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Identification of Rodent Husbandry Refinement Opportunities through Benchmarking and Collaboration

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Expanding the use of methods that refine, reduce, and replace (3Rs) the use of animals in research is fundamental for both ethical and scientific reasons. The mission of the 3Rs Translational and Predictive Sciences Leadership Group (3Rs TPS LG) of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ Consortium) is to promote sharing and integration of science and technology to advance the 3Rs in the discovery and development of new medicines, vaccines, medical devices, and health care products for humans and animals. The 3Rs TPS LG is dedicated to identifying opportunities for member companies to share practices, enhance learning, promote discussions, and advance the 3Rs across the industry. One such opportunity was a benchmarking survey, conducted by the Contract Research Organization (CRO) Outreach Working Group, designed to share practices in rodent husbandry for drug safety research and to identify potential opportunities for refinement. IQ member companies and CROs in Asia, North America, and Europe were surveyed. Areas identified for potential alignment included provision of corncob bedding and wire-grid flooring, management of the nest at cage change, approaches to social housing for male mice, evidence-based enrichment strategies, and evaluating the effects of the timing of studies in relation to the animals' circadian rhythm and light-cycle, with consideration for how such extrinsic factors influence animal welfare and scientific outcomes. This manuscript presents the results of the benchmarking survey, including general trends in mouse and rat husbandry practices in toxicology studies, considerations for social housing, enrichment selection, and potential effects of bedding substrate, emphasizing opportunities for collaboration that can help to identify refinements to rodent husbandry practices.

Abbreviations: 3Rs, refine, reduce, and replace; 3Rs TPS LG, 3Rs Translational and Predictive Sciences Leadership Group; CRO, Contract Research Organization; IQ Consortium, International Consortium for Innovation and Quality in Pharmaceutical Development.

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Introduction

The expansion of methods that refine, reduce, and replace (3Rs) the use of animals in research is important for both ethical and scientific reasons. The 3Rs Translational and Predictive Sciences Leadership Group (3Rs TPS LG) of the International Consortium for Innovation and Quality in Pharmaceutical Development (IQ Consortium) was established in 2012 as an association of senior veterinarians, scientists, and 3Rs specialists from IQ member companies, which include both pharmaceutical and biotechnology companies. The group meets regularly to discuss common scientific and regulatory issues and topics surrounding the use of animals in research and testing. The mission of the 3Rs TPS LG is to promote sharing and integration of science and technology to advance the 3Rs in the use of animals in the discovery and development of new medicines, vaccines, medical devices, and health care products for humans and animals. The group is dedicated to identifying opportunities for member companies to share practices, enhance learning and promote discussions that will advance the 3Rs across the industry. Over the past 8 y, the group has grown to over 30 member companies and has produced several deliverables aimed at advancing animal welfare and the 3Rs.

The Contract Research Organization (CRO) Outreach Working Group was formed as an initiative of the 3Rs TPS LG to allow IQ member companies to partner with CROs to strengthen collaboration in the application of the 3Rs and integrate 3Rs principles into scientific efforts. The 3Rs TPS LG recognizes that a significant percentage of animal research conducted on behalf of IQ Consortium members is contracted to business partners in the CRO industry. The CRO Outreach Working Group fosters high standards of scientific excellence and animal welfare with a focus on continued advancement in application of the 3Rs. IQ member company representatives work with CRO partners to identify opportunities for implementing 3Rs principles, examining, evaluating, and enhancing optimal 3Rs practices, and promoting refinement of animal use across the field of drug discovery and development. To this end, a benchmarking survey was designed to survey and share mouse and rat husbandry practices in toxicology studies for potential alignment, including provision of corncob bedding, use of wire-grid flooring, manage-

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ment of the nest at cage change, approaches to social housing of male mice, enrichment strategies, and evaluating the effects of the timing of studies in relation to the animals' circadian rhythm and light-cycle, and consideration of extrinsic factors that may influence animal welfare, scientific outcomes, and thus research rigor and reproducibility. The survey was intended to identify general trends in rodent husbandry practices, considerations for social housing, enrichment selection, and potential impact of bedding substrate with emphasis on the need for additional collaboration to identify opportunities for refinement and industry alignment.

Methods

The survey was divided into several categories (see Figure S1). Demographic information included company type, vivarium location, rodent census, biocontainment level, the role of the respondent, and organizational registrations, certifications, and accreditations. Much of the survey differentiated between husbandry practices for mice and rats. Information collected regarding housing included specifics regarding rodent cage space/density, strategies for social housing, addressing cage mate attrition, and the use of caging with wire-grid flooring. Several questions specific to cage changes included frequency of cage change, types of bedding substrates, and transfer of bedding substrates at cage change. Approaches to enrichment included queries on types of enrichment devices, type of nesting material for mice, and management of nesting material during study-specific animal observations and at cage change. The survey also queried approaches, strategies, and resources for assessing impact of enrichment and behavioral changes resulting from enrichment. Finally, the survey included questions about respondent attitudes toward animals and how these attitudes influenced their view of their company's commitment to animal welfare. Due to its breadth and scope, the survey focused on practices specific to rodent toxicology studies.

The survey was administered through Survey Monkey and was distributed by the IQ Consortium Secretariat (Drinker Biddle and Reath LLP) by email in August 2018 to companies in the IQ Consortium's 3Rs TPS LG and CRO Outreach WG; the distribution list included 34 global companies, of which 32 were pharmaceutical companies and 2 were CROs. Completion of the survey was voluntary, and the recipient organization selected the individuals who completed the survey. The IQ Consortium Secretariat received the responses and compiled them in an anonymous manner into a master Excel spreadsheet.

Descriptive statistics were used to summarize survey data, which consisted of summary means of single variables and the percentage of responses. Percentages were calculated based on the total number of respondents or sites per question, depending on the question format (see Figure S1). For questions for which respondents could choose more than one response option, percentages were calculated based on the overall total number of respondents or sites ($n = 32$). For questions in which respondents could only choose one response option, percentages were calculated based on the total number of respondents per question. Response options for "Other (specify in comments)" were excluded from the count of total number of respondents and comments describing those responses are reported in Figure S1.

Results

Demographic information. Responses were received from 32 sites representing 22 companies (20 in North America, 8 in Europe, and 4 in Asia). Of these 32 sites, 65% identified as pharmaceutical companies, 25% as CROs, and 19% as biotechnology companies. In addition, 93% of the sites identified as AAALAC International or Canadian Council on Animal Care (CCAC) accredited facilities with a rodent census of less than 20,000. Of the 32 sites, 45% perform studies under the Food and Drug Administration's Good Laboratory Practice for Nonclinical Laboratory Studies (GLP) and 19% under the Good Manufacturing Practice (CGMP) Regulations. Study types run by these facilities include single-dose and repeat dose toxicology (78%), toxicokinetics (69%), local tolerance (44%), reproductive and developmental toxicology (38%), genetic toxicology (35%), and carcinogenicity (31%), among others. Despite our intention to limit responses to husbandry practices used for toxicology studies, respondents also referred to breeding and efficacy studies under study type; this should be considered with regard to context when evaluating the survey results. Finally, respondents' roles ranged widely and included facility managers, veterinarians, veterinary technicians, veterinary health technicians, regulators, scientists, Institutional Animal Care and Use Committee (IACUC) Coordinators, and Behaviorist/Enrichment Coordinators. Nearly half of the respondents self-identified as holding more than one role with the company. Roles held include veterinary support (45%) (that is, veterinarians, veterinary technicians, and veterinary support staff), facility manager (33%), scientist (24%), and IACUC Coordinator or member (45%).

Default housing strategies. Survey respondents across North America, Europe, and Asia reported that pair or group housing was the primary default housing strategy for mice and rats (Figure 1). Default single housing was reported by 1 respondent in Asia for both mice and rats and by 1 repondent in North America for mice only. Respondents reported that rats are most commonly housed at a density of 3 animals per enclosure (70%), while mice are most often housed at a density of 5 animals per enclosure (63%), although social housing densities ranged from 3 to 10 rats and up to 10 mice per enclosure. The respondents' reasons for the default housing density included study size and scientific justification. Study type requirements such as the need to monitor individual food consumption and the presence of interanimal aggression were the main justifications provided for social housing exemptions. In addition, 2 respondents indicated that single housing of male mice was common practice due to social incompatibility.

Cage mate attrition was addressed similarly for both mice and rats, with a near even occurrence of rodents either remaining singly housed indefinitely (47% of facilities housing mice; 50% of facilities housing rats) or a practice of compatible cage mates continuously being sought (40% of facilities housing mice; 44% of facilities housing rats). Some respondents commented that male mice remained single-housed whereas efforts were made to find compatible cage mates for female mice. One respondent also mentioned the age of rats and 2 respondents mentioned study duration as the rationale for the housing strategy after cage mate attrition. Few facilities reported having an established policy that limits the period of single housing in mice (19% of facilities) or rats (16% of facilities).

The standard bedding substrates used by survey respondents and across geographic regions are shown in Figures 2A and 2B. Rationale for bedding selection included animal welfare, facility resources, historic data, scientific or performance needs, regulatory requirements, published literature, and cost. One respondent reported that their organization did not use corncob bedding because it contained contaminants that could potentially affect both animal welfare and study outcomes.

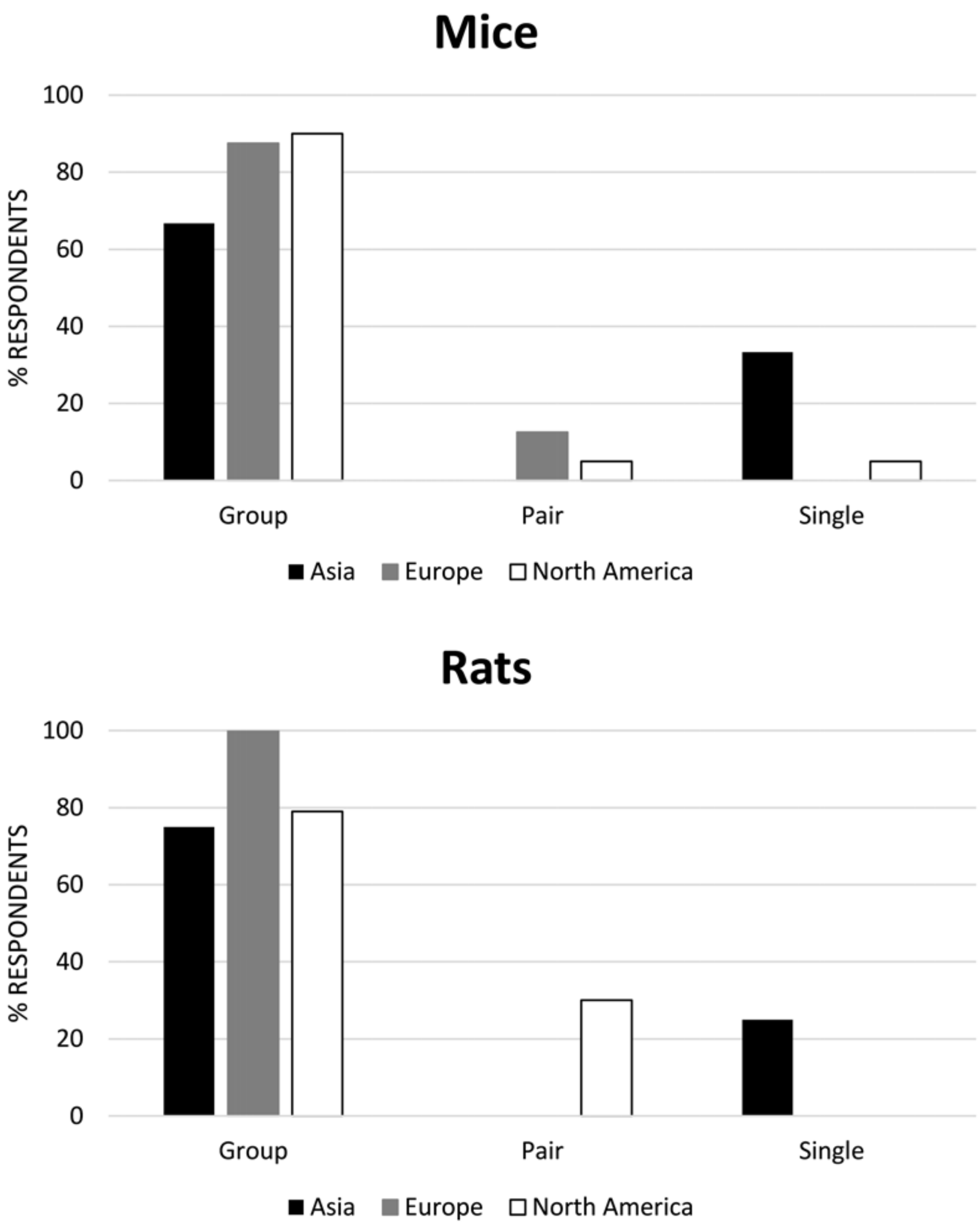

Figure 1. Default housing strategy for rodents by region.

Survey respondents across Asia, North America, and Europe reported the use of suspended wire grid flooring to house rodents (see Figure 3A). Rationale for using this caging included brief duration of housing, cost, metabolism studies, scientific justification, client request, plug monitoring on reproductive studies, and use of radioactive-labeled compounds. Most facilities allowed a maximum duration of 24 h to 1 wk on wire-grid flooring specifically for metabolism studies (see Figure 3B). Fifty percent of respondents that reported using grid flooring provide resting structures for rats, while 43% provide resting structures for mice. Justification for not providing resting structures included study type, potential interference in urine and fecal collection, and use of radioactive compounds. One European respondent reported the refinement of using perforated flooring rather than wire for metabolism studies.

Husbandry practices. In our survey, animal welfare was cited as a driver of cage change frequency for 66% of the survey respondents, with 74% of respondents changing rodent primary enclosures weekly and 26% changing them biweekly. Of the sites surveyed, 41% transfer nesting material at cage change and 56% provide new nesting material, which may or may not be added to the old nest. However, many sites (66%) completely discard the nest at cage change (differences in this practice could occur within facilities based on sex, strain, study type, and breeding status). Facility resources and other factors may be influencing these figures, but 66% of respondents cite animal welfare as a major reason for transferring nesting material at cage change.

When conducting cage-side health and welfare assessments, most survey respondents reported moving the cage until mice leave the nest so that their condition can be clearly assessed (59%), and some only observe mice that were out of the nest (34%) at the time or skip observations on cages in which mice were in nests, assuming that they are healthy (25%). While performing study-specific assessments (that is, a functional

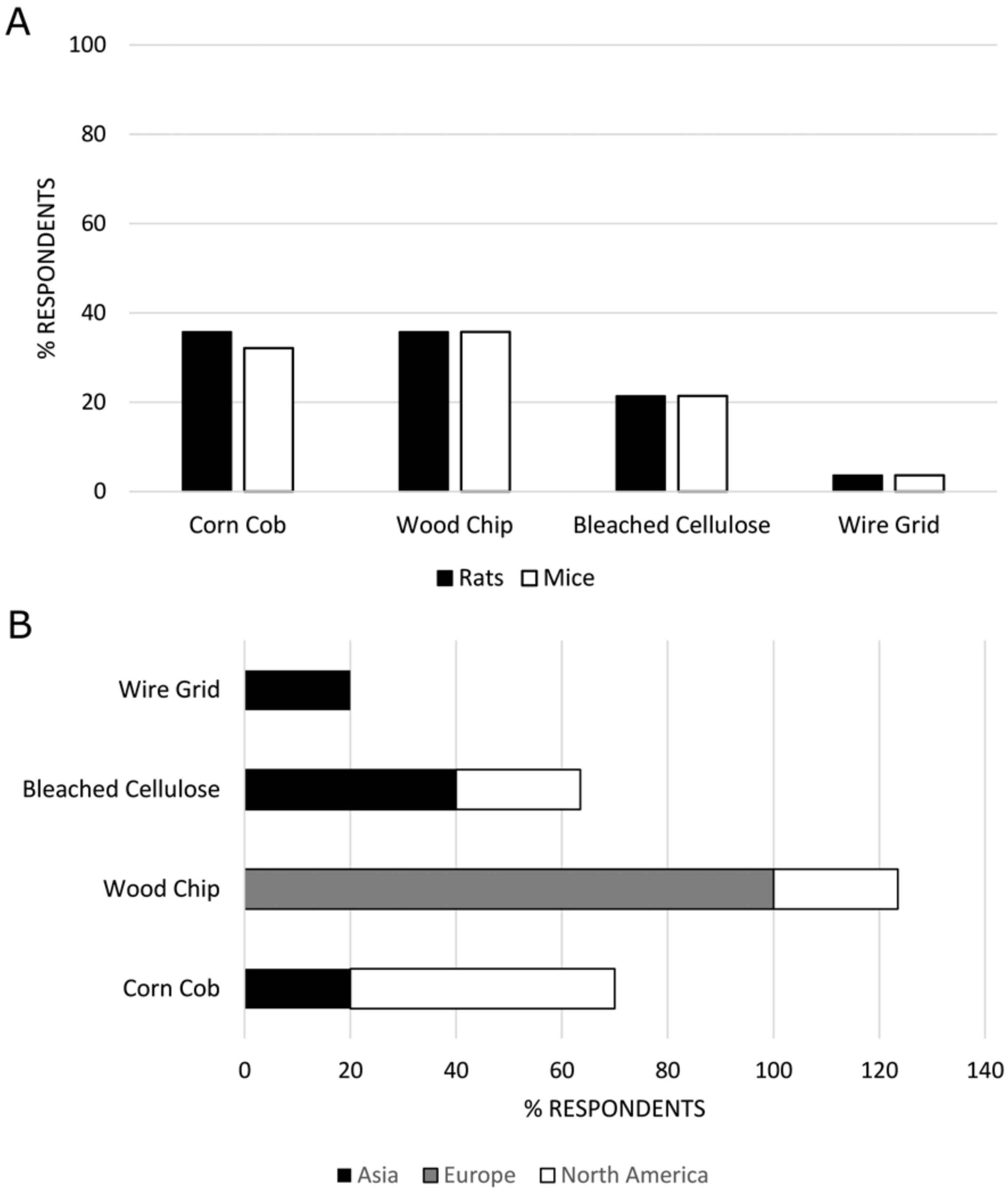

Figure 2. (A) Standard bedding substrates for rodents across all sites. (B) Standard bedding substrates for rodents by region.

observation battery), most observers (62%) move the cage until mice leave the nest and 6% of respondents perform observations only when mice are out of the nest.

Enrichment strategies. All respondents in our survey indicated that enrichment is provided as a standard practice to mice and rats, and 84% of respondents believe they are providing sufficient enrichment to rodents in their programs. Trends in enrichment strategies were also noted (see Figure 4). For example, nesting material was almost universally provided for mice (97%), with social housing (pair or group) being the second most common form of enrichment (81%) for mice. Other frequently reported forms of enrichment for mice were huts, tubes, and items for gnawing. Pair or group housing was reported as a standard form of enrichment for rats by 84% of respondents (see Figure 4). Other frequently reported forms of enrichment for rats include items for gnawing, wood blocks, nesting material, and tubes. The most common rationale for the standard enrichment was reported to be animal welfare (97%), with scientific literature also listed as a rationale by 39% of sites, facility resources by 31% of sites, and regulatory requirements by 28% of sites. Regardless of geographic region or enrichment items provided, animal welfare was almost universally reported to be the primary driver for the enrichment strategies employed.

Evaluation of enrichment strategies varied among the programs. Employment of behaviorists or enrichment coordinators to assess enrichment strategies was reported by 19% of sites, while 50% of sites identified the "animal welfare manager" as the primary individual responsible for assessment

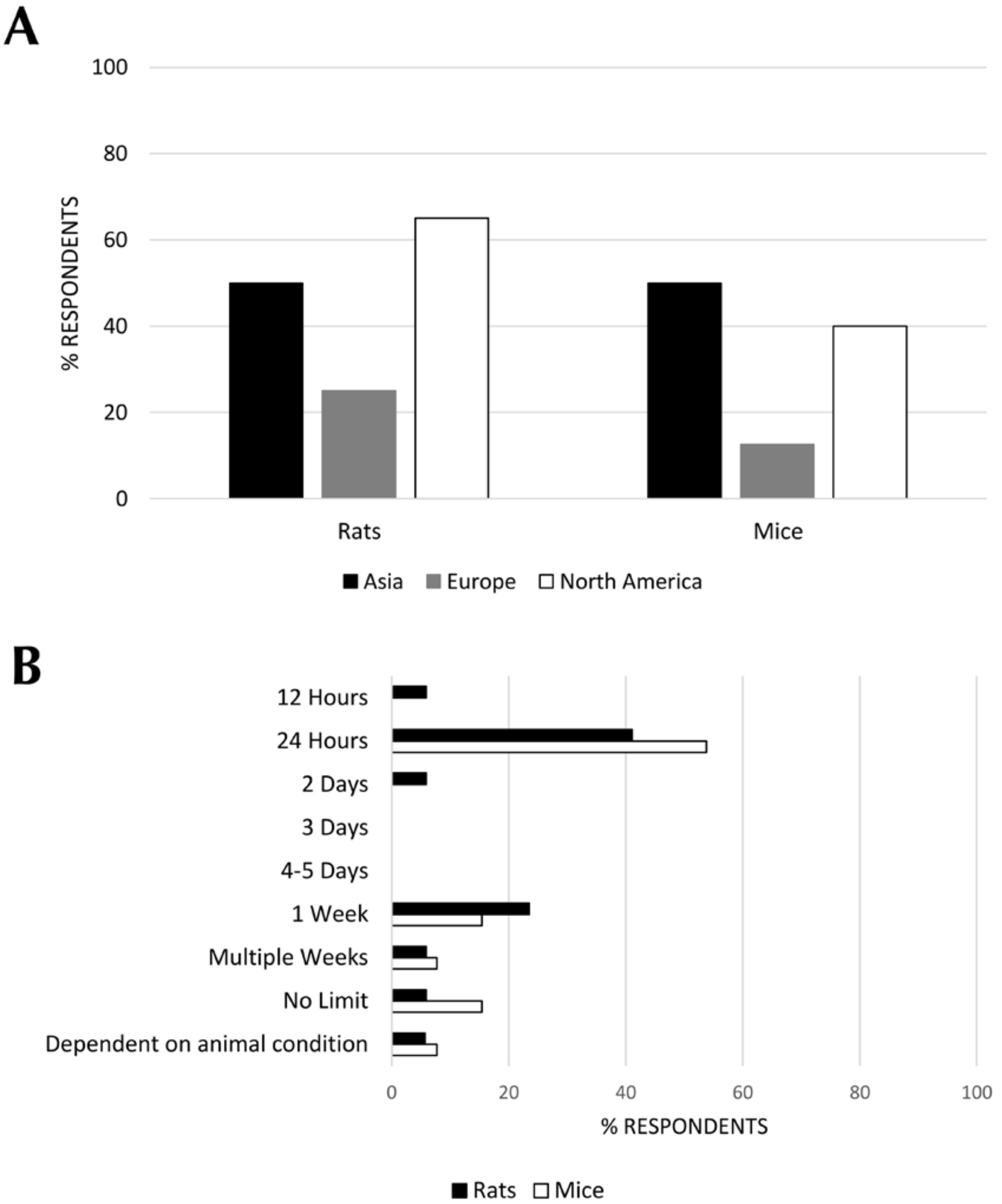

Figure 3. (A) Prevalence of suspended wire-grid flooring use. (B) Duration of use for suspended wire-grid flooring.

of an enrichment program. Regional differences were noted, with 87% of respondents in Europe reporting the use of an animal welfare manager to assess enrichment strategies as compared with 50% of respondents in Asia and 35% in North America. Some respondents (62%) reported that they have a staff member with training, education, or experience specifically in animal behavior. Over half of the survey respondents did not have a documented training program for identifying stereotypic behavior in rodents, with little variation between regions. However, 94% of respondents reported that they feel adequately trained to identify stereotypic behaviors in rodents.

Discussion

The Guide for the Care and Use of Laboratory Animals (*The Guide*) states that social animals should be housed in stable pairs or groups of compatible individuals unless single housing is required for scientific reasons or social incompatibility.[35] Survey respondents reported that they are meeting this expectation by using social housing configurations appropriate for available cage size and floor space requirements. Exemptions to social housing included the need to measure individual food consumption and interanimal aggression. Respondents did not elaborate on the types of studies requiring individual food consumption. However, in our experiences, toxicology studies often measure food consumption using group-housed rodents by using individual body weights and cage-averaged food consumption to evaluate compound-related effects.

Rodent age at animal arrival, grouping, and administration of the first study dose may alter social cohesion. However, specific rodent ages for receipt and study start

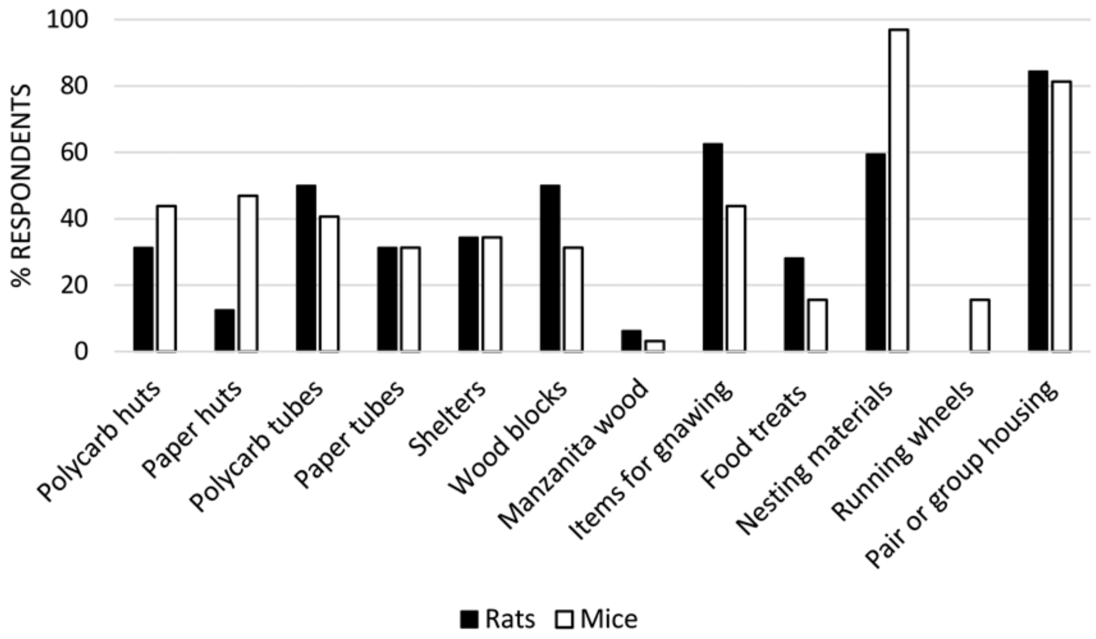

Figure 4. Standard enrichment for rodents across all sites.

in relation to social housing could not be identified from the survey responses due to its design. Housing density is known to affect the level of aggression in socially housed male mice. When directly compared, trio-housed CD-1 mice had fewer bite wounds than pair-housed mice over 14 wk.[19] Housing BALB/c mice at 3 per cage, rather than in groups of 5 or 8, has been recommended to maintain dominance hierarchy.[44] Other studies report increased aggression with trio housing[18] and prolonged success (housing duration of 433 d) with housing 4 C57BL/6 mice per cage if social groups are formed at 3 to 4 wk of age.[38] These studies demonstrate the multifactorial nature of successful social housing of male mice, with strain, cage size, and age at grouping as important determinants.

Corncob has become a commonly used bedding substrate due to its high absorbency and ability to minimize ammonia levels, thus enabling longer cage-change intervals.[21] As noted by one survey respondent, however, corncob has attributes that can confound study outcomes and affect animal welfare. These attributes include the presence of linoleic acid derivatives that act as endocrine disruptors,[31] phytoestrogens that exert estrogenic effects on the central nervous system, induce estrus, and stimulate development of the female genital tract,[41] and endotoxin.[49] *The Guide* identifies phytoestrogens and contaminants, potentially found in diets, as compounds that have the potential to affect experimental results. Rats and hamsters have been reported to prefer other bedding types over corncob.[21,23] This preference may occur because corncob is uncomfortable as a resting surface.[33] This also may account for reduced time in slow-wave sleep in rats when housed on corncob bedding as compared with aspen-chip, suggesting reduced quality sleep and altered pain response.[24,25] Recent studies have shown that pelleted cellulose, refined diced cellulose, and compressed paper perform as well as, if not better than corncob, providing alternative bedding substrates that may improve animal welfare and avoid effects on experimental outcomes.[36,40]

Similarly, suspended wire grid flooring has the potential to affect scientific endpoints and animal welfare. *The Guide* notes that if wire-mesh flooring is used, a solid resting area may help to prevent foot lesions in rodents and rabbits. The size and weight of the animal and the duration of housing are also considerations. Rodents prefer solid floors with bedding over grid or wire-mesh flooring.[35] Based on the survey, use of wire-grid flooring remains an ongoing opportunity for refinement.

Rodent cages should be changed at a frequency that preserves intracage air quality and minimizes microenvironmental disruption, allowing the animals to maintain separate spaces for living, nesting, and soiling areas.[5,35,47] Cage changes disrupt the odor cues that rodents use to maintain their social hierarchy and can therefore lead to increased activity and aggression.[45] Prolonging the cage change interval by spot cleaning has been correlated with reduced aggression, but more research is needed to guide the practice.[26] *The Guide* suggests that determination of cage change frequency be made based on professional judgement but offers the recommendation that solid bottom rodent caging be changed once weekly, while mechanically forced air changes provided by ventilated caging can extend the cage change interval to 2 wk without adversely affecting animal health.[8,35,38,39] However, while ventilated caging may improve air quality, other factors such as cold stress may be intensified, leading to welfare issues and physiologic changes that may affect study outcomes.[7] Wire lids and other accessories should also be changed biweekly.[35] Determination of cage change frequency depends on several factors including sex of the animals, housing density, type and volume of bedding material, occupational health, research objectives, caging type, etc. Most respondents change their cages weekly, with animal welfare as the primary driver. Each facility has unique animal populations, regulatory environments, research agendas, and caging inventory, and each of these factors likely contribute to cage change frequency. We did not collect specific details related to cage change from respondents; this information would be necessary to fully examine the justification for each facility's cage change frequency. However, if animal welfare is the sole driver of cage change frequency, current literature suggests that cages can be changed less frequently than once a week.[1,4,26,39,48]

Mice are often housed in same sex groups that create organized social hierarchies based to a great extent on olfactory cues. These social hierarchies rely on pheromones present in the urine and other glandular secretions.[43] The importance of maintaining olfactory cues has long been used as the rationale for transferring some component of the old cage (bedding, nesting material, and or enrichment devices) over to the new cage.[46] The rationale for this practice is that olfactory cues from the old cage will be present in the new cage and thereby prevent aggression triggered by cage change. While a logical hypothesis, only the transfer of the old nest to the new cage has been consistently shown to reduce aggression in mice.[26,43] The majority of survey respondents completely discarded the nest at the cage change, citing animal welfare as a factor. This rationale offers opportunities to educate by sharing more recently published research findings on transfer of nesting material[46] and to implement a refinement that could reduce stress and mitigate excessive aggression due to social dominance. Unlike the nest, transferring dirty bedding material increases aggression.[26,45] In alignment with the current literature, the majority of survey respondents do not transfer dirty bedding to the new cage during cage changes. However, one respondent stated "If animals are showing aggression, we will try transferring bedding at cage change before resorting to single housing." This statement seems to reflect divergence from the current literature and could present an opportunity for refinement.[46]

The aim of enrichment as described in *The Guide* is "to enhance animal well-being by providing animals with sensory and motor stimulation, through structures and resources that facilitate the expression of species-typical behaviors and promote psychological well-being through physical exercise, manipulative activities, and cognitive challenges according to species-specific characteristics."[35] How animals use enrichment may provide important information about their health and welfare. For example, when mice are thriving, they will instinctively make a robust and complex nest.[17] Nest complexity has been used as a measure of health and welfare.[12] Nest building is an important activity for rodent thermoregulation.[13] By encouraging species-specific behavior, and in an effort to minimize stereotypic and repetitive behaviors, environmental enrichment may also result in more reliable and replicable scientific data.[9] Despite these well documented benefits, nests and other forms of enrichment often increase the structural complexity of the cage and thereby impede visualization of the animals. This is a major factor for husbandry and research staff who are conducting visual assessments. In a recent study, nesting material did not impede the ability to perform routine cage-side health observations, and mice that died spontaneously were more often found outside of the nest.[3] However, to obtain a full view of the animals, the cage and or the enrichment must be moved. This disruption may lead to aggression, interruption of sleep, and detrimental effects on health.[6,10,37]

The responses we received indicate that greater consideration is given to visualizing the animals than to minimizing cage disruption. Some respondents attempt to gently encourage the mice to come out of the nest, keeping the nest intact and disrupting the nest as little as possible. Others do not provide nesting material at all, which is a poor option for animal welfare purposes as mice rely on nesting for thermoregulation.[11] Most respondents seem to apply a similar philosophy with regard to study-specific observations (that is, functional observation battery [FOB]). Most observers move the nest in order to observe the mice. Only a small number apply their FOB only to mice that are outside of the nest. In addition to disrupting the nest or not providing nesting, arousing rodents from sleep to conduct observations or behavioral assessments could affect animal welfare and behavior and may also affect study data. Because most rodents are nocturnal, observation of their full behavioral repertoire would be best done during the dark phase of the diurnal cycle as rodents are often huddled in a nest and asleep during the light phase.

One approach to this issue is to house rodents in rooms on a modified or reversed light cycle that allows behaviors to be observed during normal wake periods, unaffected by sleep interruption and/or fatigue. Daytime manipulations of rodents, whether husbandry-related or experimental, is stressful and can have a negative impact on both data quality and animal welfare.[16] None of the survey respondents indicated that they house their animals on a reverse light cycle. Perhaps this is an opportunity for refinement, but more research would be required to compare the impact of running studies at a completely different point in the animal's circadian cycle. Animal facility routines, including cage side observations and transfer of nesting material, should be well-established and controlled when planning and implementing research studies to avoid causing distress that leads to poor animal welfare and consequently, poor experimental results. Choosing enrichment options appropriate for the species can be challenging, especially for mice. Regulatory and accreditation standards set general expectations for provision of enrichment,[35] but the details can vary. In our survey, 84% of respondents believe they are providing sufficient enrichment to the rodents in their programs, which appears to conflict with results of a recently published survey in which most laboratory animal personnel reported a desire to provide more enrichment.[22]

Many enrichment options for mice are available, such as plastic huts, hiding shelters, paper structures, and materials for nesting and shredding. While some of these items can offer shelter, assist with thermoregulation, and possibly encourage nesting behavior, there are some disadvantages to placing these devices in the cage.[7] While more pronounced in certain strains, 'fighting' or aggression, especially in male mice, is a normal hierarchal behavior. In fact, the absence of fighting may be abnormal.[45] Male mice will display many postures of aggression and submission and spend a great amount of time marking and defending territories; this may ultimately lead to more aggressive encounters and severe wounding of other mice in the cage.[14,15,27,28,32] Our survey results indicated that use of paper huts, polycarbonate huts, polycarbonate tubes, and paper tubes was quite common. However, when objects such as shelters are placed in a cage (specifically with males), the mice may regard these as a physical territory. The addition of specific caging enhancements may therefore increase the potential for fighting and further aggression.[18,20,30,43,45] Thus, consideration of strain, sex, and enrichment type, together with careful monitoring of intracage dynamics should be an integral part of enrichment practices.

Our survey showed that provision of nesting material was almost universal in mouse cages (none of the facilities increased room temperature to accommodate thermoregulation). Proper nesting is critical for mouse behavioral and clinical health. Nesting is the optimal form of enrichment for mice and should be plentiful enough for the mice to construct a proper nest (recommended amount: 8+ g).[2] Nesting allows mice to thermoregulate, especially in cages in which they have no control over their environment (for example, in individually ventilated cages [IVCs]) in which frequent air changes can potentially create a 'windy' environment.[7] A healthy mouse has the drive and ability to build a proper nest, which, depending on strain, should consist of a dome like structure with multiple entry and exit holes.[17] Mice will attempt to organize their living space so that the nest and resting place are in a different location from urine and feces.[29] Ideally, the nest is kept free from urine and feces, providing a place for mice to properly thermoregulate.[12] In essence, the nest is the 'safe place.'[2] Mice need long stranded paper or wood substrate to construct a proper nest.[12] Cotton, hemp, or other organic nesting material can be added, but are relatively short fibers and are not always adequate on their own to support the construction of a fully domed nest with multiple entries/exits.[17]

Evaluation of enrichment strategies varied among the programs that participated in this survey. Some respondents reported the employment of behaviorists or enrichment coordinators to assess enrichment strategies, while most identified that the "animal welfare manager" was the primary individual responsible for assessment of the enrichment program. However, although the term "animal welfare manager" was used to refer to the individual responsible for animal welfare oversight of the facility in this survey, the term was not defined and could have been interpreted differently across respondents.

While training programs were not a primary focus of the survey, we were interested in whether approaches, strategies, and resources were in place to assess the impact of enrichment and behavioral changes resulting from enrichment. Sixty-three percent of the respondents indicated that they have a staff member with specific training, education, or experience in animal behavior. Although over half of the survey respondents did not have a documented training program for identifying stereotypic behavior in rodents (with little variation between geographic regions), most of the respondents felt that they had been adequately trained to identify such behaviors. Personnel trained to evaluate the effectiveness of enrichment and the behavioral issues caused by inappropriate enrichment are valuable as such behaviors can indicate poor animal welfare and potentially influence study outcomes. In addition, personnel trained to evaluate potential stressors and recognize abnormal behaviors in mice should be used to maintain optimal clinical and behavioral health for study subjects. Such expertise is also relevant to consideration of environmental stressors such as light, sound, and vibration.[6,7,42] Enrichment should be evaluated on an ongoing basis to ensure it is relevant and supported by scientific evidence or ongoing and regular performance and use-based assessments.

The goal of the current initiative was to recognize opportunities for refinement by identifying areas in which industry stakeholders and CRO partners have successfully refined husbandry and enrichment practices, including strategies for successful social housing. Due to the potential for negative health and welfare outcomes related to intermale aggression, male mice are often singly housed for toxicology studies. However, with single housing, alleviation of aggression must be balanced against the potential effects of social isolation, both on animal welfare and study data. Likewise, using husbandry practices that prioritize advancement of animal welfare must always be balanced with valid data collection and scientific outcomes. To gain insight on effective practices for social housing of male mice, other routine husbandry practices must also be considered, such as cage type, age of pairing, handling, randomization practices, nesting material, and handling of the nest, among others. Creating the survey was challenging due to the vast variation present in routine rodent husbandry. The working group recognized that husbandry practices may differ based on therapeutic area or study type. Because animals often remain on preclinical toxicology studies for months, loss of animals due to aggression-related wounding could be detrimental to study outcomes and regulatory filings. A general preconception among the working group members was that male mouse social housing would be the most prevalent issue in the surveyed fields. Due to the breadth and scope of the topic, the group focused the survey on rodent husbandry practices specific to toxicology studies to identify strategies with immediate benefit that could then potentially be applied to other areas of research.

One issue that we encountered was that while the survey was focused on identifying husbandry and enrichment practices in toxicology, some survey responses were related to husbandry practices in areas other than toxicology or preclinical safety studies. Despite efforts to frame questions that would allow for natural alignment of responses specific to husbandry in toxicology, reviewing responses to questions and specific comments provided revealed that some respondent sites did more than just toxicology work and the responses were not limited to toxicology husbandry practices. However, based on how the survey was distributed and blinded, excluding results that were specifically relevant to areas of research other than toxicology was not feasible and a full understanding of the rationale behind strategies and approaches was not possible. Respondent organizations each support multiple types of work that are performed under country-specific guidelines with varying amounts of resources.

While the survey identified opportunities for potential alignment, creating the survey and framing questions that would provide insight around common trends was challenging. Another issue encountered with survey development was the inability to gain full clarity on some of the survey responses due to how the questions were asked and/or interpreted by the respondents. Finally, while survey respondents represented a broad range of roles in the laboratory animal husbandry space, our results may be limited by the perspectives of individuals responding to the survey for their site rather than being representative of the broader company or laboratory animal community. Overall, however, this survey revealed trends in husbandry practices, general considerations for social housing, enrichment strategies, and the potential effects of bedding substrates in the fields of preclinical safety and toxicology.

Optimal animal welfare is critical to the production of quality scientific data. A tempting approach is to have human observers make subjective determinations of which husbandry practices provide for good animal welfare based on anecdotal evidence and personal observations of animal behavior. However, recommendations for standard rodent husbandry practices must be based on evidence obtained from well-designed studies that produced valid data. To promote such guidelines, a necessary first step is an understanding of current husbandry practices commonly employed in toxicology studies. In addition, some husbandry practices are not published in a way that makes the information accessible, and adopting new approaches into standard practice requires resources. Keeping abreast of the current literature and trends in the animal welfare can be challenging, even with dedicated resources. While published information is available on many of these practices, including the potential impact on research and challenges to implementation, confusion and disagreement still exist on how to apply this information to establish acceptable standard practice. Examples include how wood blocks affect research outcomes, whether resting platforms with wire-grid flooring affect metabolism studies, husbandry strategies to reduce male mouse aggression, and how the type of bedding affects animal welfare and experimental outcomes. As such, extrinsic factors that may influence the animal should be considered in experimental design to optimize research rigor and reproducibility across studies and facilities. A recent report by the National Institutes of Health (NIH) Advisory Committee to the Director (ACD) Working Group on Enhancing Rigor, Transparency, and Translatability in Animal Research recommends that the NIH dedicate funds for the investigation of high-value extrinsic factors to raise awareness of their importance in animal care.[34] An additional recommendation is that "all the extrinsic factors be measured and evaluated for further considerations on how to harmonize these factors to support increased study reproducibility."[34] In addition, investigators should measure and report environmental and husbandry data, including deviations from standard housing and husbandry.[34]

Collaborative communication is necessary to overcome the disparities in the application of husbandry practices, which should be based on animal welfare, scientific rigor, and the 3Rs. Groups like the IQ Consortium's 3Rs Translational and Predictive Sciences Leadership Group, the North American 3Rs Collaborative (NA3RsC) and the National Centre for the Replacement, Refinement, and Reduction of Animals in Research (NC3Rs), are some of the organizations for contribution, collaboration, and accessing 3Rs-related resources. With improved communication and networking, strategies and acceptable standard practices can be shared more broadly. This survey provided a snapshot of common global rodent husbandry practices, identified commonalities and differences in practices, and highlighted areas in which future collaboration could drive refinement in rodent husbandry.

Supplementary Materials

Figure S1. Survey questions, response options, and responses by region.

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