Handling mice using gloves sprayed with alcohol-based hand sanitiser: acute effects on mouse behaviour

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Abstract

Alcohols are commonly used in laboratory animal facilities to disinfect hands, equipment and laboratory environments. The effect on mice is unknown, so we observed male and female C57BL/6J and BALB/c mice during and after handling with nitrile gloves that were either sprayed with 70% alcohol sanitiser (~67% ethanol, ~3% methanol, and 30% water), or not sprayed. We hypothesised that, if mice perceived this hand sanitiser as aversive, its application to gloves before handling would increase behavioural indicators of fear or defence; it could also affect social interactions and grooming. Handling mice with sanitised gloves increased wall rearing, self-grooming, allogrooming, sniffing of cagemates and eating/drinking in one or both strains of mice. In males, it also reduced initial home-cage aggression, replaced by grooming but it is unclear whether aggression was truly decreased or simply delayed. There were no statistically significant effects of treatment on avoidance behaviours shown in a hand interaction test. Defensive burying occurred with both sanitised and control gloves during the first-hand interaction test and significantly declined over the 4-week study, suggesting a novelty effect. Findings indicate that handling mice with alcohol-based hand sanitiser affects mouse behaviour, including social interactions, although replication is required because we could not blind the observer to the treatment. Further research is required to assess the long-term effects of using alcohol-based hand-sanitiser and alternative disinfectants when handling laboratory mice in order to make recommendations for refinement.

Keywords: animal behaviour; animal welfare; disinfectant; handling; hygiene; mice

Introduction

To ensure that mice are free from undesirable or pathogenic microorganisms, laboratory animal units put in place strict biosecurity practices for example Shek *et al* (2015).[1] These procedures vary between facilities depending on the level of microbiological exclusion but they generally involve keeping mice in micro-isolation cages, controlling animal imports and routinely monitoring the health status of the colony. Additionally, personal protective equipment is worn, and consumables, equipment and surfaces are decontaminated.[2,3] Although these practices are important to safeguard the health of laboratory animals, their impact on animal behaviour and welfare is rarely investigated. Additionally, recent reports suggest that keeping laboratory animals in extremely hygienic facilities impairs their immunological response and compromises the reproducibility and translation of the results to humans.[4,5] Garner *et al* (2017)[6] outline the importance for experimental designs to take into account animal biology, husbandry, and welfare (representing three of six key considerations) if research is to produce valid and reproducible results.

In a survey of 51 UK mouse facilities, 22-30% of respondents reported using 'alcohol' to disinfect a variety of items such as work surfaces, anaesthetic equipment, behavioural apparatus and surgical equipment, and 12% specifically reported using it as a hand sanitiser.[3] In that survey, a subset of respondents suggested that alcohol-based disinfectants caused skin problems (4/9 respondents) respiratory problems (1/9) and behaviour changes (1/9) in mice, with 1/9 suggesting there were no adverse effects. This demonstrates that some mouse facility staff are concerned by the use of alcohols as sanitisers. Here, we focus on the acute behavioural effects of handling mice using alcohol-based hand sanitiser which usually contains one or more types of alcohol: ethanol, methanol, isopropanol, and/or n-propanol. In the authors' experience of working in multiple animal facilities in England, it is common practice to use 70% ethanol hand sanitiser immediately before handling rodents. Providing alcohol-based sanitisers has been recommended for use when handling laboratory animals to help prevent infections, even when using gloves because gloves are permeable and can otherwise easily become contaminated.[7] LeMoine and colleagues (2015)[8] found that 48% of non-sterilised standard nitrile or latex gloves (the two most common glove types in UK laboratory animal facilities)[3] tested positive for microbial growth after donning; however, a 30 seconds soak with 70% isopropyl alcohol reduced microbial contamination to 25%. Keen *et al* (2010)[9] also found that using 70% isopropyl alcohol was significantly more effective at preventing microbial contamination of gloves during mouse laparotomy than not using it. However to our knowledge, no research has been carried out to assess the impact of this practice on the animals themselves.

In humans, frequent use of alcohol-based hand sanitisers by health professionals can cause transient, low, but detectable concentrations of ethanol in the breath, due to inhalation of the vapour.[10,11] Such use does not seem to increase alcohol concentrations in the blood,[12] nor does it appear to detrimentally affect the skin.[11] Overall, the internal concentrations observed via inhalation and dermal routes are well within the range of those occurring with ingestion of non-alcoholic foods, such as fruit juices or 'alcohol-free' beers and are well within safe limits.[13] However oral ingestion of larger doses of alcohol-based sanitiser can cause intoxication ('drunkenness'), alcohol poisoning, coma or even death.[14-16]

It is difficult to extrapolate these findings to mice, especially because of potential species differences in pharmacology, and in body size; mice are orders of magnitude smaller than an adult human relative to the volume of sanitiser likely to be applied to a human hand. In rodents, effects of alcohol have mainly been investigated in the context of modelling alcoholism. Most experiments have thus investigated the effects of oral ingestion of ethanol but when rodents are unwilling to ingest it, forced inhalation of ethanol vapour intermittently over a 2 week period can increase voluntary consumption of ethanol by rats 2-8h following withdrawal of the vapour.[17] Oral ingestion of large enough doses of ethanol by mice can cause ataxia, aggression, cognitive deficits and other effects consistent with intoxication and alcoholism in humans for example.[18]

This alcoholism-related research has limited relevance with respect to the effects that alcohol-based sanitiser could have on mice in applied contexts, for a number of reasons. Firstly, the quantities that mice are likely to inhale or ingest after handling by sanitised gloves are likely to be much lower than those administered to animals modelling alcoholism. However hand sanitisers contain much higher concentrations of alcohol (usually 70%) compared with those used in alcoholism research (10-20%), so it is unclear how the exposure would compare. Secondly, whilst alcohol-based sanitisers in the laboratory commonly contain ethanol, this may be mixed with other alcohols unsuitable for consumption such as methanol or propanol, or other substances entirely. Mice[19] and rats[20] are less sensitive to the toxic effects of methanol than are humans.[20] Nevertheless relatively high doses of methanol can cause skeletal and neural defects in rodents exposed as embryos or juveniles, and chronic methanol ingestion and – to a lesser extent – inhalation can cause pathology of the liver, pancreas and possibly other organs (reviewed in[21]). Finally, the route of exposure to hand sanitiser is complex with inhalation and oral consumption influenced by factors such as: the amount of product used; the delay between dispensing the product onto the hand and handling the mouse; and the animals' behaviour following contact (e.g. whether or not they sniff or lick the product from the hand or their fur).

We thus aimed to investigate whether use of an alcohol-based hand spray when handling mice has any acute behavioural effects on the animals in an applied context with a view to refining husbandry protocols. We hypothesised that mice handled using gloves sprayed with 70% alcohol could show differences in behaviour compared to handling without the sanitiser, indicating effects on mouse welfare. We used both sexes, and two strains, of mice to increase external validity of any findings;[22] C57BL/6 mice will drink ethanol relatively willingly, whereas BALB/c mice have high avoidance of it.[18,23,24] We used the 'cupping' method of handling, to follow the example of refined practice and to represent a handling method that would involve contact between the hand and the mouse's body, with relevance to activities such as health checking, cage-cleaning, or manual restraint.[25]

Methods

Animals

We used off-study stock or breeding mice that were lent by colleagues at the Royal Veterinary College. The resulting sample comprised 22 cages containing:

- Adult C57BL/6J mice aged 20-24 weeks (n = 13 cages: 16 males, two cages holding two mice each and two cages holding three mice each; 14 females, four cages holding two mice each and two cages holding three mice each), and
- Adult BALB/c mice aged 9-28 weeks (n = 9 cages: ten males, five cages holding two mice each and two cages holding three mice each; 12 females, three

cages holding two mice each and two cages holding three mice each).

Mice were kept in standard open-top cages (Tecniplast, 32cm x 16cm x 14cm). Cages contained bedding (Litaspen premium, Datesand Ltd), a cardboard tube and additional nesting material (Sizzlenest, Datesand Ltd). Animals had *ad libitum* access to water and food (Rat & Mouse # 1 Diet, Special Diet Services) and were maintained at constant room temperature (~21°C) and humidity (~45%) and were kept under a regular light/dark schedule with lights on from 08:00 to 20:00 h (light = 270 lux). Cage cleaning was carried out by a member of the animal unit staff once a week on a different day than behavioural observations were recorded.

Housing and care were in compliance with the Code of Practice for housing and care of laboratory animals used in scientific procedures and the experiment was approved by the Royal Veterinary College Ethical Committee (URN 2014 1261).

Experimental procedures

Two experimenters (both female) carried out all the observations in this experiment between 9:00 and 12:00h. Only one of the experimenters (NLS) handled the animals. We used two treatments representing realistic husbandry alternatives: handling using nitrile gloves that were either left unsprayed (Control), or first sprayed with 70% alcohol (Sanitised). The 70% alcohol (methylated spirits, Fisher Scientific Ltd[26]) comprised 66-68% ethanol, 2-4% methanol, and 30% distilled water. A new pair of gloves was used for each cage to prevent contamination.

The mice in our sample were unlikely to have previously experienced glove sanitation with alcohol, so 1 week before the experiment, all mice were gently handled in cupped hands using alcohol-sanitised gloves, to help reduce novelty-induced behaviours the following week. Mice within each cage were then exposed to one of the treatments per week, alternating them each week for a period of 4 weeks; mice therefore experienced both treatments twice during the experimental period. Testing order was randomly allocated to cages balancing across strains and sexes (using an online random number generator), such that half the cages experienced the sequence: Week 1 = Control, Week 2 = Sanitised, Week 3 = Control, Week 4 = Sanitised; the other half received the opposite order, starting with Sanitised in Week 1 and alternating thereafter.

Handling effects were observed in four stages: (1) Voluntary interaction with the sanitised or control gloved-hand, similar to the hand interaction test used by Hurst and West;[25] (2) Handleability during handling; (3) Voluntary interaction with the hand after handling; and (4) Home-cagebehaviour both immediately after returning to the rack and 20 minutes later.

Voluntary interaction with the hand before handling

Each cage was placed on a bench and aligned such that an adhesive tape on the bench delineated the cross-sectional midline of the cage. The cage was opened on the bench by the non-handling observer, who removed housing and all nesting material, allowing mice to habituate for 1 minute before behavioural observations. After donning a fresh pair of nitrile gloves, the handling experimenter either sprayed the gloves with 70% alcohol or left them unsprayed. The experimenter then placed one hand inside the near corner of the cage and kept it motionless to allow mice to voluntarily interact (Figure 1; the other hand was used to write down behavioural observations). Observations were recorded by both experimenters for 3 minutes to measure any difference in approach/avoidance or defensive behaviour towards the hand.

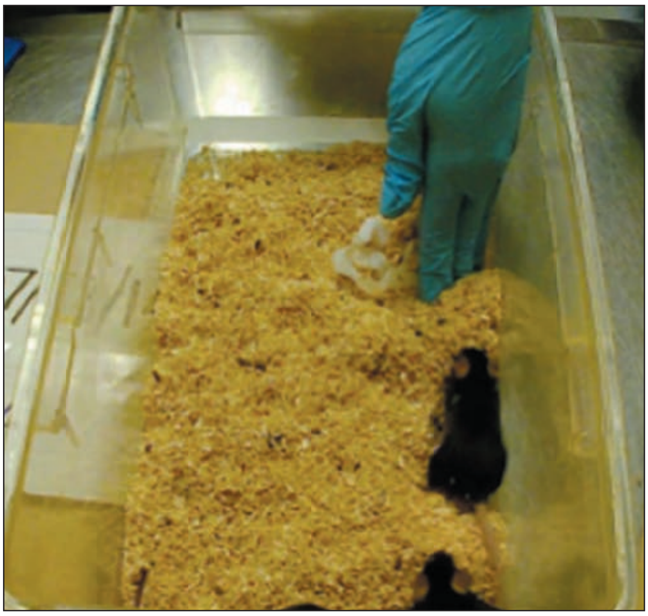

Figure 1. The hand interaction test. This screenshot shows a C57BL/6J mouse displaying defensive burying of the hand.

Handleability

After recording hand interaction, for the sanitised treatment, the handling experimenter re-sprayed the glove with the alcohol because many volatiles would have dissipated during the previous 3 minutes observation. To mimic normal handling in applied situations, when there can be little delay between sanitation and handling, the mouse was then almost immediately lifted using its home-cage tunnel following re-spraying and loosely cupped in the hands for 20-30 seconds to measure handleability. The respraying and handling were repeated for each mouse in the cage. Cage was the experimental unit, so all mice within a cage experienced the same treatment on a given day.

Voluntary interaction with the hand after handling

Once all mice in the cage had been handled, the observer returned their hand to the corner of the cage and kept it motionless, whilst the behaviour of the mice was observed for a further 2 minutes.

Home-cage behaviour after handling

Finally, the cage was relocated to the rack and home-cage behaviour was observed for an initial 2 minute period. Approximately 20 minutes later, home-cage behaviour was again observed for a final 2 minutes.

Behavioural observations

Behaviours were defined according to an Ethogram (Table 1) and recorded in real time by the two observers. It was not possible for us to blind the observers to the treatment; consideration was given to using water spray as a control but this would have been an unrealistic treatment within this applied study and the volatile odour of the alcohol would have continued to make the treatment obvious. (Blinding would still have been possible, via video recordings analysed by an additional treatment-blind observer but these options were unavailable to us during this study, which had limited coverage of research costs.)

Voluntary interaction with the hand before and after handling

The number of mice were located in the half of the cage nearest to, and furthest from, the hand according to the midline of the cage was noted simultaneously by both observers at 15 defensive instances. All other behaviours were allocated to either one of the observers (because there were too many behaviours for a single observer to record); these were recorded on a one-zero schedule, i.e. whether or not they occurred during each 15 seconds interval. Additionally, when mice showed defensive burying, the height of the resulting sawdust 'wall' was measured with a ruler by the non-handling observer after the observation was complete.

Handleability

The non-handling observer recorded handleability on a subjective scale (no struggle; minor struggle; vigorous struggle or escape), vocalisation or biting, and whether urination or defecation occurred.

Home-cage behaviour after handling

Each observer was allocated a time point (NLS: immediately after return to the rack; NC: 20 minutes later). All behaviours (Table 1) were recorded on a one-zero schedule every 15 seconds for a period of 2 minutes.

Behaviour	Definition	Recording stage(s)
Aggression	Biting (using the teeth to pierce the skin), pinning (grabbing recipient mouse's flank and holding down), boxing (movements of the body towards the opponent combined with alternated kicking of the forepaws), tail rattling (fast waving movements of the tail).	Homecage
Allogrooming	Licking the fur of another mouse or using the forepaws to smooth it.	Voluntary interaction with hand and Homecage
Bar-biting*	Chewing the cage grid.	Homecage
Chasing	Rapidly following a fleeing mouse.	Homecage
Chewing glove	Using the teeth as if to pierce the glove material.	Voluntary interaction with hand
Climbing bars	Hanging from the cage grid, without chewing the bars.	Homecage
Defensive burying	Displacing bedding material towards the gloved hand with alternating forward pushing movements of their forepaws and shovelling movements of their heads.	Voluntary interaction with hand
Eating or drinking	Consuming food or water. The animal rears up and licks the nozzle of the drinker or gnaws at food pellets through the bars of the food hopper.	Homecage
Grooming (caudal)	Self-cleaning of the body, legs and tail/genitals.	Voluntary interaction with hand and Homecage
Grooming (rostral)	Self-cleaning of the paws, snout and head.	Voluntary interaction with hand and Homecage
Kick digging	Displacing bedding material with fore paw movements alternated by backwards kicking of both hind legs simultaneously.	Voluntary interaction with hand and Homecage
Location: close to hand	Mouse is in the half of the cage closer to the hand, relative to the midline of the cage.	Voluntary interaction with hand
Location: far from hand	Mouse is in the half of the cage further to the hand, relative to the midline of the cage.	Voluntary interaction with hand
Nesting	Manipulating nesting material	Homecage
Paws on hand	Mouse places one or both paws on the gloved hand.	Voluntary interaction with hand
Rearing	Standing upright on hind legs, without the two front paws touching any surfaces.	Voluntary interaction with hand and Homecage
Sleeping or resting	Lying immobile for at least 5 seconds.	Homecage
Sniffing cagemate (anogenital)	Rapid twitching movements of the nose towards the anogenital area of another mouse.	Voluntary interaction with hand and Homecage
Sniffing cagemate (body)	Rapid twitching movements of the nose towards the head or body of another mouse.	Voluntary interaction with hand and Homecage
Sniffing hand	Rapid twitching movements of the nose towards the gloved hand, with the nose at < 1 body length from the gloved hand.	Voluntary interaction with hand
Vocalizing	Emitting vocal sounds audible to humans.	Voluntary interaction with hand and Homecage
Wall rearing	Standing upright on the hind legs and resting one or both front paws on a cage wall.	Voluntary interaction with hand and Homecage

Table 1. Ethogram of the behaviour recorded.

The behaviours are adapted from an existing mouse ethogram.[27] The behaviour categories are arranged in alphabetical order and the stage at which they were recorded is given. *Other escape or stereotypic behaviours (circling, jumping, barbering and somersaulting) were included but are not shown here because they were never observed.

Statistical analysis

IBM SPSS Statistics 22 was used to perform the statistical analyses. Cage was the experimental unit. Behavioural data across the 15 second time intervals were summed for each cage at the relevant time points. A mean handleability score was calculated per cage.

Normally distributed data were analysed using a Linear Mixed Effects Model with Treatment, Strain, Sex and Week set as fixed factors and Cage as a random factor. The two-way interactions between Treatment and Strain, Treatment and Sex and Treatment and Week were initially included but removed if not statistically significant. If interactions were statistically significant, post-hoc pairwise comparisons were conducted to discover which categories the significant differences lay between. Normality was checked by visual inspection of the histograms of the residuals of the models and by carrying out Kolmorov-Smirnov and Shapiro-Wilk tests of normality. When normality assumptions were not met, the original outcomes were transformed and assumptions checked again. If the residuals did not fit a normal distribution after transformation, the relevant behaviour was converted to a binary variable (presence/absence of the behaviour). Binary responses were analysed using a Generalised Linear Effect Mixed Model (Binary Logistic Regression) with the same random and fixed factors as described above. The standard errors of the resulting coefficients were checked for inflation that could indicate multicollinearity.

As both observers recorded the location of mice during voluntary interaction, an interrater reliability analysis using the Kappa statistic was performed to determine consistency among raters. The interrater reliability was 'substantial' (Kappa = 0.697; P <0.001)[28], allowing only one set of observations (the one with fewer missing values) to be analysed for this variable.

Results

Statistically significant effects are shown in Table 2. In the hand interaction test, the sanitiser significantly increased wall-rearing (during Week 1), self-grooming and – in BALB/c mice – sniffing and grooming of the cagemate (Figure 2).

There were no significant effects observed during handling itself and urination/defecation during handling was recorded three times only, in BALB/c mice (twice with sanitiser and once with the control). Only two mice squeaked or bit with sanitiser versus three with control gloves. Six mice struggled vigorously with sanitiser, versus three with the control.

Immediately upon return to the home-cage, aggression frequency showed a statistically significant reduction after handling with sanitised gloves compared with control gloves, being observed at least once in four cages immediately after handling with sanitiser, versus seven

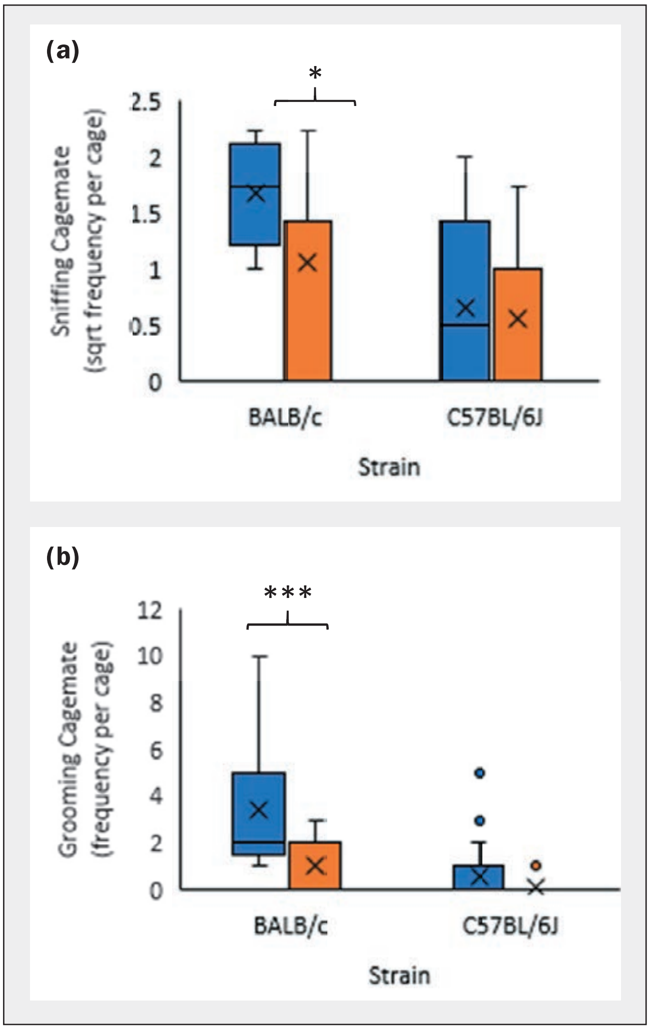

Figure 2. Treatment and strain effects on social behaviour in the hand interaction test. Use of hand sanitiser (blue bars) when handling mice increased (a) sniffing the cagemate and (b) grooming the cagemate compared with controls (orange bars). This reached statistical significance in BALB/c mice: *P<0.05; ***P<0.001. X indicates mean values, the boxes indicate the inter-quartile range, and the whiskers indicate the 95% range.

cages in the control treatment. This difference disappeared 20 minutes later. Handling with sanitiser increased food/water consumption in C57BL/6J mice at the 20 minute time point. Bar-biting was seen at least once in five of the cages immediately after handling with sanitiser, compared with just one cage after control handling but this difference was not statistically significant (P = 0.343).

Some of the effects were interactive, reaching statistical significance in only one of the mouse strains or sexes. Alcohol spray significantly increased wall rearing and home-cage eating/drinking compared with the control in C57BL/6 mice only. In the case of caudal grooming 20 minutes after being returned to the home-cage, the sanitiser seemingly masked a sex difference whereby males groomed more than females only in control conditions.

Handling stage	Behaviour affected	Effect direction	Odds ratio/ †Coefficient (95% CI)	Statistic	P-Value
Hand interaction before handling	Wall rearing	Sanitised > Control only during the first week of the study (statistical interaction)	†5.842 (1.432-10.252)	$F^{3, 76} = 5.281$	0.002
Hand interaction after handling	Sniffing of cage-mate	Sanitised > Control in BALB/c only (statistical interaction)	†0.645 (0.061-1.228)	$F^{1, 80} = 4.836$	0.031
	Grooming of cage-mate	Sanitised > Control in BALB/c only (statistical interaction)	†2.903 (1.453-4.354)	$F^{1, 79} = 15.869$	<0.001
	Self-grooming	Sanitised > Control	†0.660 (0.370-0.949)	$F^{1, 81} = 20.557$	<0.001
	Proportion of rostral versus total grooming	Sanitised > Control in C57BL/6J only (statistical interaction)	†0.142 (0.005-0.278)	$F^{1, 80} = 4.276$	0.042
	Wall rearing	Sanitised > Control in the 1st and 3rd weeks only (statistical interaction)	†Week 1: 5.459 (1.770-9.149); Week 3: 4.904 (1.214 - 8.594)	$F^{1,78} = 3.594$	0.017 (Week 1: Post-hoc P = 0.004; Week 3 Post-hoc P = 0.010)
Home-cage immediately after return	Aggression (seen in males only)	Control > Sanitised	0.251 (0.109-0.574)	$F^{1, 58} = 11.605$	0.001
	Self-grooming (caudal)	Sanitised > Control	2.318 (1.440-3.732)	$F^{1, 58} = 9.141$	0.004
Home-cage 20 minutes after return	Self-grooming (caudal)	Males > Females only in Control conditions (statistical interaction)	2.921 (1.366-6.245)	$F^{1, 58} = 4.737$	0.034 (Post-hoc P < 0.001 in females; P = 0.014 in males)
	Consumption of food/water	Sanitised > Control only in C57BL/6J (statistical interaction)	3.142 (2.106-4.688)	$F^{1, 57} = 4.467$	0.039 (Post-hoc P < 0.001)

Table 2. Statistically significant differences in behaviour between mice when handled using gloves sanitised with 70% alcohol *versus* unsprayed gloves.

†Indicates that, rather than an odds ratio, a coefficient from a Linear Mixed Effects Model is provided, i.e. where the response was able to produce normally distributed residuals. The Control treatment was used as the reference category, so when a behaviour increased with the alcohol spray, the odds ratio is >1 and when a behaviour decreased with alcohol, the odds ratio is <1. The post-hoc p-value is given for statistically significant pairwise comparisons when overall two-way interactions were statistically significant.

Some of the behaviours measured were also significantly affected by mouse strain, sex and week (see supplementary Table S1). For example, BALB/c mice contacted the glove more and were less aggressive towards each other, compared with C57BL/6J mice; the same was true for female mice compared with males. Across strains and sexes, the frequency of defensive burying during the hand interaction test declined week by week (Before handling: $F_{3, 81} = 3.433$, $P = 0.021$; after handling: $F_{3, 81} = 3.379$, $P = 0.022$).

Discussion

Our aim was to evaluate whether handling mice after use of an alcohol-based hand sanitiser acutely affects mouse behaviour and welfare. The results suggested that mice could perceive the sanitiser and it affected their behaviour but there were no clear acute effects on their welfare or on handleability. The effects of the sanitiser at each stage of the experiment are discussed in turn, and then alternative hand-sanitisers are briefly explored, but more research is needed.

Voluntary interaction with the hand

Although the mice showed no significant avoidance of sanitised gloves, the increased frequency of wall-rearing both before and after handling in the sanitised treatment during the first week, suggests that mice could smell the alcohol and were responding to it. Rearing can occur in response to threatening[29] or novel[30] stimuli. We had attempted to avoid interference from novelty by handling the mice with the alcohol-based sanitiser the week before the experiment but the fact that the treatment effect occurred mainly during the first week (the second time of exposure) suggests that novelty still played a role at that time point. Defensive burying also occurred during the hand interaction on the first week, regardless of treatment, but declined thereafter, suggesting that novelty of the test situation itself affected behaviour. However, the sanitiser effect on wall-rearing became significant again (after handling) during Week 3, so novelty was perhaps not the only cause, even if it did contribute.

After handling mice with sanitised gloves we observed increased self-grooming and – in BALC/c mice – sniffing of cagemates and allo-grooming, suggesting that mice could detect residual alcohol on themselves and on other mice. The increased grooming could have occurred for several reasons. It could serve to re-establish each mouse's own odour by removing the alcohol odour. However Kemble *et al.* (1995) showed that neither chocolate odour, nor sheep's wool odour, increased grooming in mice, so it is not the case that mice groom all foreign odours away.[31] Alternatively, the grooming could be a displacement behaviour in response to anxiety or aversion to the alcohol.[32-34] Anxiety-related grooming would usually be identifiable as a disruption of the normal cephalocaudal progression of the grooming process and this is supported by the increase in the relative proportion of rostral grooming with sanitiser in the C57BL/6J mice.[32] Finally, it is worth considering whether mice increased grooming in order to ingest the alcohol, especially in the C57BL/6J mice because that strain has a preference for drinking (sweetened) ethanol over other fluids.[18, 23, 24] Observation of the facial expression microstructure when grooming could confirm whether mice were gaping (as with unpalatable substances), or lip-licking (as with palatable ones)[35] and whether they showed 'taste rejection behaviour'.[36]

The increased sniffing and grooming is of potential concern because it makes it highly likely that the mice were both inhaling and ingesting some alcohol. The long-term effects of this are unknown, especially in animals that are frequently handled using sanitised gloves. Rats administered 1.0g/Kg bodyweight of methylated spirits on alternate days for 4 weeks showed increased plasma aldosterone and lost significantly more sodium in their urine, compared with controls, which indicates renal damage; this was despite showing no significant behavioural changes.[37] In humans, consumption of methylated spirits can cause signs of intoxication as with ethanol, along with methanol-induced metabolic acidosis, which causes impaired vision, neurological damage and even death.[38] However, this acidosis does not occur in mice or rats, because their metabolism of methanol does not cause accumulation of folate.[19,20] The implications of this for the long-term effects of mice inhaling and consuming small doses of alcohol-based sanitiser are unclear.

Home-cage behaviour after handling

Grooming continued in the home-cage, with caudal grooming still being almost significantly more frequently observed even 20 minutes after handling with sanitised gloves than with the control. In the 2 minutes after the cage was returned to the rack, the sanitiser significantly reduced the frequency of aggression. This could be an artefact of increased grooming. However, in a previous study, even without increased grooming, male mice rubbed with novel odours (chocolate or sheep's wool) showed decreased aggression in a resident-intruder test compared with mice rubbed with familiar sawdust.[31] Masking of male mouse scent, even without the distraction of grooming, can therefore sometimes reduce aggression. Aggression is an important welfare problem in male mice, which is notoriously difficult to manage.[39,40] Refining husbandry procedures to reduce aggression would greatly improve mouse welfare, avoiding injuries derived from agonistic interactions, reducing chronic social defeat stress and preventing the need to separate and isolate aggressive males. However, even though the sanitiser reduced immediate aggression frequencies in the current study, the effect disappeared after 20 minutes, so we cannot know whether aggression was prevented or merely delayed. To better understand the impact of alcohol-based sanitiser on social dynamics, it would be interesting to measure scent marking behaviour (e.g. urination frequency and patterning) associated with the establishment of hierarchies in group housed male mice.[41,42]

Lastly, the increase in feeding/drinking observed in C57BL/6J after being handled with sanitised gloves could indicate that the mice settled to normal behaviour more quickly than when handled with control, unsprayed gloves. Alternatively, it could have been a displacement activity. For example, after acute restraint, rats increased their drinking behaviour in the first 15 minutes, followed by increased feeding.[43] Furthermore, if the alcohol had tasted bitter to the mice during grooming, eating/drinking could have served to rid the mice of the unpalatable taste, and the bitterness would have increased salivation, so it could have increased thirst.[36] Again analysis of the microstructure of the oral behaviours could help elucidate whether the mice were gaping as they do with bitter substances.[35,36]

Alternative hand sanitisers

The current study appears to be the first investigation of the behavioural effects of hand sanitisers in an applied context on animals, so if users wish to avoid the behavioural effects of methanol-ethanol-based sanitisers, it is difficult to suggest an alternative at present. Alcohol-based sanitisers that contain isopropanol and/or n-propanol instead of methanol are unlikely to be beneficial because toxicity tests reveal that adverse effects occur at lower doses (reviewed in Patocka J and Kuca K. 2012).[44]

Many alcohol-free hand sanitisers also exist. A UK survey revealed that at least seven different hand sanitisers were used for handling laboratory mice across 51 different facilities, with 46% of respondents reporting generically that they used 'soap', followed by 24% reporting that they used Hibiscrub™ and 12% reporting 'alcohol'.[3] Hibiscrub™ is thus the most widely reported single brand of sanitiser reported for mouse handling in the UK. Its active ingredient is chlorhexidine gluconate (4.0%) but it also contains a low concentration of n-propanol (4.0%) as a solvent. Whilst use of 80% ethanol as an antiseptic on the ear of an allergic dermatitis mouse model significantly worsened inflammation of the skin, use of 0.5% chlorhexidine gluconate showed no significant difference from the control which suggests that the latter may be less irritant.[45] No compound will be entirely free of adverse effects, depending on factors such as dose, form and characteristics of the animals themselves. For example, chlorhexidine compounds can occasionally cause allergic reactions in humans and are irritants of the eye in humans and rabbits at least (reviewed in[46]) whilst another alcohol-free alternative, benzalkonium chloride, is an irritant of the eyes, skin and mucosa of many species (including some limited data on mice reviewed in[47]).

Further research will be necessary to ascertain, under treatment-blind conditions, the hand sanitiser that causes least harm to animals whilst being effective, practical and safe for humans. In the meantime the current results lead us to make the following recommendations. Researchers and other staff working with animals should:

- Consider whether hand sanitisers need to be used at all, given that some gloves are initially sterile when first worn and/or, given that mouse immune systems are more normal with a full microbiome.[4,5]
- If alcohol-based sanitisers are required, wait as long as feasible for alcohol to dry before handling mice.
- When using a hand-sanitiser for rodent handling, monitor the effects on animals carefully, initially checking at the very least for avoidance, defensive behaviour, excessive grooming and effects on aggression. As several products have irritant properties, it will also be necessary to monitor skin condition and scratching behaviour, eye irritation such as excessive blinking and respiratory signs such as nose-rubbing and sneezing.

Conclusions

This experiment suggested changes in mouse behaviour resulting from sanitising gloves with 70% alcohol. Although the reduction in aggression when the cage was returned to the rack could be interpreted as a positive finding, it was temporary and is likely to be the result of the increase in grooming to remove the sanitiser. Finally, the increased sniffing and grooming following alcohol sanitisation implies that the mice both inhaled and ingested some of the methylated spirits. Rodents are less sensitive to methanol than humans are, so long-term effects may be negligible but are currently unknown. Further work should be carried out under treatment-blind conditions to investigate the suitability of alternative glove sanitisers with different mouse strains using longer term animal health and welfare indicators.

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