratories agreed on an effect on urination, and only two where

most laboratories agreed on either decreased defecation (acute bis-acrylamide) or increased defecation (repeated acrylamide). In general these measures contributed little or no information regarding the chemical treatment.

Spontaneous motor abnormalities sometimes did not show good agreement. This may have been due to mis-interpretations in descriptions of the observations, as discussed earlier (Data Exclusions). Consistent effects were obtained with a single dose of DDT and parathion, with somewhat less agreement on the effects of TET and lead acetate. Some laboratories rarely recorded any types of movements, whereas others reported a low incidence of certain types (primarily mild tremors, smacking) in almost all studies.

The most consistent decreases in excitability were produced by single administration of TET and parathion; in addition arousal was decreased by acute bis-acrylamide, and lead acetate (both single and repeated). While most of the changes that were reported consisted of decreased excitability, there were also many instances of increases as well (most consistently by a single dose of acrylamide and DDT). Some of the less consistent effects may have depended on baseline values, since it was evident that the laboratories which had low baseline scores on these measures generally did not obtain treatment-related decreases. Furthermore, those for which the control groups varied greatly over time showed the most inconsistent changes; there were often increases and decreases obtained at different times in a single study, and also instances where the significant effects did not show a dose-response.

The neuromuscular test measures were generally the most reliable, both in chemical effects and in stability across time. Landing foot splay was most consistently increased with acrylamide (single and repeated dosing) and a single dose of parathion, and was most consistently decreased with bis-acrylamide (repeated dosing). Both forelimb and hindlimb grip strength were most consistently decreased with repeated dosing of acrylamide and bis-acrylamide, and by single dosing of parathion and TET. In addition, forelimb grip strength was more often affected by one dose of toluene and repeated exposure to DDT, whereas hindlimb grip was more often affected by a single dose of DDT and repeated lead acetate.

Gait score was affected most frequently of any measures in the FOB, and the largest effects were observed in the studies of acrylamide and TET (both single- and repeated-dose), and parathion, bis-acrylamide, and DDT (single). Righting reflex was not affected as often, but there were consistent effects of single-dose TET and parathion, and of repeated acrylamide.

The approach and touch responses generally showed few effects at the times of maximal effects in these studies. Of these two measures, the only treatment-related change was a decreased touch response following single administration of parathion. Interestingly, for these measures increases in response were almost as common as decreases. The less selective nature of the stimuli may be a factor in the inconsistency of responses seen with these parameters. The click and tail-pinch responses were more stable than approach and touch. The click response was most consistently decreased by single-dose TET and parathion, and increased by DDT; effects were not usually obtained with the other chemicals. The tail-pinch response was most consistently decreased by one dose of parathion, and both single- and repeated-exposure to TET. Baseline values (Moser *et al.*, 1997b) appeared to influence this measure: the laboratory with the highest tail-pinch scores reported the most effects, all of which were decreased response, while the laboratories with the lowest control responses were the only ones which obtained increased response.

Thus, these data provide important information regarding the reliability, sensitivity, and robustness of neurobehavioral screening methods over a range of laboratory conditions. The results of the chemical tests indicated that all participating laboratories generally could detect and characterize the effects of known neurotoxicants, with one exception (toluene) that may have been related more to dosing parameters. As expected, there was some variability in the data on specific endpoints, but many of these differences could be related to time of assessment, dose, or baseline/control values. These data illustrate the amount of variability that may be expected across laboratories; this finding emphasizes the need for tight experimental control and well-trained personnel, which will decrease variability and improve sensitivity. Reliability estimates (see Catalano *et al.*, 1997) can also be used to evaluate specific endpoints and lead to refinement of the test battery. Thus, this study provides extensive data regarding the use of neurobehavioral screening methods over a range of laboratory conditions as well as the ability of the tests to detect neurotoxic potential of chemicals.

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