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Effect of Addition of Artificial Flavour on Rodent Bait Attractiveness

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ABSTRACT: Some ready-to-use rodenticide baits against synanthrophic mice and rats exhibit an intense scent of vanilla, chocolate, peanut, or hazelnut. The effects of these additives on bait palatability are unclear. The aim of this study was to test whether flavoured food is more attractive to naïve rodents than natural (untreated) food. The attractiveness of flavoured against natural oat flakes was tested on wild strain groups of rodents. We tested vanilla, chocolate, hazelnut, and peanut flavour. House mice (group size 13 to 23 mice), roof rats (group size 5 to 8 rats) and brown rats (group size 5 to 7 rats) were introduced to test chambers (rats: 6.2 m², mice: 5 m²) for choice experiments. Flavoured and natural food as well as water was offered *ad libitum* for 12 days. Each day, the amount of consumed food was determined by weighting the remaining food, and food and water were replenished. All flavours that were tested on house mice had a positive effect on bait uptake, and the mice took up more flavoured than untreated food (hazelnut 57.7%, chocolate 61.2%, peanut 62%, and vanilla 73.1% of total food uptake). The effect on food uptake was not significantly different between flavours. Roof rats were repelled by chocolate and hazelnut (7.2% and 30.4% of total food uptake, respectively), whereas vanilla flavour had no clear effect on food consumption (51.6% of total food uptake). Similar effects were observed for bait consumption of brown rats, where chocolate flavour had a negative effect on food uptake (28.2% of total food uptake) and hazelnut flavour was neutral in terms of bait attractivity (50.0% of total food uptake). We conclude that the addition of an artificial flavour may increase the attractiveness of rodent baits to house mice, whereas it can reduce bait uptake in both rat species and has thus a detrimental effect on bait attractiveness. The different reaction of mice and rats to flavoured food is explained by the fact that house mice are neophilic, whereas both rat species avoid new stimuli and are neophobic.

KEY WORDS: attractants, bait acceptance, commensal rodents, flavours, food preference, house mice, *Mus musculus*, rats, *Rattus norvegicus*, *Rattus rattus*, rodenticide efficacy

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INTRODUCTION

The success of rodent control operations with toxic chemicals for oral uptake relies heavily on palatability of the bait that carries the active substance. Rats are particularly peculiar in this respect, since they are neophobic and avoid new stimuli or objects (regardless if harmless or dangerous), including bait introduced to their habitat (Meehan 1984, Berdoy and Drickamer 2007). Producers of rodenticides and pest control operators have long tried to increase bait uptake through the addition of attractants and lures. These attractants may include flavours, and ready-to-use rodenticide baits against synanthrophic mice and rats often exhibit an intense smell of vanilla, chocolate, or hazelnut. Clapperton (2006) compiles a list of studies on lures and attractants that may increase bait palatability for commensal rodents. This thorough review covers mostly food ingredients, and contains less information about flavours. Shumake and Hakim (2000) tested rat urine, preputial gland extract, and carbon disulfide and found that the latter is an attractant for brown rats, confirming earlier findings of Galef et al. (1988).

Generally, brown rats (*Rattus norvegicus*) prefer food they are accustomed to. Even rat foetuses that are exposed to specific food compounds of their mother's diet in utero may prefer these over other food after birth (Smotherman 1982, Hepper 1988). Brown rats, roof rats (*Rattus rattus*), and house mice (*Mus musculus*) show social learning of food preferences. Food eaten by a demonstrator will also be preferred by a naïve observer, and food preferences that emerge through social learning can persist over many generations in rat groups (Valsecchi and Galef 1989, Galef 2007). In contrast to behaviourally acquired food preferences, no sound data on inherited food preferences are available, and since brown rats as well as roof rats are omnivorous and show a high degree of plasticity in their food choices (Meehan 1984), it is probably not very likely that such genetically based preferences exist.

The addition of flavours that are also attractive for humans may increase the risk of accidental ingestion, especially by children, although this risk can be mitigated through the addition of aversive agents (i.e., bittering agents). However, it is not clear if flavours actually increase bait palatability. A probable advantage of adding flavours to the bait could be a better bait uptake, resulting in more efficient pest control together with a positive effect for the environment through decrease of amounts of rodenticides used for rodent eradication. The addition of flavours to rodent baits may also have a beneficial commercial aspect, since flavoured bait will probably increase human user expectations that the bait is palatable for rodents.

However, the aim of our study is to evaluate practical advantages or disadvantages of addition of flavours and attractants to rodent bait in respect to rodenticide efficacy, and is not about increase of commercial attractiveness of baits to customers, which would be itself a certainly interesting study in consumer psychology. We tested attractivity of artificially flavoured rodent food with groups of brown rats, roof rats, and house mice under semi-natural conditions.

METHODS

Animals

Experimental animals were obtained from wild strains of house mice, roof rats, and brown rats reared in laboratory colonies at the Federal Environment Agency. Rat and mice colonies are offspring from animals caught in natural habitats in Northern and Eastern Germany. Prior to the experiments, mice and rats were raised and kept with a diet from 80% oat, 5% oat flakes, 5% sunflower seeds, 5% corn, and 5% dry cat food. The animals were also regularly provided with pieces of dry bread.

Flavours

As test food, oat flakes were treated with flavour and a dye. The flavours used are commercially available and were vanilla (Dr. Oetker Nahrungsmittel KG, Bielefeld, Germany), chocolate, peanut, and hazel (all Aromaplus, Günter Aroma GmbH, Beinwil am See, Switzerland). Chocolate, hazelnut, and peanut were concentrated natural aromas and dissolved in alcohol and propylene glycol. The vanilla flavour was a nature identical aroma, also dissolved in alcohol and propylene glycol. For bait with vanilla flavour, 1 g undiluted flavour was added per 100 g oat flakes, and for bait with chocolate, peanut, and hazel, 0.25 g undiluted flavour was added per 100 g oat flakes. The concentrations were chosen according to the compositions of commercial available products. The flavour was well detectable and of about the same intensity as in ready-to-use baits. To distinguish treated and untreated oat flakes in the experiment, flavoured as well as unflavoured flakes were dyed with red (E 124) or dark yellow (E 102) food colouring (Ringe + Kuhlmann GmbH & Co.KG, ERKA Type S 3060 and 1060, Hamburg, Germany). Preliminary tests showed that dye colour had no influence on bait attractiveness.

Test Setup

Test for attractivity of flavoured vs. unflavoured food were conducted as choice experiments with groups of wild strain rodents. For this end, groups of house mice, roof rats, and brown rats were introduced to test chambers (rats: 6.2 m²; mice: 5 m²) with tiled walls, floor, and ceiling. Three wooden nest boxes (18 × 25 × 40 cm) were installed inside the chambers with rats and one nest box in experiments with mice. Table 1 gives an overview of all experiments. To avoid stress while handling the wild strain rodents, the male/female ratio was only estimated prior to the beginning of the trials and then exactly determined after the trials, and was thus subject to slight variation. The male/female ratio amounted to 0.8 (1.quartile 0.7; 3.quartile 0.9; $n = 19$) in trials with house mice, in trials with roof rats to 1.0 (1.quartile 0.7; 3.quartile 1.2; $n = 8$), and in trials with brown rats to 0.8 (1.quartile 0.5; 3.quartile 1.0; $n = 7$). Group sizes also varied slightly, depending on the numbers of rodents that were available from the laboratory colonies at the time of experiments (see Table 1). Each test chamber had two windows and was not artificially illuminated. The test chambers were empty except for the nest boxes, a water supply, and feeding dishes. Prior to the beginning of the tests, the animals were given 3 days of familiarization with the test chamber and were fed dry bread *ad libitum*. Wooden nest boxes were installed inside the chambers. During the experiments, the animals had *ad libitum* access to both types of food (treated/untreated) and water. Each day, the amount of food consumed was determined by weighting the remaining food. Food and water were replenished daily. Flavoured and unflavoured oat flakes were offered for 12 days, each in an open food tray. The two trays with flavoured and unflavoured oat flakes were interchanged daily to avoid spatial conditioning. The distance between both food trays was 120 cm in experiments both with rats and mice.

Table 1. Overview of experiments. In all trials, mice and rats were familiarized with the experimental chambers for 3 days. Treated (flavoured) and untreated food was offered for 12 days in all experiments. For calculation of E, see text.

Species	Flavour	No. of Trials	Number of Individuals Tested		Effect of Flavour (E) (%) Median (max; min)
			Total	Per Trial	
House mouse (*M. musculus*)	hazelnut	5	101	15-23	+7.7 (+22.4; +2.9)
	peanut	5	86	15-20	+12.0 (+14.9; +4.6)
	chocolate	4	56	13-15	+11.6 (+13.7; +10.4)
	vanilla	5	97	17-22	+23.1 (+25.4; +13.1)
Roof rat (*R. rattus*)	hazelnut	2	12	5 and 7	-19.6 (-14.2; -25.1)
	chocolate	3	24	8	-42.8 (-32.6; -44.0)
	vanilla	3	18	6	+1.6 (+19.0; -20.6)
Brown rat (*R. norvegicus*)	hazelnut	5	35	7	0.0 (+8.6; -7.1)
	chocolate	2	10	5	-21.8 (-20.3; -23.3)

Evaluation

For test evaluation, the amount of treated and untreated food, respectively, taken up during 12 days of bait offering was summed. Calculation of the mean effect of flavour on food consumption is based on the assumption that if the flavour had no effect on food consumption and both types of food are offered in equal quantities (to rule out effects of food rareness, Greenwood et al. 1984), the fraction of both treated and untreated food taken up is equal (i.e., 50% of total food consumption). The effect of flavouring is thus calculated as

$$E = P_F - 50, \text{ with } P_F = M_F/(M_F + M_U) \times 100$$

where E is the effect of flavouring expressed as fraction plus or minus of 50% of food uptake, P_F is the percentage of flavoured food related to total food consumption, M_F is the total mass of flavoured food taken up during the trial, and M_U is the total mass of unflavoured food taken up during the trial.

Comparison with Rodenticide Efficacy Data

In order to compare the results of our tests with flavourized bait, we compiled exemplary data from efficacy tests that are undertaken routinely at the Federal Environment Agency for rodenticide product evaluation after the *Infectious Diseases Protection Act* (2001; *Infektionsschutzgesetz, IfSG*) of the Federal Republic of Germany, which prescribes the publication of a list of officially tested and approved biocides against pest organisms. For listing, the respective biocide has to be examined for efficacy and attractiveness; the biocide must be functionally capable of leading to the eradication of the target organism population. Among others, the Federal Environmental Agency of Germany examines rodenticides, and the methods described here are regularly used for practical tests of rodenticide efficacy and attractiveness. Details of the test methods have been described and published as official test guidelines (BBA 1992, 1994). Tests organisms, procedures, and test chambers are identical to the trials with flavoured oat flakes presented in this study. The main difference from the trials with flavoured food was that the bait offering period in choice-trials was 28 days for house mice and 14 days for brown rats and that poison bait was offered, whereas the bait was non-poisoned in our flavour tests, and the bait offering period was 12 days for all rodent species. The length of a test for poison baits was chosen according to the respective test guidelines and adjusted to the mode of action of the active ingredient (chronic or acute poison; BBA 1992, 1994). The main parameter for evaluation of choice tests for bait efficacy after these guidelines is mortality. The test length for our tests on flavour attractivity tests is shorter, since it was a test without poison. We did some preliminary experiments with non-poison bats over a longer period, but the test outcome was identical, and for practical reasons we chose a shorter time of 12 days.

We compared only commercial ready-to-use products that were flavoured. Since the product evaluation of the FEA is confidential, all products are anonymized. Bait consumption was calculated as fraction of rodent bait product consumption in relation to total food uptake (= rodent bait + placebo bait). Since in the FEA efficacy tests the bait products are not compared to unflavoured bait of the same composition as the rodenticide product, but to a challenge diet (oat flakes for house mice and wheat for brown rats), E was not calculated.

RESULTS

House Mice

All four flavours (hazelnut, chocolate, peanut, and vanilla) that were tested with groups of house mice had a positive effect on bait attractiveness (Figure 1, Table 1). The effect had a range from E = +7.7% (hazelnut) up to +23.1% (vanilla), but food consumption of flavoured oats was not significantly different between the flavours (Kruskal-Wallis, $p = 0.081$). House mice preferred flavoured oat flakes over untreated oat flakes within the first 2 days in all trials (Figure 2A). Mean individual food consumption of house mice in all trials was 2.5 g/d/individual (SD ± 0.3 g/d/individual, $n = 19$ trials).

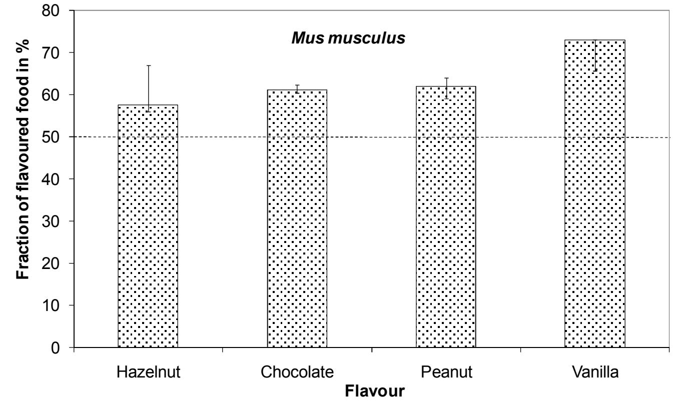

Figure 1. Effect of flavouring on food uptake of groups of house mice. The average fraction of the sum of flavoured food consumed by mice during a baiting period of 12 days is given as median, upper bars indicate 3rd quartile, and lower bars 1st quartile. For number of experiments, see Table 1.

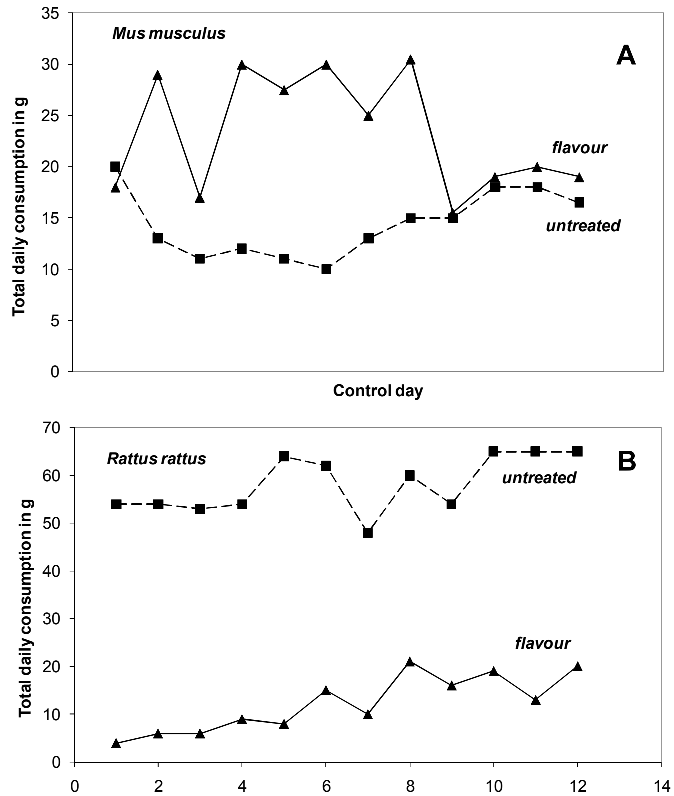

Figure 2 A&B. Examples for trials for food uptake of chocolate flavoured vs. untreated oat flakes. Punctuated lines and squares indicate daily consumption of untreated oat flakes, triangles and solid lines indicate daily consumption of chocolate-flavoured oat flakes. House mouse group size in A was 13 (6 males, 7 females) and roof rat group size in B was 8 (4 males, 4 females).

Roof Rats

Three flavours (hazelnut, chocolate, and vanilla) were tested on groups of roof rats. Treatment of oat flakes with hazelnut and chocolate flavours had a negative effect on

Table 2. Attractiveness of flavoured commercial rodent baits. All products have been tested in routine rodenticide efficacy testing at the Federal Environment Agency. Since the product evaluation of the FEA is confidential, all products are anonymized. Bait consumption was calculated as fraction of rodent bait product consumption in relation to total food uptake (= rodent bait + placebo bait). Active ingredient in all products was difenacoum (0.005%).

House Mice (*M. musculus*)						
Product	**Flavour**	**Bait Type**	**No. of Animals**	**Eradication After (days)**	**Bait Consumption (%)**	**Placebo Bait**
A	vanilla	paste	20	19	32.4	oat flakes
A	vanilla	paste	20	17	31.8	oat flakes
B	vanilla	block	21	17	15.0	oat flakes
C	hazelnut	paste	19	22	15.6	oat flakes
C	hazelnut	paste	19	24	34.4	oat flakes
Brown Rats (*R. norvegicus*)						
A	vanilla	paste	7	14	14.0	wheat
A	vanilla	paste	7	14	6.3	wheat
B	vanilla	block	8	*n.e.*	0.4	wheat
B	vanilla	block	8	*n.e.*	0	wheat
D	chocolate	block	8	*n.e.*	9.6	wheat
D	chocolate	block	6	*n.e.*	5.3	wheat
D	chocolate	block	7	*n.e.*	11.4	wheat

n.e. = no eradication of test population was achieved within baiting period

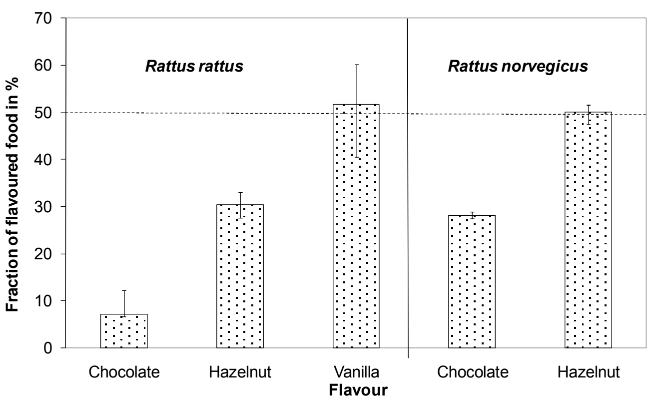

Figure 3. Effect of flavouring on food uptake of groups of roof rats and brown rats. The average fraction of the sum of flavoured food consumed by rats during a baiting period of 12 days is given as median, upper bars indicate 3rd quartile, and lower bars 1st quartile. For number of experiments, see Table 1.

bait attractiveness, i.e., the rats preferred untreated food (Figure 3, Table 1). E (hazelnut) was -19.6% and E (chocolate) was -42.8%. However, the effect of vanilla flavour was less clear. In each of the three trials, the outcome was different: Trial 1 revealed a negative effect (E= -20.6%), in Trial 2 the effect of flavouring was neutral (E = +1.6%), and in Trial 3 more flavoured than untreated food (E = +19.0%) was consumed by the rats. In all three trials with vanilla, the rats initially preferred flavoured food for the first two days, even in Trial 1 with a negative value for E. In trials with hazelnut and chocolate, this effect could not be observed (Figure 3). Mean individual food consumption of roof rats in all trials was 8.1 g/d/individual (SD ± 1.6 g/d/individual, n = 8 trials).

Brown Rats

Two flavours (hazelnut, chocolate) were tested on groups of brown rats. Treatment of oat flakes with chocolate flavour had a negative effect on bait attractiveness (E = -21.8%), whereas the effect of hazelnut flavour was neutral (Figure 3, Table 1). The value for E in all 5 trials with hazelnut flavour had a range from E = -7.1% up to E = +8.6% (median E = 0.0 %). Mean individual food consumption of brown rats in all trials was 15.5 g/d/individual (SD ± 2.4 g/d/individual, n = 7 trials).

Comparison with Rodenticide Efficacy Data

Of a total of 17 products against brown rats and 13 products against house mice that were tested for rodenticide efficacy at the FEA from 2005 to 2009, 5 products against rats (29%) and 5 products against mice (38%) were flavoured and exhibited an intense smell of vanilla, hazelnut, or chocolate. Bait consumption of Product A with vanilla flavour was lower in tests with brown rats (6.3% and 14% of total food consumption) compared to bait consumption of house mice (31.8% and 32.4% of total food consumption) (Table 2). This effect was even more pronounced in tests with Product B (vanilla), where the rats refused the bait. Bait consumption was also low in tests with Product D, which was strongly flavoured with chocolate. The rats did not take up sufficient amounts of the bait, and a total of 7 of 20 rats survived the trials.

DISCUSSION

Generally, mice were attracted by flavoured food, whereas most rats avoided it. Food uptake of mice was increased up to 25% through addition with flavour, with no significant differences between the flavours. The effect of flavoured bait on brown rats and roof rats was negative or neutral in the majority of cases. Only in two trials (one trial with roof rats/vanilla flavour, and one trial with brown rats/hazelnut flavour) of a total of 15 trials was the effect of flavoured bait positive.

Both rat species and house mice were raised and kept with a diet that contained oat grain in significant amounts. The effect of flavouring oat flakes was therefore that a known food component was altered, and flavoured oat presented a new stimulus for the rodents. The different results for rats (negative/neutral) and mice (positive) reflect natural differences in foraging behaviour between rats and mice. Unlike rats (*R. rattus* as well as *R. norvegicus*), *Mus musculus* are typically described as neophilic with regard to their feeding habits, and neophilia is a heritable trait in mice (Bolivar and Flaherty 2004). Rats, in contrast, are neophobic and cautious against new foods (Berdoy and Drickamer 2007). House mice did not react specifically to a new flavour, as no significant differences between flavours were found. It is thus likely that the presence of a new stimulus, regardless which flavour it had, led to an increased bait consumption.

Since the limited numbers of experiments with rats preclude a statistical evaluation, it remains unclear if the negative or neutral effect of flavours on their bait uptake can be correlated with a specific flavour or is a general reaction against a novel stimulus. The evaluation of commercial rodenticide products at the FEA revealed that flavouring of baits had no observable positive effect on mice and in some products a negative effect on rats. The palatability and thus efficacy of rodent baits is being influenced not only by its scent but also other factors, and our study is limited to grain bait. In this regard, it would be interesting to test also other bait types than grain, e.g., paste or block baits. Block baits contain a large proportion of paraffin, which encapsulates odorants, and rodents may have different responses to flavours, depending on whether these are smelled or tasted upon ingestion. The acceptance of rodenticide bait on a site infested by rodents depends much on the composition and texture of the bait (Meehan 1984). Rats as well as mice are able to determine the nutritional value of their diet (e.g., Rose 1931, Frazier et al. 1947). Therefore, bait composition has a strong influence on bait palatability.

The results from efficacy testing at the FEA show that flavours, at least, do not increase the attractivity of bait that is unattractive due to other reasons not investigated in this study. Our study indicates that although mice are attracted by new stimuli that are unrelated to the nutritional value of their food, rats may react neutrally or are even repelled by these stimuli.

CONCLUSION

Our results give evidence that under certain circumstances, the addition of flavours may increase bait attractivity for *Mus musculus* and can have a negative effect on bait attractivity for rats (*Rattus rattus* and *Rattus norvegicus*). Our conclusions are limited to the flavours and flavour concentration we have tested so far, and further test should be made with other flavour types, concentrations, and bait types. The emphasis of such tests should be the investigation of differences in the response of rats and mice to these attractants.

Many rodenticide products with additional flavours are sold for use against rats as well as mice, and the addition of such flavours to products that are used for both mice and rat control may have no overall benefits regarding bait consumption and efficacy when one of the target species is probably repelled by an unknown scent. Bait products that act specifically against only one or a few target species may have a commercial disadvantage over those that act against a wider range of target species (i.e., rats as well as mice). Therefore, in regard to flavours, differences in the foraging behaviour of rats and mice should be considered during development of new bait products, since flavours may have unintended negative effects on palatability for one of the target species.

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