# VALIDATION OF CNS SAFETY PHARMACOLOGY ASSESSMENTS IN RATS

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## ABSTRACT

The validation of CNS Safety Pharmacology assessments in rats included a complete functional observational battery (FOB) developed at ITR Laboratories Canada Inc. and automated monitoring of locomotor activity. The FOB assessment was developed through the evaluation of the general behavior of Sprague-Dawley rats subsequent to the administration of CNS-active substances (Alertonic®, Diazepam and Carbaryl). The FOB results obtained for each technician were compared with the results from the Trainer and the other technicians to establish reliability between observers. Discrepancies were therefore assessed for each technician and for each observation occasion according to the level of discordance. Since such evaluations are subjective in nature, the technicians were not aware of the treatment administered to the rats. Three teams of three technicians and one Trainer were assigned to work together (with individual records). The inter-observer reliability of the FOBs (conducted both before and after administration of negative [water] or positive controls) was considered very good for all technicians (overall more than 85% in agreement with the Trainer and among themselves). The greatest discrepancies were seen for parameters in which there was more potential for subjectivity. Quantitative measurement of hindlimb foot splay and grip strength were validated after the technicians had developed a consistent and appropriate method of holding the animals. The effect of Diazepam on the locomotor activity of freely moving rats was assessed using Opto-Varimex monitors that can register horizontal, ambulatory and vertical activity.

## INTRODUCTION

This validation study was designed to develop the functional observational battery (FOB) procedure at ITR Laboratories Canada Inc. through the evaluation of the general behavior of Sprague-Dawley rats subsequent to the administration of CNS-active substances. This method development focused on the abilities of designated technicians to recognize the general behavior of rats in order to confirm the effectiveness of the FOB training program. The FOB included both qualitative and quantitative assessments. The method for monitoring the locomotor activity of the Sprague-Dawley rats was also validated using Opto-Varimex activity equipment. Locomotor activity was assessed prior and subsequent to the administration of a positive control substance, Diazepam.

## VALIDATION TEAM

The validation team included: Study Director, Technical Team Leader, 9 technicians, QA Inspector, Representative of Senior Management.

## METHODS - FOB

Qualitative and quantitative evaluations following administration of control or positive substances were noted by each involved technician and the designated Trainer. The evaluations included the following:

| Domain | Behavioral observations performed |
|---|---|
| Behavioral | Posture and activity in home cage |
| | Ease of removal from the cage |
| | Handling reactivity |
| | Arousal |
| | Rearing |
| | Exploratory activity |
| | Touch response |
| | Abnormal or stereotyped behavior |

| Domain | Behavioral observations performed |
|---|---|
| Neurological / Neuromuscular | Involuntary motor movements (such as convulsion and tremors) |
| | Gait |
| | Righting reaction |
| | Hindlimb foot splay |
| | Forelimb and hindlimb grip strength |
| | Vision test |
| | Auditory test |
| | Tail pinch response |
| | Pinna reflex |

| Domain | Behavioral observations performed |
|---|---|
| Autonomic | Lacrimation |
| | Salivation |
| | Pupil response to light |
| | Palpebral closure |
| | Defecation |
| | Urination |
| | Piloerection |
| | Exophthalmus |
| | Body temperature |

## TREATMENTS

| Treatment | Dose Level | Dose Conc. | No. of Animals | |
|---|---|---|---|---|
| | (mg/kg) | (mg/ml) | Males | Females |
| Water[a] | 0 | 0 | 6 | 6 |
| Alertonic® [b] | 10 | * | 6 | 6 |
| Diazepam [b] | 20 | 2.0 | 6 | 6 |
| Carbaryl [b] | 100 | 10 | 6 | 6 |

FOBs are somewhat subjective in nature, and therefore to avoid bias, the observers performing the FOB were not aware of the treatment administered to the rats. The dosing formulations were differentiated randomly as Formulations A to D. Only the Pharmacy Department was made aware of the correspondence between the composition of the dosing formulations and their identification.

*Was used as supplied.

[a] Negative control substance

[b] Positive control substances

## COMPARATIVE PERFORMANCE

**Data Analysis:**

- The data generated from this study were analyzed by comparing the scores of each technician per FOB evaluation with that of the Trainer, and with the overall performance of other technicians.
- This analysis was done for all qualitative observations and was examined generally according to the order for the ranked observation within the FOB list.
- The discrepancies in scoring were assessed for each technician and for each observation occasion according to the level of discordance.
- In general, the total of discrepancies per technician and per occasion for the subjective observations should not exceed 25% when compared with the Trainer and other technicians (i.e. should not exceed a discrepancy in more than 7 out of the 28 observations).
- For quantitative measurements and where it involved repeated manipulation of the animals by the same technician (i.e. foot splay and grip strength), means and standard deviations were reported per time points.

Ex.: Females – 1 hour post treatment (Diazepam, 20 mg/kg)

| | Rat #7 | | | Rat #2 | | | Rat #3 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Trainer | Tech 7 | Tech 2 | Trainer | Tech 4 | Tech 3 | Trainer | Tech 7 | Tech 8 |
| **HOME CAGE MEASUREMENT** | | | | | | | | | |
| POSTURE | L | P | L | L | P | P | L | P | P | P | P |
| PALPEBRAL CLOSURE | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 |
| INVOLUNTARY MOTOR MOVEMENTS: | | | | | | | | | | | | |
| CLONIC | - | - | - | - | - | - | - | - | - | - | - | - |
| TONIC | - | - | - | - | - | - | - | - | - | - | - | - |
| **MEASUREMENTS MADE WHILE HANDLING ANIMALS** | | | | | | | | | | | | |
| EASE OF REMOVAL | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| REACTIONS TO BEING HANDLED | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 1 |
| BITES | - | - | - | - | - | - | - | - | - | - | - | - |
| PALPEBRAL CLOSURE | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **OPEN-FIELD MEASUREMENTS** | | | | | | | | | | | | |
| REARING | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| LIMB-FLICK | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| DEFECATION | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| INVOLUNTARY MOTOR MOVEMENTS: | | | | | | | | | | | | |
| CLONIC | A | - | - | - | - | - | - | - | A | A | B | - |
| TONIC | - | - | - | - | - | - | - | - | - | - | - | - |
| GAIT | - | - | - | - | D | D | B | D | D | - | - | - |
| EXPLORATORY ACTIVITY | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| ARCUSAL | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 1 |
| **STIMULUS REACTIVITY** | | | | | | | | | | | | |
| VISION TEST | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| TOUCH RESPONSE | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| AUDITORY TEST | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| TAIL PINCH | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| PUPIL RESPONSE | + | + | + | + | + | + | + | + | + | + | + | + |
| HYBLIMB RESPONSE | + | + | + | + | + | + | + | + | + | + | + | + |
| FLEXOR REFLEX | + | + | + | + | + | + | + | + | + | + | + | + |
| EXTENSOR THRUST REFLEX | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| PINNA REFLEX | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| PROPRIOCEPTION POSITIONING | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 1 | 1 |
| SURFACE RIGHTING REACTION | 2 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 2 | 1 | 2 |
| AERIAL RIGHTING REACTION | 1 | 1 | 1 | 1 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 1 |

## QUANTITATIVE PARAMETERS

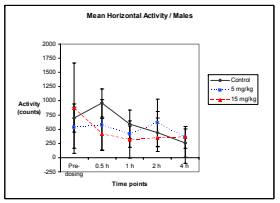
- Hindlimb splay
  - Grip strength
  - Body temperature

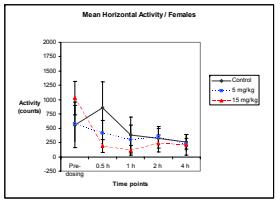
The training success of the quantitative FOB was assessed by the evaluation of the technician's ability to perform consistent measurements in manipulative tests.

## LOCOMOTOR ACTIVITY MONITORS

- Rodents were placed in motor activity cages equipped with infra-red beams.
- Monitoring:
  - Horizontal activity (for static activity)
  - Ambulatory activity (for mobility assessment)
  - Vertical activity (for exploratory activity)
- Performed before and after dosing (typically 3 or more timepoints post dose)

## EFFECT OF DIAZEPAM ON LOCOMOTOR ACTIVITY




## CONCLUSION

**From the Comparative Performance of the FOB:**

- The FOB consisted of 28 qualitative observations which were divided among: the Home Cage, While Handling Animals, the Open-Field and Stimulus Reactivity observations. The performance of the technicians (9 in total) in making these qualitative behavior observations varied from good to very good.

- The number of discrepancies with the Trainer's observations was generally between 1 to 3 per observation occasion (and at some occasions, up to 4 or 5). These discrepancies were in general within one level of discordance according to the order for the ranked observations within the FOB list. Occasionally, higher number of discrepancies was noted. These cases were often seen when a specific behavior of the animal was difficult to define within the pre-established behavioral definitions.

- The observations recorded by the technicians per FOB evaluation were overall more than 85% in agreement with the Trainer and among themselves.

- The discrepancies (from 4 to 11%, corresponding to 1 to 3 discrepancies over 28 qualitative observations) were mainly seen at the same observation occasions where subjectivity could occur, and were considered of minor magnitude.

- The techniques of the hindlimb foot splay and the grip strength require consistency in the method of holding the animals.

**From the Motor Activity Assessment:**

- Following the administration of 15 mg/kg of Diazepam, a significant decrease in mean horizontal, ambulatory and rearing activities (not shown) was noted in both male and female rats in comparison to pre-dosing values. The effect was observed up to 2 h for the males and up to 4 h for the females when compared to control animals from water treatment.

In summary, the results demonstrated that the behavioral and activity affects of the positive control agents could be detected, in a dose-related manner, using the FOB and locomotor activity procedures defined in the validation protocol. Furthermore, the inter-observer scores confirmed that there was only minor variation between and among observers. Therefore it was concluded that the FOB and motor activity assessments had been successfully validated within this laboratory and that the FOB training program was suitable and effective.