Factors Affecting People's Acceptance of the Use of Zebrafish and Mice in Research

Elisabeth H. Ormandy, Catherine A. Schuppli and Daniel M. Weary

Animal Welfare Program, The University of British Columbia, Vancouver, British Columbia, Canada

Summary — The species of laboratory animal used is known to influence people's willingness to support animal-based research. An online experiment was used to test people's willingness to accept the use of zebrafish or mice, two of the most commonly used species, in research involving either induced mutation (specifically, ethyl-*N*-nitrosourea [ENU] mutagenesis) or genetic modification, with and without regulatory oversight. Participants who were willing to support research on zebrafish (31.9%) were also willing to support the same research on mice. The participants expressed low levels of support for research involving ENU mutagenesis of zebrafish in both unregulated (30.7%) and regulated (38.5%) research programmes. A reason for the rejection of ENU mutagenesis was the perception that the procedure is painful. Some participants expressed a preference for the use of genetically-modified (GM) animal models over ENU mutagenesis, based on the belief that the former involves less pain and improves both the accuracy and efficiency of the animal models. Better informing the public about scientific practice, and scientists about public attitudes, may help reduce the disconnect between scientific practice and societal values.

Key words: *ENU mutagenesis, genetic modification, laboratory animals, public attitudes.*

Address for correspondence: *Daniel Weary, Animal Welfare Program, The University of British Columbia, 2357 Main Mall, Vancouver V6T 1Z4, British Columbia, Canada*.
E-mail: *ehormandy@gmail.com*

Introduction

Public attitudes toward animal research range from a desire for complete abolition to strong support (1–4). A wide variety of factors are known to influence public attitudes toward animal research, including: a) people's personal and cultural characteristics, such as age (4), gender (5), and country (6); b) animal characteristics, such as species (7, 8) or animal sentience (9, 10); and c) research characteristics, such as the level of invasiveness (11).

In this study, we focused on three factors: species of animal, genetic modification techniques, and the regulatory framework (i.e. whether or not the presented research was subject to formal regulations to control or oversee how the animals were used). The most commonly used research species are the mouse, zebrafish and rat (12), and the number of animals used is now greater than ever, due to an increase in the use of genetically-modified (GM) animals — the majority of which are mice and zebrafish (12). People's attitudes toward research animal use tend to differ, depending on the species involved (7, 8, 13). For example, members of the public tend to be less willing to accept the use of companion animals (e.g. dogs or cats), partly because these animals are viewed as having higher mental abilities compared to many other species used in research (14). Similarly, Animal Care Committee members have been shown to be less comfortable with research on companion animals and non-human primates (10), due to their beliefs about the sentience of these species. It has been shown that animals that are closer relatives of humans tend to evoke more-positive feelings (14), and those animals perceived as 'cute' tend to be preferred (15–17). Species such as fish and invertebrates are typically rated below mammals, and, as such, are often considered an appropriate replacement for mammals in research (18–20). However, the welfare of laboratory fish has received little academic interest, despite the mounting evidence that fish are sentient and have the capacity to feel pain (21).

Relatively new scientific technologies, such as genetic modification, can also affect attitudes toward animal research. Several studies have shown that support for either the creation or the use of GM animals is low (e.g. 22–24). People have expressed fundamental moral opposition to genetic modification (25), and concern regarding its 'unnaturalness' and its potential to lead to unknown consequences (e.g. 26–28). The bulk of the research on public attitudes has focused on consumer views toward the use of GM animals for food production, but there is evidence that the use of GM animals for biomedical applications might be more acceptable than for food-related applications (24). However, there is public concern about the methods employed for the development of GM animals, including concerns about the increased numbers of research animals used due to the relatively inefficient and unpredictable nature of some genetic modification techniques (22, 25). Attitudes toward other genetic technologies, such as ethyl-*N*-nitrosourea (ENU) mutagenesis, have yet to be evaluated. ENU mutagenesis is a common practice — a filtered search of the Mouse Genome Informatics (MGI) database for mutant mice that have been created by using ENU yielded thousands of results, and a PubMed search with the terms 'mouse' and 'ENU' retrieved 836 articles and 75 reviews. ENU mutagenesis is also commonly used on zebrafish (29).

The presence of an appropriate regulatory framework also affects people's willingness to accept animal-based research. For example, participant support for research involving pigs increased when this was conducted within a regulated environment (25); regulation had less of an effect on people's willingness to accept the use of GM pigs in the research, which reflects overriding concerns about genetic modification (25).

Based on the literature concerning how public attitudes toward animal research are affected by species, genetic modification and regulation, we predicted that: a) support for the use of zebrafish would be higher than support for the use of mice, even if the research was unregulated; b) support for the use of GM zebrafish would be low (compared to the use of zebrafish in ENU mutagenesis), even if the research was regulated; and c) support for research involving zebrafish or mice would increase if the research was regulated. To test these predictions, we designed an online experiment in which participants were presented with different research scenarios, all focused on creating animal models for biomedical research on skin cancer. The study was designed to explore where and why people would draw the line in terms of what they would be willing to accept, with a view to informing animal welfare policy.

Materials and Methods

Experimental design

The participants (n = 467) were randomly assigned to either an *unregulated* or *regulated* research scenario (Table 1). The experiment used a contingency design: how participants answered each question determined the next question they received. The 14 different paths through the questions (Figure 1) allowed for the manipulation of the variables of interest and provided a means to examine where people draw the line in terms of what they are willing to accept.

The participants presented with the regulated research scenario were told that the proposed research was subject to independent ethical review, to third party facility inspection and that researchers were required to accurately report the number of research animals used.

The first question for all the participants described a research proposal in which zebrafish were to be exposed to chemicals that cause mutations in germline cells (Table 1). The research scenario involved ENU mutagenesis, a common technique that is used to create mutant animal models (30). It was made clear that the intention of ENU mutagenesis was to create animal models for the study and treatment of skin cancer. Participants were asked whether they were willing to support the use of 100 zebrafish in this research. Those who responded, "yes" were then randomly assigned to either a question involving ENU mutagenesis on mice rather than zebrafish (species treatment), or a question involving the use of GM zebrafish (GM treatment), and asked again about their support. The species treatment proposed the use of 100 mice exposed to ENU mutagenesis; the GM treatment proposed the use of 100 GM zebrafish. Both treatments proposed the use of animals to create models of skin cancer and were based on real research projects (31–33).

The participants presented with the unregulated research scenario, and who expressed opposition at any point by responding "no", were asked the same question again, but with regulation in place. The participants who were initially assigned to comment on the regulated research, and who supported either the use of mice (species treatment) or the use of GM zebrafish (GM treatment), were asked the same question again, but with the regulation removed.

In addition to "yes" or "no" answers, the participants were asked to provide reasons for their answers. Participants who did not want to comment could leave an "x" in the text field, but most respondents (93%) commented on their "yes" or "no" response.

To further characterise the participants, a series of demographic questions were included (Table 2), allowing us to examine the influence of various demographic factors on participant support. In addition, participants were asked their views on the sentience of these species, genetic modification, and regulation of animal-based research (Table 3). These questions allowed us to better understand responses to the three variables manipulated in this experiment.

Recruitment

Participants were recruited for the experiment by using *Facebook*. Four *Facebook* stakeholder groups were targeted: an animal advocacy group (1049 members), an anti-vivisection group (2055 members), a pro-research group (4095 members), and an environmental advocacy group (8021 members). Assumptions were made about the characteristics of these groups (for example, that animal advocates would pay more attention to animal welfare,

Table 1: The independent variables manipulated in the experiment

Variable	Levels / treatment groups	**Research scenario** (text as it appeared to the participants)
Regulation	Unregulated	These researchers are *unregulated*. That is to say there are no formal regulations to control or oversee how animals are used in research.
	Regulated	The use of animals in this research is *regulated*. Attributes of this system include: — regulation that aims to promote ethical use of animals; — formal review of a) research team members, b) the research facility, and c) experimental proposals by an independent panel including scientists and members of the general public; — accurate reporting of numbers of animals used; and — random and routine inspection of the research facility by a third party.
Species	Zebrafish	The researchers propose the use of 100 zebrafish to study skin cancer. Zebrafish are a species of small, tropical freshwater fish, commonly used in scientific research. To study skin cancer the zebrafish will be immersed in a chemical solution that causes tumours.
	Mice	To further their understanding of skin cancer the researchers now propose the use of 100 mice. To study skin cancer the mice will be injected with a chemical that causes tumours to grow at the site of the injection.
GM	Non-GM	The researchers propose the use of 100 zebrafish to study skin cancer. Zebrafish are a species of small, tropical freshwater fish, commonly used in scientific research. To study skin cancer the zebrafish will be immersed in a chemical solution that causes tumours.
	GM	To further their understanding of skin cancer, the researchers now propose the use of 100 genetically modified zebrafish. To study skin cancer, the zebrafish will be genetically altered so that they carry a gene that activates tumour growth.

Initially, all respondents were randomly assigned to either the unregulated or regulated research scenario and then asked whether they would approve research that proposes "the use of 100 zebrafish to study skin cancer". Participants who answered "yes" to this research scenario were then randomly assigned to either the species or the genetic modification (GM) treatment group.

and that participants that were pro-research would pay more attention to the benefits of research), and the groups were targeted for recruitment based on these assumptions (purposive sampling; see Tashakkori and Teddlie [34]). The environmental advocacy group was included in the recruitment, since an interest in environmental issues has been shown to influence people's attitudes toward animal research.

Statistical analysis

Univariable logistic regression was used to test the effect of each demographic variable on support for ENU mutagenesis in zebrafish. Regulation was also included in each logistic model. Demographic effects that were significant (i.e. $p < 0.05$) in the individual models were then included in a final multivariable logistic regression model. The multivariable model also included regulation, and the two-way interactions between regulation and each demographic variable.

Comment analysis

Qualitative analysis focused on understanding why participants did or did not support the proposed research and why they switched when conditions changed.

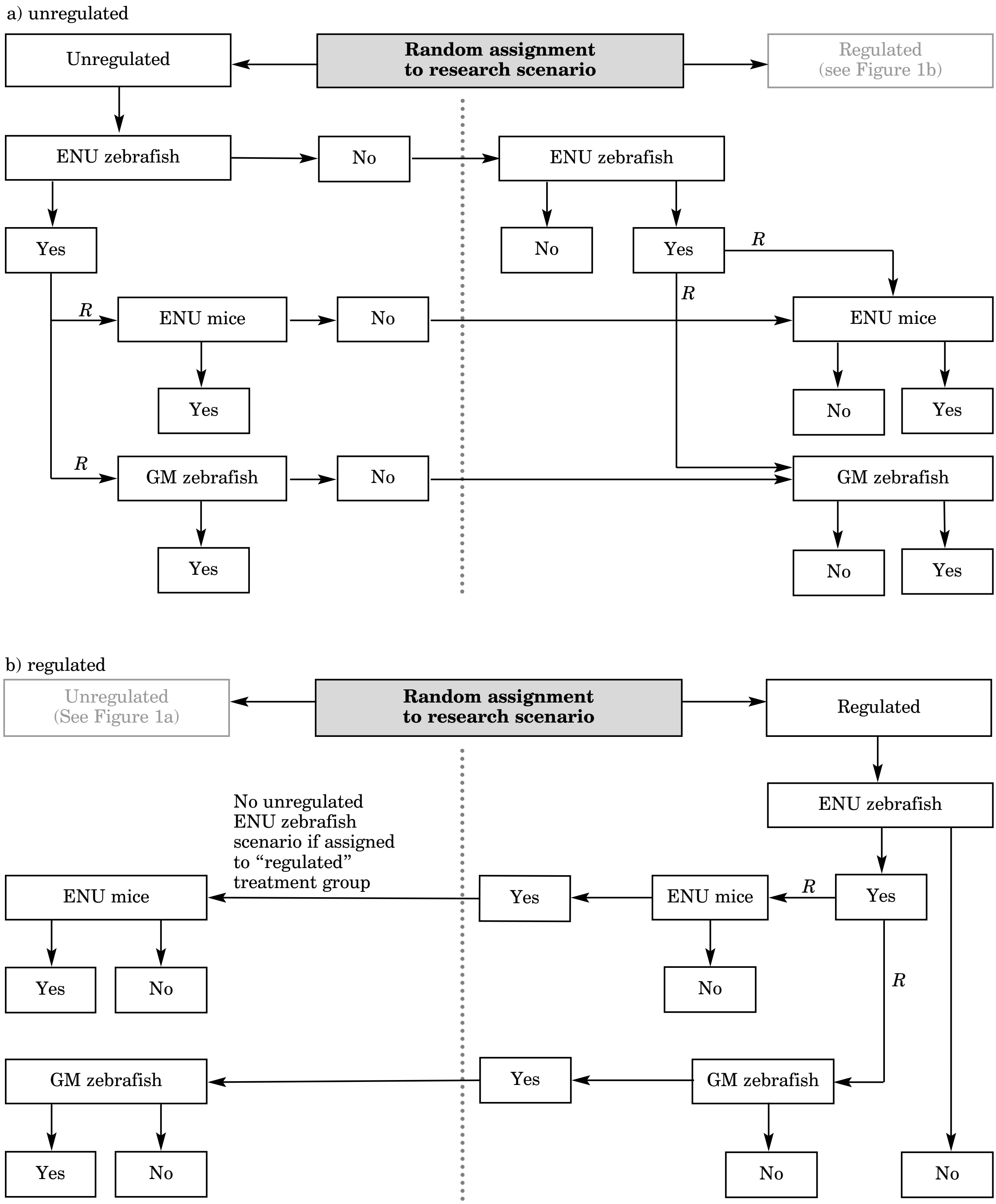
The first stage of the comment analysis involved the reading and assigning of codes — i.e. "tags or labels for assigning units of meaning to the descriptive or inferential information compiled during a study" (35). The comments were then read again, and the codes were checked for consistency and altered slightly as the comment data were interpreted (36).

Quantitative Results

Demographics

Many of the participants (36.9%) were relatively young (19–29 years old), and the majority (74.8%)

Figure 1: An illustration of pathways through the questions



Participants are first presented with unregulated (a) or regulated (b) research scenarios. 'R' indicates random assignment of the participant to either the species treatment group or the genetic modification (GM) treatment group.

Table 2: Participant demographics

Demographic question	Response options	% Participants
Age	19–29	36.9
	30–39	20.0
	40–49	16.9
	50–59	17.4
	60+	6.6
	—	2.2
Sex identity	Male	23.8
	Female	74.8
	—	1.4
Level of education	Secondary	7.6
	College/university	56.1
	Masters	14.8
	Doctorate	19.1
	Other	1.4
	—	1.0
Have you ever been a member of, or supported, the animal advocacy/ protection movement?	Yes	44.3
	No	54.7
	—	1.0
If so, please rate your level of involvement:	Minimal	26.4
	Occasional	36.0
	Frequent	37.6
How would you rate your familiarity with animal welfare?	Not familiar	6.3
	Somewhat familiar	36.6
	Very familiar	54.9
	—	2.2
Have you ever been a member of, or supported the environmental movement?	Yes	54.7
	No	43.4
	—	1.9
If so, please rate your level of involvement:	Minimal	15.1
	Occasional	47.1
	Frequent	37.8
Are you directly involved with some aspect of animal research (i.e. research team member, technician etc)?	Yes	33.3
	No	64.5
	—	2.2
How would you rate your familiarity with animal research?	Not familiar	14.0
	Somewhat familiar	41.1
	Very familiar	42.3
	—	2.6
Do you consider yourself to be vegetarian/vegan?	Yes	23.7
	No	73.9
	—	2.4
Do you currently own a pet?	Yes	78.0
	No	20.3
	—	1.7
Do you come from a rural or an urban background?	Rural	31.9
	Urban	64.4
	—	3.7
Politically, how do you consider yourself to be?	Liberal	36.5
	Somewhat liberal	23.9
	Neutral	25.1
	Somewhat conservative	8.2
	Conservative	2.9
	—	3.4

n = *415; — = indicates that no answer was given.*

Table 3: Additional questions put to the participants to establish attitudes toward species sentience, genetic modification and regulation of animal-based research

Question	Response	% Participants
Dogs can experience pain, suffering, happiness and pleasure:	Totally agree	85.1
	Mostly agree	7.7
	Mostly disagree	0.7
	Totally disagree	3.9
	Undecided	0.7
	—	1.9
Mice can experience pain, suffering, happiness and pleasure:	Totally agree	15.9
	Mostly agree	29.4
	Mostly disagree	14.0
	Totally disagree	27.2
	Undecided	11.3
	—	2.2
Fish can experience pain, suffering, happiness and pleasure:	Totally agree	10.1
	Mostly agree	35.3
	Mostly disagree	20.8
	Totally disagree	24.4
	Undecided	8.0
	—	1.4
Genetic modification of animals is an acceptable practice:	Totally agree	63.0
	Mostly agree	26.6
	Mostly disagree	2.2
	Totally disagree	3.1
	Undecided	2.7
	—	2.4
Public authorities (i.e. governments) can be trusted to regulate the use of animals in research:	Totally agree	31.9
	Mostly agree	36.0
	Mostly disagree	13.4
	Totally disagree	2.4
	Undecided	13.0
	—	3.1

n = *415; — = indicates that no answer was given.*

were female and had at least some level of post-secondary education (56.1%; Table 2). The participants were from Canada (69.6%), the USA (18.2%), the United Kingdom (7.7%), Australia (1.0%), and other countries (all less than 1%), including Afghanistan, Colombia, France, Germany, India, Italy, New Zealand, Portugal, Singapore, Turkey and the United Arab Emirates.

Many (44.3%) of the participants stated that they had been involved in the animal advocacy/protection movement, with 11.8% expressing "frequent" involvement. Similarly, 54.7% stated that they had been involved in the environmental movement, with 8.7% expressing "frequent" involvement. The majority of the participants (54.9%) considered themselves to be "very familiar" with animal welfare, 33.3% were directly involved in animal research, and 42.3% claimed to be "very familiar" with animal research.

Approximately one quarter (23.7%) of the participants indicated that they were vegetarian, 78.0% owned pets, 31.9% were from a rural background, and 36.5% considered themselves to be politically "liberal".

Statistical analysis

Of the 467 participants, 415 answered all the demographic questions. Logistic regression analysis included only those participants who provided their demographics, but the descriptive and qualitative analysis of participant responses included all the participants.

Results from the univariable logistic regression analysis showed that pet owners, vegetarians, females, and those involved in animal or environmental advocacy, were less likely to support the use of zebrafish in ENU mutagenesis research. In contrast, people who were involved in animal research, considered themselves familiar with animal research, and those who had a higher level of education were more likely to support animal use (Table 4a). These results formed the basis of a multivariable model to test the relative effects of each significant variable on the level of support for ENU mutagenesis in zebrafish (Table 4b).

The multivariable model showed that the odds of participants supporting ENU mutagenesis in zebrafish were decreased by 60% if they were animal advocates. The odds of participants supporting ENU mutagenesis in zebrafish were decreased by 40% if they were younger (19–29 age bracket), or were vegetarian. In contrast, those participants who claimed to be familiar with animal research, were 2.1 times more likely to support the ENU mutagenesis in zebrafish. There were no interactions between regulation and any of the demographic variables in this model.

Participant responses

The level of opposition to ENU mutagenesis of zebrafish was high in both the regulated (61.5%) and unregulated (69.3%) research scenario groups (Tables 5 and 6, parts i and ii combined). Of the 158 participants who were initially opposed to unregulated ENU mutagenesis research involving zebrafish, 124 (78.4%) maintained their opposition, even after regulation was added (Tables 5 and 6, parts iii combined).

Due to low levels of support in the opening questions, sample sizes for the subsequent questions where the participants were randomly allocated to either the species or the GM treatment, were relatively small. In general, those participants who had supported research involving the use of zebrafish in ENU mutagenesis tended to maintain their support when the species was switched to mice, or when the GM treatment was added, regardless of whether regulation was in place or not.

Qualitative Results and Discussion

Costs and benefits of ENU mutagenesis

The opponents of ENU mutagenesis (for both zebrafish and mice), tended to focus on the harm to the animals. A proportion (34%) of the participants, commenting on welfare costs, expressed the belief that ENU mutagenesis was painful. For example, one participant commented that the research *"clearly will cause harm to the fish"*, while another stated that their opposition to ENU mutagenesis, even if it was regulated, was because *"it is still hurting the fish because they are still put in the chemicals that cause tumours"*. The concern for pain was related to sentience by one participant:

Table 4: Results from the logistic regression analyses

a) Demographic variables and their effect on the participants' willingness to support ENU mutagenesis in zebrafish

Demographic variable	Odds ratio	CL (95%)	R^2	*P* value
Animal advocacy	0.3	0.2–0.4	0.12	< 0.0001
Involvement in animal research	3.4	2.3–4.9	0.10	< 0.0010
Vegetarianism	0.4	0.3–0.6	0.05	< 0.0001
Familiarity with animal research	2.3	1.7–3.3	0.06	< 0.0001
Sex identity	2.0	1.3–3.0	0.03	0.0009
Environmental advocacy	0.6	0.4–0.8	0.03	0.0017
Level of education	1.6	1.2–2.1	0.03	0.0030
Age	0.6	0.5–1.0	0.02	0.0200

Individual logistic regressions were carried out for each demographic variable (as reported in Table 2), with 'regulation' factored into the model. Only those that were significant predictors of support ($p \leq 0.05$) are included here. CL = confidence limits.

b) Results from the multivariable logistic regression for the ENU mutagenesis in zebrafish question

Demographic variable	Odds ratio	CL (95%)	*P* value
Animal advocacy	0.4	0.3–0.7	0.0003
Familiarity with animal research	2.1	1.2–3.9	0.0049
Vegetarianism	0.6	0.4–1.0	0.0360
Age	1.7	1.1–2.5	0.0132

Regulation was factored into the model. CL = confidence limits; $R^2 = 0.19$

Table 5: Quantitative results for the species treatment group

a)

ENU mice **regulated**	ENU zebrafish **regulated**	ENU zebrafish unregulated	**ENU mice** unregulated	ENU mice **regulated**
(viii) (n = 14) Yes = 9 No = 5	**(iii)** (n = 79) Yes = 14 No = 65	**(i)** (n=116) Yes = 37 No = 79	**(iv)** (n = 37) Yes = 35 No = 2	**(v)** (n = 2) Yes = 1 No = 1

b)

ENU zebrafish regulated	**ENU mice** regulated	ENU mice **unregulated**
(ii) (n = 120) Yes = 46 No = 74	(vi) (n = 46) Yes = 43 No = 3	(vii) (n = 43) Yes = 12 No = 31

*Number of participants per question responding "yes/no". A vote of "yes" indicates support for the proposed research. Text in bold at the top of the table columns represents the changes in treatments. Underlined values in the table represent the number of participants that changed their "yes/no" response based on changes in species or regulation. ENU = ethyl-*N*-nitrosourea-induced chemical mutagenesis procedure. Roman numerals (i–vii) mark where specific results are discussed in the main text of the paper.*

"There is no difference between sentient creatures being immersed in a chemical solution unregulated or sentient creatures being immersed in a chemical solution regulated. The issue is the ability to feel pain." The belief that zebrafish or mice can feel pain is one aspect of 'belief in animal mind' (or animal sentience), which has been shown to affect attitudes toward research animal use in some, but not all, cases (9, 37).

For some opponents to ENU mutagenesis (15%), concerns about animal suffering were framed in relation to the Three Rs (38), although the actual terms — *Replacement*, *Reduction* or *Refinement* — were not necessarily used. For example, one participant implicitly referred to *reduction* by stating that *"I think the research is using too many mice, fewer should be used"*; another similarly referred to *refinement* by saying that *"the pain should be alleviated with analgesia and anaesthesia"*. Some opponents (11%) raised questions about whether non-animal alternatives are available. For example, one participant wrote, *"It is still unethical to use any animal in this way — are there no alternatives?"*, while someone else commented, *"I would prefer to see grant money used to find alternatives to research using animals."* Some opponents to ENU mutagenesis (5%) were of the opinion that human tissue should be used instead. For example, one participant commented, *"It is not relevant to get data from fish. The scientists should use human data with toxicogenomics and cell culture."* Another opponent stated that *"better results would be obtained by using human skin cells rather than inflicting cruelty to animals."* These comments suggest that opposition to ENU mutagenesis is rooted in two beliefs. First, that the method causes pain to the animals involved, and second, that the use of animals in the proposed research is not necessary and that use of human tissue would provide a more relevant test.

Supporters of ENU mutagenesis in both zebrafish and mice tended to focus on the benefits of the research to human and animal health. For example, one participant indicated that their support was rooted in the belief that this research should proceed because of the *"potential value of the project to human/animal health"*. Another supporter expressed a similar view by stating that *"I believe in medical research that will save and improve the quality of life for all humans."* However, 31% of ENU mutagenesis supporters also stipulated conditions to their support, which were primarily related to concerns about animal pain, suffering and the Three Rs. For example, one supporter stated several conditions: *"I don't like the idea of animal testing but feel it is necessary to* *do so at times, so yes, [but] ONLY if there is no pain caused to the mice AND if there is no alternative to using live animals AND if they are provided with living conditions and nutrition that are good for them and cause them no stress AND if they are to be euthanised, that it is done in a humane manner"* (emphasis added by the participant). Such results provide support for decision-making processes proposed by Serpell (13), Stafleu (39) and Ideland (40), who postulate that when making decisions about animal use, a common strategy is to trade off the utility and necessity of using animals (in this case, the benefits to human health, under the premise that there are no alternatives), against concern for animal welfare (in this case, by minimising pain and providing humane care).

Species sentience

The species used had little effect on participant support: 94% of participants who supported ENU mutagenesis in zebrafish also supported ENU mutagenesis in mice (Table 5, parts iv and vi combined). However, 11 of the 70 participants who were supportive of the use of zebrafish in ENU mutagenesis, commented that species sentience, or the location of zebrafish on the phylogenetic scale, was an important factor. For example, one participant stated that *"it is necessary to use an animal model to study cancer, and zebrafish are lower on the evolutionary chain than other larger species, so if they can be used instead it is preferred."* Other comments included: *"I've heard fish do not have pain receptors, I figure the fish are unaware";* and *"Again, medical research is important. I'm glad that more intelligent animals such as monkeys are not being used".*

Ten participants did switch from support to opposition when the species changed from zebrafish to mouse. One participant explained that *"mice are very intelligent so I consider this far more serious than using fish",* illustrating that, for some people, the perceived difference in sentience between species is important. The validation questions about animal sentience (Table 3) also support this interpretation — more of the participants totally agreed that mice are sentient (i.e. they can experience pain, suffering, happiness and pleasure) compared to zebrafish (15.9% and 10.1%, respectively). In contrast, 92.8% considered that dogs are sentient.

Supportive of genetic modification

Participants who were initially supportive of the use of zebrafish for ENU mutagenesis often main-

Table 6: Quantitative results for the genetic modification (GM) treatment group

a)

GM zebrafish regulated	ENU zebrafish **regulated**	ENU zebrafish unregulated	**GM zebrafish** unregulated	**GM zebrafish regulated**
(viii) (*n* = 20) ← Yes = 19 No = 1	**(iii)** (*n* = 79) ← Yes = 20 ← No = 59	**(i)** (*n* = 112) Yes = 33 → No = 79	**(iv)** (*n* = 33) Yes = 30 No = 3 →	**(v)** (*n* = 3) Yes = 3 No = 0

b)

ENU zebrafish regulated	**GM zebrafish** regulated	**GM zebrafish unregulated**
(ii) (*n* = 119) Yes = 46 → No = 73	**(vi)** (*n* = 46) Yes = 44 → No = 2	**(vii)** (*n* = 44) Yes = 11 No = 33

*Number of participants per question responding "yes/no". A vote of "yes" indicates support for the proposed research. Text in bold at the top of the table columns represents the changes in treatments. Underlined values in the table represent the number of participants that changed their "yes/no" response based on changes in procedure (ENU mutagenesis or GM) or regulation. ENU = ethyl-*N*-nitrosourea-induced chemical mutagenesis procedure. GM = genetically modified. Roman numerals (i-vii) mark where specific results are discussed in the main text of the paper.*

tained their support when the GM treatment was proposed (94% continued support; Table 6, parts iv and vi combined). These participants commented that GM animal models are more accurate, reduce animal numbers, and are preferable to ENU mutagenesis. For example, one participant commented, *"I agree with the use of genetically modified research species as it increases the probability of successful research and also allows for a reduction in the numbers used as you can use less fish and have a greater chance of all (or almost all) generating tumour growth."* Another participant stated a preference for GM over ENU mutagenesis because it may be less painful — *"The genetically-modified fish may grow tumours without the pain of being dipped in chemicals."*

In our experiment, the support for genetic modification concurred with results from the additional questions asked at the end of the survey (Table 3), where a high proportion (63.0%) of the participants totally agreed that genetic modification is an acceptable practice. The question on the general acceptability of genetic modification as a practice came at the end of the experiment, after the participants had given their responses to the specific research scenarios — many of them had judged the GM treatment to be less painful, more accurate and more efficient than ENU mutagenesis. This might have framed the issue in a way that made the participants more accepting of genetic modification for this particular experiment. In addition, if the participants' answers were specific to the biomedical research applications given in this experiment, rather than to a more-general application of GM animals, this result may well be an indication of different views toward the genetic modification of laboratory animals, compared to the genetic modification of food animals. A difference in attitudes based on the end use for GM animals (i.e. food *versus* biomedicine) and the resulting benefits has been found in other studies (25, 41, 42).

Against genetic modification

A relatively small percentage (6%) of the participants who were initially supportive of ENU mutagenesis on zebrafish switched to opposition when the GM treatment was proposed (Table 6, parts iv, vi and viii combined). These participants explained that they were fearful of unknown consequences. For example, one opponent to GM commented, *"I can't put it into words very well, but the idea of genetic mutations that we create scares me. Maybe a latent, perhaps irrational, fear that the mutated genes will have unexpected results that aggressively invade the species or other species or somehow upset a delicate balance…"*

Opinions on regulation

Regulation was mentioned by 88 of the 105 (84%) participants who changed their answer in response to the shift in regulation. For example, a participant who switched from support to opposition when regulation was removed commented that *"regulations are important to ensure humane use of animals (for example humane endpoints), adequate project review, inspections, etc. These things are important not only for humane reasons but also to help ensure sound scientific results."* Another participant who switched from opposition to support when regulations were put in place stated that *"before, it was not regulated and the health and welfare of the animals was not the first priority… Also, having a third party inspect the facility is a great method for maintaining a professional and properly run facility. This method will decrease the chance of animals being mistreated."* This type of response indicates that regulation is an important component affecting some people's willingness to support animal-based research. Specific components of regulation that participants identified as being valuable include the ethical review, inspection of facilities, animal welfare monitoring programmes, reporting of animal numbers, and openness (in order to maintain public accountability).

Influence of demographics on participant support

Vegetarianism was a predictor of decreased support, which confirms previous findings (43, 44). Typical rates of vegetarianism in the UK, US and Canada are roughly 3–4% (45–47). The over-representation of vegetarians among our participants (24%) permitted a stronger test of the effect of this variable, and may have influenced the significance of vegetarianism in our study.

Animal advocacy was also associated with decreased levels of support. This also fits with previous findings (e.g. 4), suggesting that people's underlying beliefs about animals (i.e. their willingness to advocate for protecting them) influences their willingness to support this type of animal-based research. Our participant population had a high percentage of animal advocates (44%), which may have skewed the results, leading to the high levels of opposition to animal use.

In previous research on public attitudes to animal research, age has been an influential factor, with younger people generally being less supportive of such research (4). Our results also showed that age affected the likelihood of support, with older participants showing greater levels of support. The over-representation of younger people in our participant population is likely to have allowed for a stronger test of the effects of this variable, and may have influenced the significance of effects of age on support.

Familiarity with animal research was associated with higher levels of support. Some studies have also shown a positive association between familiarity (or knowledge) of a particular topic and positive attitudes (23, 47). However, this pattern is not consistent across studies. In a previous experiment that asked participants about their willingness to support the use of pigs in research (25), familiarity with animal research was associated with lower levels of support, which concurs with some previous findings (43, 48, 49). An explanation for the difference in the results between the agricultural model used in the previous experiment and the biomedical model used here might be the difference in research settings. We suggest that general preconceptions about the day-to-day life of the animals are different for laboratory and farm animals, whether or not they are being used for research. The general image of life in the laboratory setting may often be worse than the reality (explaining why knowledgeable individuals are more willing to support this use), while the reverse may be true for pigs and other farm animals.

Sex identity and the level of education also influenced participant support, with females less likely to support animal-based research than males, and those with higher levels of education being more likely to show support. The effect of sex identity and education on attitudes toward animals has been well documented, and our findings match those reported in other studies (e.g. 12, 50–52).

A consistent theme in the public attitudes literature is that participants often draw a distinction between different types of animal research (28). The use of animals for medical experiments that will benefit human health tends to be regarded more positively than their use for cosmetics testing (53, 54). On this basis, we had expected the majority of the participants to support research intended to aid the study of skin cancer. The low levels of support might be related to the perception that ENU mutagenesis is a painful procedure — research that is perceived as painful or invasive is often less supported (4, 55). For example, our previous work on farm animals showed that non-invasive research on pigs was more highly supported, but this support declined when the research required an invasive procedure (25). However, in the present study, only 10.1% of the participants totally agreed (35.3% mostly agreed) that fish can experience pain, suffering, happiness, and pleasure, suggesting that there might be something else about the ENU mutagenesis procedure that the participants found objectionable, despite the perceived benefits to human health from the research.

Conclusions

Many of the participants in this study (65%) said that they were opposed to ENU mutagenesis in zebrafish. The primary reasons for this opposition were concerns: a) about animal welfare (i.e. pain and suffering); b) about *reduction* and *refinement* implementation (i.e. too many animals being used, or lack of *refinement*); and c) that the use of animals in this type of research is unnecessary (due to the belief that the use of human tissue would provide a better means of researching skin cancer). When asked about genetic modification, most participants were supportive.

The results suggest that biomedical applications with GM animals are acceptable, provided that animal welfare concerns are taken into consideration and that there is sufficient regulation. However, many participants perceived ENU mutagenesis to be painful, suggesting that research that exposes animals to this procedure is not well supported, regardless of whether mice or zebrafish are used. This result calls into question the assumption that research deemed to cause pain will be considered more acceptable, if performed on ‘lower’ species, such as fish, that sometimes serve as replacements for mammals in animal research. It also suggests that more research is required on the welfare effects of ENU mutagenesis and how these effects might be mitigated. Greater effort may also be required to inform the public about scientific practice and to permit feedback via public engagement, to reduce the gaps between common scientific practice and societal values.

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