

## METHOD FOR EFFICACY TESTING OF RODENT BAIT STATIONS UNDER LABORATORY CONDITIONS

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**Abstract** The use of bait in a bait station may significantly alter bait acceptance, and consequently its efficacy for rodent control. Here, we describe a method for efficacy testing of rodent bait stations under laboratory conditions. Bait stations are tested in an experimental setup of three test chambers (A, B, and C, each 5 m<sup>2</sup>) which are connected with passage holes. Nest boxes are placed in chamber A, an open tray with food as well as a water supply are placed in chamber C. The bait stations to be tested are placed in chamber B between A and C. Non-poisonous food is offered in the bait stations and an open tray to equal amounts for 10 days. Each day, the amount of consumed food is determined and food and water are replenished. Exemplary results are presented for absolute and relative efficacy of rodent bait stations: for evaluation of absolute efficacy, 3 bait station products for use against mice were tested with groups of 20 to 22 wild strain house mice (*Mus musculus*) in each experiment. In the first experiment (product I), the mice did prefer food from the open tray (62% of total food consumption), in the experiment with product II the amount taken up from the open tray was about equal to the amount taken up in bait stations (52% from open tray) and in a test with product III, the mice preferred food from bait stations (58% from bait stations). In all cases, more than 25% of all food consumed by the mice was taken up from the bait stations. The relative efficacy of two products with same design but with material of different optical properties (translucent vs. opaque) was tested with house mice and brown rats. From all food taken up in the bait stations in an experiment with a group of 8 wild strain brown rats (*Rattus norvegicus*) only 26% were consumed in translucent bait stations, clearly indicating that opaque bait stations are preferred. Similar results were achieved in a test with a group of 24 wild strain house mice, although the preference for opaque bait stations was less pronounced: 38% of all food consumed in bait stations was taken up in translucent bait stations.

**Key Words** *Rattus norvegicus*, *Mus musculus*, rodenticides, risk mitigation

### INTRODUCTION

House mice (*Mus musculus*) and brown rats (*Rattus norvegicus*) are pest organisms which cause considerable damage in crops and stored products, and are a threat to human and livestock health. The need to control rats and mice with rodenticides is undisputed. However, all rodenticides currently available on the market do not act specifically against the target organisms and are a health risk for non-target organisms and humans when accidentally taken up. A well-established and widely distributed means of risk mitigation are rodent bait stations, which prevent free access for non-target organisms (including humans) to rodent baits. During the course of the biocide authorization process after EU directive 98/8 as well as biocide authorization in the U.S., in two recent risk mitigation proposals for anticoagulant rodenticides by the EU commission (EC, 2007) and the U.S. Environmental Protection Agency (U.S.EPA, 2007), use of bait station is recommended (EU) or prescribed (U.S.).

Rodent bait stations have positive as well as negative effects on bait acceptance, depending on the target species as well as the bait station design: Bohills et al. (1982) found that house mice (*Mus musculus*) prefer bait placed in small bait stations to bait in large bait stations or open trays, probably because the mice felt more secure against predators when hidden inside the stations. However, rats prefer larger stations, where they can adopt their normal posture for feeding (sitting on their haunches) (Clapperton, 2006). Especially in rats, social behaviour may have an influence on the acceptability of bait stations, since subdominant rats will probably prefer stations with two entrances from which they can more easily escape when encountering dominant conspecifics. Rats are neophobic, and bait stations introduce a new situation for food uptake which will not easily be accepted.

In order to mitigate risks connected with rodent bait use, the bait stations should be tamper-resistant: they must be resistant against manipulation through non-target species (including humans, especially children)

and typical non-catastrophic weather (e.g. rain, moisture). Moreover, the bait must be placed in a way that it can not be spilled or removed from the inside of the stations. Rodent bait stations are made from a variety of materials, including plastic, cardboard, wood or metal. The material can be opaque, translucent or (partly) transparent. Material and material properties have an influence on bait station attractivity, e.g. wood is often preferred to other material (Lund, 1988; pers. observation of the authors).

In our paper, we do not test the mechanical properties and its safeness of bait stations for non-target organisms, but rather their attractivity and impact on rodent control efficacy. To this end, we have developed a standard test method to assess the attractivity of rodent bait stations under controlled laboratory conditions. The aim of this paper is to describe the test method and present some representative results.

## MATERIAL AND METHODS

The basic idea of our method was a test under controlled conditions which simulated the practical use of the bait stations. To this end, we used groups of animals exhibiting a typical wild rodent behaviour. Since in practice rodent baits are usually placed on passageways and trails between the mice or rats nests and their food sources, we simulated these conditions in our test chambers. In order to eliminate all other parameters which could have an influence on rodent behaviour, we tested the bait stations only with poison-free (placebo) food.

### Animals

For rodent bait station testing, we used wild strains of house mice (*Mus musculus*) and brown rats (*Rattus norvegicus*) from laboratory colonies kept at the Federal Environment Agency. The rat and mice colonies consist of animals which have been caught in natural habitats in Northern and Eastern Germany. New wild animals are regularly introduced to the colonies at least every two years. The animals exhibit typical wild behaviour and are kept and reared in social groups with a minimum of handling and disturbance.

### Experimental Setup

Rodent bait stations were tested in an experimental setup with three test chambers (A, B, and C) which were connected with passage holes (diameter 9.5 cm). Walls, floor and ceiling of the test chambers were tiled. The floor space of each chamber amounted to 5 m<sup>2</sup>. In chamber A, three wooden nest boxes (Length 40 cm, Width 25 cm, Height 18 cm; Length, Width and Height are hereafter denoted as L,W,H) were installed in experiments with rats and one nest box in experiments with mice. An open tray with food as well as a water supply was placed in chamber C. The bait stations to be tested were placed in chamber B between A and C. Each test chamber had two windows and was not artificially illuminated. The test chambers were empty except for the nest boxes, bait stations, or water supply and feeding dishes, respectively (for a sketch of the experimental setup). The bait stations were provided with placebo bait, and as a challenge food station an open tray with food *ad libitum* was placed in chamber C for 10 days. During the experiments, the animals had *ad libitum* access to water.

For testing the absolute or relative efficacy of rodent bait stations, groups of house mice (20 to 24 individuals per group, male/female-ratio 1/1) and brown rats (8 individuals per group, male/female-ratio 2/1 to 1/2) were removed from their laboratory colonies to the test chambers. Prior to the beginning of the tests, the animals were given 3 days of familiarization with all three test chambers. During this period, they were fed with dry bread *ad libitum* placed on an open tray in chamber C. During this period, chamber B was left empty. The bait stations were only introduced in this chamber at the first day of the testing period. For testing the absolute efficacy, 4 bait stations of the same type in chamber B were tested against the open tray in chamber C. Relative efficacy was determined by placing two different types of bait stations (two of each type, e.g. translucent vs. opaque material) in chamber B. The position of the different types was interchanged each day to avoid spatial conditioning of the test animals; the position of the open food tray in chamber C remained unchanged. Each day, food, bait and water were replenished and the amount of consumed placebo bait and food was determined with an electronic fine balance (PG5002-S Delta Range, Mettler Toledo GmbH, Giessen, Germany, exactness = 0.1 g).

## RESULTS

Here, we present exemplary results for absolute and relative efficacy of rodent bait stations. Since the aim of this paper is to demonstrate the method and not to evaluate specific products, all tested products are anonymized, and a product description is given.

Three bait stations for mice were tested. The material of all three products was duroplastic plastic. Product I had two entrance holes (diameter 2.3 cm) at opposite ends, so that the mice were able to see the respective other (exit) hole when entering the bait station. The station was made from opaque black material with an external dimension of length 26 cm, width 16 cm and height 7.5 cm, and a removable inlet (L 20 cm, W 14 cm, H 3.5 cm) which was subdivided in a vestibule (L 20 cm, W 3.5 cm) and a bait compartment (L 10.5 cm, W 5.5 cm). The bait stations were secured with a lock.

Product II and III had the same design and dimensions, except that product II was opaque black and product III was translucent with transparent parts at the position of the bait. Both stations had external dimensions of L 13.8 cm, W 4 cm, H 3.5 cm with only one entrance hole (diameter 2.3 cm). The bait stations were subdivided in a vestibule (internal dimensions L 6.5 cm, W 3.6 cm) connected to the bait compartment (entrance diameter 2.3 cm; internal dimensions L 5.5 cm, W 3.6 cm). The backside of the bait compartment could be opened with a specific opening tool (miniature crowbar).

Two bait stations for rats were also tested. Like product II and III, these bait stations had the same design and dimensions but differed in their material. Product IV was opaque black and product V was translucent with transparent parts at the position of the bait. The external dimensions were L 31 cm, W 19 cm, H 9 cm. The bait stations had two entrance holes at their opposite ends, a vestibule (L 28 cm, W 6.5 cm, H 9 cm to 7.5 cm) and a bait compartment (L 18.5 cm, W 6.5 cm, H 5.5 cm to 7 cm). The bait stations had a children-safe lock mechanism.

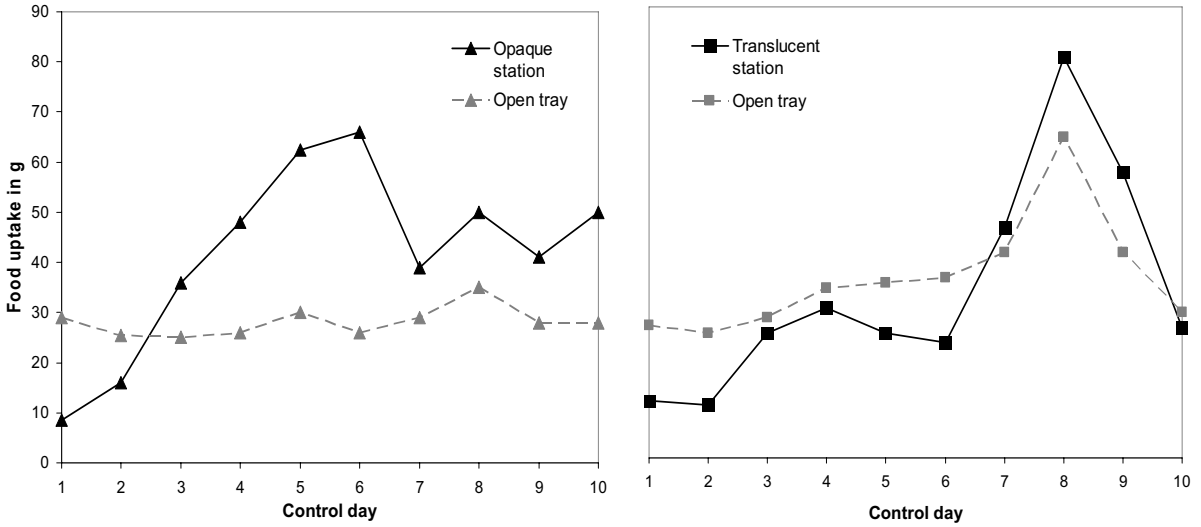
### Absolute Efficacy

Products I, II and III were tested for absolute efficacy on house mice. Our results are presented in detail in Table 1. Oat flakes were offered in bait station product I as well as an open tray to a group of 20 wild strain house mice. The mice preferred food from the open tray (62% of total food consumption), although the bait boxes were sufficiently attractive (38% of total food consumption) with an average food consumption of 0.9 (SD 0.3) g/day/individual.

Products II and III were not suitable for grain bait, since preliminary experiments revealed that the mice spilled the oat flakes. Therefore, we used placebo wax block bait (Detex Blox, Bell Laboratories Inc., Madison, Wi, USA) which fitted exactly in the bait compartment. Two groups of 22 mice each did not show a feeding preference between product III (translucent) and an open tray (48% vs. 52%) but did clearly prefer the opaque black bait stations (product II) to the open tray. In some cases, the mice were found resting or sleeping inside the bait stations during our daily inspections.

**Table 1.** Amount of food taken up in 10 days from bait stations in comparison to a challenge food resource (open tray). For explanation of threshold value, see discussion.

Product	Species	Amount of food consumed from		Individual food consumption from bait station		Percentage of food consumption from	
		bait Station (g)	open tray (g)	measured value (g/d/individ.)	threshold value (g/d/individ.)	bait station (%)	open tray (%)
I	<i>Mus musculus</i>	179	294	0.9	0.2	37.8	62.2
II	<i>Mus musculus</i>	334	360	1.5	0.2	48.2	51.8
III	<i>Mus musculus</i>	417	282	1.9	0.2	59.7	40.3

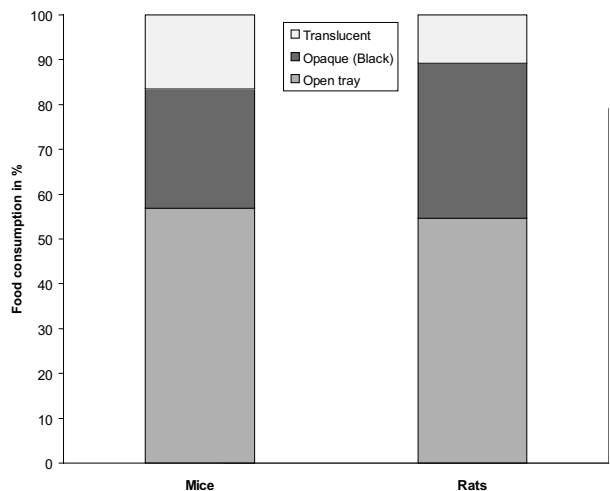


**Figure 1.** Daily food consumption in two experiments used for absolute efficacy evaluation of mouse bait stations. In each experiment, a group of 22 wild strain house mice was introduced to the test chambers. Figure A shows food consumption from bait stations with opaque material vs. an open tray, and figure B food consumption from translucent bait stations vs. an open tray.

**Relative efficacy.** Products II and III for mice and products IV and V for rats were tested for relative efficacy. As described above, products II and III are identical except for the material, which was opaque black (Product II) and translucent (product III). The same holds good for products IV (opaque black) and V (translucent), respectively. Figures 2 and 3 illustrate our results.

Translucent bait stations (product V) were tested against opaque bait stations (product IV) in an experiment with wild strain brown rats (*Rattus norvegicus*). The bait stations were tested with a group of 8 rats. Oat flakes were offered to the rats in the bait stations as well as an open tray. The boxes proved to be attractive (45 % of total food consumption from bait stations versus 55% food consumption from open tray). However, from all food taken up in the bait stations, only 26% were consumed in translucent bait stations, clearly indicating that opaque boxes were preferred. The same results were obtained with a group of 24 house mice and products II and III. In total, the mice took up 56.9% of all food from the open tray. A comparison of the amount of food taken up from the bait stations shows that in this experiment, 61.6% of all food taken up in bait stations was consumed from opaque bait stations and 38.4% from the product with translucent material.

**Figure 2.** Food consumption of a house mouse and a rat group in a choice test with translucent and opaque bait stations and an open tray. The sum of all food consumed after 10 days feeding is shown in relative proportions. The total amount of food consumed by a group of 24 wild strain house mice amounted to 723 g, and by a group of 8 wild strain brown rats to 1128 g.



## DISCUSSION

In our experiments, bait consumption from bait stations was low during the first days. With one exception house mice preferred bait boxes to an open tray after only 3 days (opaque) or 7 days (translucent). Rats did prefer bait stations (translucent and opaque combined) over an open food source after 8 days. This delayed reaction is typical for rodent behaviour against unfamiliar objects or food introduced to their habitat (Berdoy and Dickramer, 2007)).

Our experiments could show that translucent bait stations are less attractive for house mice and rats than opaque stations. This finding was somewhat surprising since most of the food from bait stations is taken up at night, when illuminance is low. Our results indicate that mice as well as rats seek for sheltered food places where they cannot be easily detected by predators. In one case, house mice preferred opaque boxes to an open food tray, confirming the findings of Bohills et al. (1982). However, our results with product I clearly show that mice do not prefer bait stations to open food places in all cases. Translucent bait stations can have advantages for the pest control operator because handling with the stations can be minimized with much time saving for the operator since it can be determined visually from outside if bait was taken up or not, and if an animal is still sitting inside the station. In this case, the advantages must be compared with the drawbacks. To do so, the absolute efficacy gives a good estimate if a bait station will be sufficiently attractive for successful rodent control. In order to evaluate the efficacy, we propose two threshold values which must be excelled: 1) Relative criterion: Food uptake from bait stations must not fall below a value of 25 % of total food consumption (= sum of food consumed from bait stations as well as open food sources); 2) Absolute criterion: The average food uptake per individual and day must not fall below 0.2 g/d/individual in house mice and 1.0 g/d/individual in brown rats.

All threshold values are empirically derived from 20 years of rodent bait efficacy testing at the Federal Environment Agency.

Because recent authorization processes for rodenticides demand high safety standards and measures for risk mitigations, the need for efficient rodent bait stations will increase. Our proposal for a standard test method for efficacy testing of rodent bait stations is a robust and simple system. It allows an objective estimate if a bait station product is sufficiently attractive for target rodents for a successful rodent control operation and also presents a method for comparative test of bait station characteristics. For example, we have recently tested if the introduction of an electronic device for rodent detection in a bait box increases or decreases the attractiveness of the bait stations. The comparative testing approach (relative efficacy) can be an important tool for development of bait station improvements. The development for test systems concerning other properties of bait stations related to risk mitigation, e.g. safety for children and non-target organisms as well as mechanical robustness are currently under way.

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