



Voice of the Voiceless

GAIA Investigation into animal experiments at Vrije Universiteit Brussel: shocking revelations of intolerable animal suffering

Between March and June 2016, an undercover investigator of GAIA worked in the Animalarium of the Free University of Brussels (Vrije Universiteit Brussel, Jette Campus) to reveal how research animals are treated. This report contains the results of our investigation.



November 2016

Background to animal experimentation in Belgium

This report details the findings from our undercover investigation between March and June 2016 at Vrije Universiteit Brussel, considered one of Belgium's leading academic institutions. Our investigation is the first of its kind in Belgium.

There is a vital need for investigation into the pain, fear and distress of laboratory animals; to expose the truth which the public is shielded from on an on-going basis; not only the suffering which is intended or expected in the procedures the animals are used in, but also the neglect, disregard for welfare (including basic needs in food and water provision) and failure in administering appropriate after-care and compassion.

Introduction to VUB

Vrije Universiteit Brussel (VUB) is one of Belgium's main universities. It has three campuses, located at Etterbeek, Jette and Anderlecht. Our investigation was carried out at the VUB Animalarium, in the college academic building at the Jette campus ⁱ.

The Animalarium is an extensive facility, with a layout of 30 rooms, many of which contain pens and cages separated by corridors. There is a main entrance for the researchers, with a smaller additional entrance to the rear of the site. There is also an entrance used to bring in animals transported to the facility (e.g. mini pigs). The facility houses pigs, mice, rats and rabbits. Specific rooms keep groups of animals undergoing certain experiments segregated as required. There are rooms for animals allocated to certain research groups; for example a room for mice which have been induced to deliberately suffer from cancer; mice used in fertility studies, as well as rooms for breeding genetically modified (GM) mice and mice under quarantine. Two rooms house the mice in stacked IVCs (Individually Ventilated Cages). There is also a specific room for carrying out procedures and areas for killing animals ('euthanasia stations' with a shelf for placing animals which are 'to be euthanized').

The facility also has areas for food stocks, bedding (e.g hay and wood shavings) washing areas (for bottles and cages) and individual rooms/offices for the Chief Veterinary Officer (CVO) and other researchers, as well as an area for a large freezer, which was seen to be used to kill live baby mice during our investigation.

The Animalarium serves an extensive network of research departments at VUB. To name just a few examples; experiments carried out by the LMCT (Laboratory of Molecular and Cellular Therapy); the HEIM group (Haematology and Immunology) and the diabetes research group known as BENE (Beta Cell Neogenesis). More detail on these and other animal research departments at VUB are provided later in this report.

The facility and research groups carry out many different types of experiment, many by senior individuals and some by students, for example carrying out animal testing for PhD projects. Recently published examples include MRI scanning in pregnant miniature pigsⁱⁱ; mice injected with cancer cells to develop enormous tumours; injection of rats to cause deliberate heart damage, weight loss, anxiety, pain and deathⁱⁱⁱ and damaging the livers of mice to test the benefits of chilli pepper extract.^{iv}

VUB's Chief Veterinary Officer of the Animalarium facility has provided recent lectures claiming that '*animal experiments are more than what we intuitively think*' and that '*not all animals are slain*

after an experiment'. While it is true that not all animals will necessarily be killed after procedures, this misleading statement is certainly no guarantee of high welfare or the protection of any animal from harm, especially as some animals may be re-used in further procedures. The CVO also claims that *'not all animals experience a form of suffering'*.^v

However, findings during our investigation clearly prove otherwise, as well as reflecting the complacency and negligence towards animal welfare and care by the Chief Veterinary Officer and other researchers at VUB. This report includes comprehensive diary logs made by our investigator - the diary logs are available upon request - from both conversations with and observations of numerous research staff, as well as detailed interviews and direct conversational evidence from extensive video footage. The vet also speaks about how the researchers need to improve on check ups on the animals and critically, that the researchers are already aware of this.

Given the negligent attitudes of the vet and research staff, this means that there is an establishment-wide failure to check on animals properly at VUB.

Key findings from the investigation

Through three months of detailed and comprehensive diaries, video footage and interviews provided by our dedicated undercover investigator, ***a catalogue of pain, suffering and distress, along with routine complacency, neglect and disregard for animal suffering*** at the VUB Animalarium has been revealed. Further detail is provided in the relevant sections of this report but for clarity, some key examples of suffering and cruelty revealed in a wealth of evidence collected between March and June 2016 are summarised under the headings below;

Inhumane methods of killing and breaches of legislation:

- **Dislocation of the necks of mice with pens and scissors (also shown clearly in video evidence) and incompetency in checking if the animals were dead**
- **Very young mice that did not die by carbon dioxide gas (CO₂) asphyxiation were decapitated-** the national and EU legislation states that decapitation is *'only to be used when other methods are not possible'*. CO₂ asphyxiation is also known to cause severe welfare problems for rodents, with studies showing acute pain in rats and fear in mice at specific concentrations^{vi}
- **Live, new born mice put into freezer to die- freezing is not specified as a permitted method of killing;** The regulation only states that 'confirmation of rigor mortis' is a method of killing. There is no reference to freezing being permitted in national legislation
- **Decapitated baby piglets.** This appears to be a possible breach of the Belgian animal experiments regulation and Annex IV of Directive 2010/63/EU on 'Methods of Killing', which only permits decapitation of birds and rodents. Decapitation of piglets may be legal when the animals are unconscious but it is not clear this is the case. **Key video evidence collected also shows piglets being killed**
- **More than one female pregnant mouse in a cage,** leading to females becoming pregnant by related male animals (father/brother) which aside from the serious breaches of ethics, was recognised by research staff to impact on scientific results of experiments
- **Cutting the heads off baby mice (pups) with scissors, as also shown on video evidence. This method is only allowed when no other methods are available.**

'Methods of Killing' as documented in national legislation (Annex 7) and directive 2010/63/EU are as follows – numbers in brackets in the table are explained in footnotes underneath;

BIJLAGE 7

Methoden voor het doden van dieren

1. Voor het doden van dieren worden de methoden van onderstaande tabel gebruikt.
Er kunnen methoden worden gebruikt die niet in de tabel staan:
 - a) voor bewusteloze dieren, op voorwaarde dat het dier voor zijn dood niet weer bij bewustzijn komt;
 - b) dieren die in landbouwkundig onderzoek worden gebruikt, wanneer het doel van het project vereist dat de dieren worden gehouden onder soortgelijke omstandigheden als dieren in de commerciële landbouw, kunnen worden gedood overeenkomstig de voorschriften in bijlage I van Verordening (EG) nr. 1099/2009 van de Raad van 24 september 2009 inzake de bescherming van dieren bij het doden
2. Het doden van de dieren wordt voltooid met één van onderstaande methoden:
 - a) bevestiging dat de circulatie definitief is gestopt;
 - b) vernietiging van de hersenen;
 - c) dislocatie van de nek;
 - d) leegbloeden; of
 - e) bevestiging dat rigor mortis is ingetreden.

Dieren-opmerkingen, methoden	Vissen	Amfibieën	Reptielen	Vogels	Knaagdieren	Konijnen	Honden, katten, fretten en vossen	Grote zoogdieren	Niet-menselijke primaten
Overdosis anesthesie	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)	(1)
Penschieettoestel			(2)						
Kooldioxyde					(3)				
Cervicale dislocatie				(4)	(5)	(6)			
Percuterende slag op de kop				(7)	(8)	(9)	(10)		
Onthoofding				(11)	(12)				
Elektrische	(13)	(13)		(13)		(13)	(13)	(13)	
Inerte gassen (Ar, N ₂)								(14)	
Afschot met passend geweer of pistool en passende munitie			(15)				(16)	(15)	

Vereisten

- 1) Waar passend met voorafgaande sedatie.
- 2) Alleen bij grote reptielen.
- 3) Alleen te gebruiken bij geleidelijke blootstelling aan het gas. Niet gebruiken voor foetale en pasgeboren knaagdieren.
- 4) Alleen voor vogels van minder dan 1 kg. Vogels van meer dan 250 g worden verdoofd.
- 5) Alleen voor knaagdieren van minder dan 1 kg. Knaagdieren van meer dan 150 g worden verdoofd.
- 6) Alleen voor konijnen van minder dan 1 kg. Konijnen van meer dan 150 g worden verdoofd.
- 7) Alleen voor vogels van minder dan 5 kg.
- 8) Alleen voor knaagdieren van minder dan 1 kg.
- 9) Alleen voor konijnen van minder dan 5 kg.
- 10) Alleen voor pasgeboren dieren.
- 11) Alleen voor vogels van minder dan 250 g.
- 12) Alleen indien andere methodes niet mogelijk zijn.
- 13) Vereist speciale apparatuur.
- 14) Alleen voor varkens.
- 15) Alleen te gebruiken door ervaren schutter in veldomstandigheden.
- 16) Alleen te gebruiken door ervaren schutter in veldomstandigheden wanneer andere methoden niet mogelijk zijn.

Negligent behaviour, disregard and neglect for animal welfare, poor environment and breaches

- Out of date medicines (expiry in 2013) being given to pigs
- Investigator told not to bother checking all cages as this is the responsibility of researchers, *yet it is known that they fail to check properly*
- Research staff deliberately removing and ignoring warning labels on cages about overcrowding and injury
- Conversations with staff and technicians that researchers may not know all details they should on the animals (welfare, pain levels, cage and housing conditions)
- Lack of staff availability when called on to come into the facility to check on sick and injured animals. Paragraph 2 of article 31 of the Royal Decree of 2013 stipulates that: “when the health and welfare of laboratory animals are incriminated, the end user, breeder or supplier have to be informed by knowledgeable staff members. They have to immediately act upon this, investigate the cause and take all necessary measures.”
- Sluggish attitude; when asked about settings on machines used to monitor/kill animals, CVO says she 'should look that up'
- Conversations between research staff explaining lack of standardised protocols and how unorganised the facility is
- The CVO and researchers laughing when notified about sick animals; at stereotypical behaviour in mice and mummified dead pigs. **Key video footage also shows stereotypical behaviour in mice, including jumping up and down frantically**

- Loud music playing Under point 2.5 of Annex 4 of the Royal Decree on the protection of laboratory animals it is stated that “noise levels must not have a negative effect on animal welfare”
- As recorded in key diary evidence, the CVO admonished the investigator for adding enrichment(wood) to some mouse cages, claiming that it may impact on results of experiments.
- A staff member told our investigator how a major animal welfare crisis occurred approximately 6 months before at the facility, under the supervision of Karine Breckpot, one of the key responsible persons at the Animalarium.
- Incompetency in checking if animals are dead

Direct evidence of suffering, cruelty and neglect in the animals (further details are provided later in this report), and breaches

- Overcrowded pens and cages on many repeat occasions- in one case, 17 mice and further babies were crammed into one cage (meant only for 5 mice) with no food or water, resulting in animal deaths. The charts in annex 4 of the Royal Decree of 2013 clearly outline the minimum surface requirements per animal. It seems the VUB does not respect these minimum requirements. The same annex of the Royal Decree states that water and an appropriate diet always have to be provided. This was known by staff to be against regulations and would result in warnings from inspectors if present
- Suffering as a result of intense breeding and genotyping for example enlarged heads in mice ('hydrocephaly' due to fluid collection in the brain). The researcher responsible for these animals thanked the investigator for raising concerns *and admitted that she wouldn't have noticed.*
- A very sick mouse with one foot eaten away, seen to need urgent euthanasia by several research staff and technicians, yet left by lazy staff to suffer for several days later until after the weekend as the CVO could not be reached
- **Mice with huge tumours, as shown clearly on video footage.** Some tumours were so large they had burst under the poor animals' skin but considered 'no problem' as they were 'part of a survival experiment'
- Sick and distressed mice attacking/eating each other (cannibalism), visibly severe suffering and bite marks on some mice; tails hanging off
- Conversations recorded with research staff reveal how the quality of care, animal welfare and monitoring has declined; particularly that the previous Chief Vet used to have a better system of regular monitoring of animals (e.g. once weekly checks). However the current CVO does not do this, instead merely asking staff to update a list of sick animals 'in the kitchen' which may be ignored for long periods of time. In support of this evidence, our investigator, who was in post as a research assistant/technician was just one employee who had not even been made aware of the list.
- A pig used in cancer experiments suffering from a catheter infection, eventually having to be killed;
- Another pig suffering from open wounds- again from a catheter and rubbing against the walls, straw and floor of her pen with severe wounds and dried blood. The CVO had appeared to have gone home early without notifying others and when telephoned, merely advised to put minor wound dressings on the pig. Our investigator, despite not being trained, was asked to inject this pig with painkillers, via conversations with other incompetent/untrained staff. Again, senior vet staff were unavailable, leaving the poor

animal to suffer even more.

- Mice left too long in a CO₂ chamber as the CVO neglected or forgot to push the button to kill them.
- Severe signs of suffering and distress in mice (including sick mice with canulas (tubes) implanted in their brains) used in neurological and anxiety experiments, such as hair loss from over grooming, hunched posture and poor fur condition, moving in circles and jumping up and down frantically being dismissed merely as 'expected stereotypic behaviour'
- Mice found trapped by tails in parts of cages and other mice with blood in their eyes
- Researcher confirms they will check on an extremely sick animal, yet the animal is found dead in their cage the next morning. Multiple incidents of further animals found dead in cages
- Failure to check on animals, with research staff leaving lab early without notifying others, or going on holiday and leaving very sick animals uncared for, with no system in place to ensure others check on them. In other cases, researchers fail to check on wounded mice for over a week. One researcher states how they try to check on the animals every two weeks but 'almost never make it'. **Perhaps unsurprisingly, the day before an announced inspection, many research staff are present in the facility and are checking on cages and labels at the last minute, after an email from the CVO. Article 31 of the Royal Decree of 2013 imposes that the end-user, breeder or supplier has to make sure that all laboratory animals and their housing areas are checked daily.**
- Repeated failure to provide basic needs for animals – food , water, regularly clean cages, despite having designated facilities to do so
- Reports of previous errors, resulting in animals left suffering in 35 degrees heat after an air conditioning failure. According to annex 4 of the Royal Decree of 2013, temperatures in the housing areas must meet the species specific needs. Temperatures must be measured and recorded on a daily basis.
- Rabbits, bred with heart defects and used in experiments over a year ago, still left in the laboratory, in such a fragile and highly stressed state that transporting them or even just moving or cleaning their cages could kill them from stress/panic – several animals had already died like this. However, in accordance with paragraph 2 of article 27 of the Royal Decree of 2013, it is decided at the end of each experiment whether or not an animal should live or die, keeping into consideration that an animal should not be kept alive when it is very likely to suffer from severe and lasting damage, even if its general health appears to have returned to normal
- Incompetence and negligence of researchers who fail to know which animals they are responsible for – e.g. one staff member was unaware of a mouse in their care who had died (in an aging experiment in which animals were to die spontaneously) and they had intended to remove the brain for analysis, but it was too late
- **Key video footage also shows procedures being performed in mice, including injections and force feeding by tubes down their throats ('gavaging')**

Animal welfare and potential breaches of regulations at VUB

Rodents

Mice and rats are highly intelligent and sensitive animals, requiring housing in socially compatible groups dependent on their age, gender and reproductive condition. Aside from the obvious basic requirements of suitable food and water, which should be cleaned and refreshed regularly, mice also need nesting and burrowing materials, enrichment (such as wood blocks for gnawing, cardboard tubes and soft paper) as they prefer to nest close to walls and make small spaces of their own. Mice are also highly sensitive to too much noise. Given that the CVO during our investigation at VUB was seen to deny use of enrichment materials (wood) in mouse cages due to potential impact on experimental results, this lack of enrichment and poor welfare is likely to have heightened their suffering.

The VUB animal facility also makes extensive use of IVCs for mice (Individually Ventilated Cages) yet welfare problems are associated with these. In particular, use of high level IVCs can cause poor air quality, draught, heat loss and chronic stress in mice if not adequately insulated and temperature controlled, as well as lacking natural environment enrichment.^{vii}

Despite public perception, rats have high levels of intellect, cognition, affection for their young and social skills. Their skills are such that, despite being intensively bred for many decades, so called 'laboratory rats' still try to retain some of the behaviours they possess in the wild, including skills in foraging and protecting their young and their sensory perception is very high.

Despite being a commonly used method of killing, CO₂ asphyxiation is known to cause severe welfare problems for rodents, with studies showing acute pain in rats and fear in mice.^{viii}

Decapitation of rodents was also found to be routinely used as a method of killing at VUB and legislation states that this should only be used when other methods are not possible .

The use of freezing of live rodents, as also observed frequently during our investigation is a serious breach of welfare as the animals would have experienced severe distress, associated with heat loss, as they succumbed to dramatically falling temperatures.

Mice and rats clearly show pain, distress, discomfort and anxiety by displaying a wide range of stereotypical behaviours which include sitting in a hunched position; over grooming (leading to baldness seen in some animals at VUB); frantic paw and gnawing movements which can resemble nail biting; tail chewing or chewing one's own limbs; jumping up and down and climbing walls of cages.

Given not only the impact of suffering due to experimental procedures but the neglect and incompetency of staff in allowing stereotypical behaviour to continue 'as it's OK' or 'expected', serious welfare issues are present at this facility. Furthermore, deliberate failure to address overcrowded cages led to aggression, fighting and extensive injuries in some mice, including severe bite marks and tragically, cannibalism of weaker animals. Our investigator observed larger animals crawling over smaller ones and dead pups, likely to have suffered and died due to suffocation and overcrowding

Pigs

Sows and baby pigs like to be in social groups when established and can suffer from separation anxiety when moved to isolated pens. They also have specific needs for flooring, foraging and access to water to cool down when needed. Stereotypical behaviour can ensue when pigs are bored, frustrated or in pain, including biting the bars of the cage/pen, biting and chewing tails and rubbing against the floor and walls. Aside from basic welfare needs, pigs are highly affectionate and intelligent animals and respond well to gentle and compassionate routine care and handling. This is highlighted by the fact that research staff at VUB were aware of the sensitivity of the pigs to associating certain researchers with causing pain.

The VUB animal facility makes extensive use of pigs (including minipigs), for example in fertility and diabetes experiments. Very concerning findings were made with the housing and care of pigs, with one animal in particular suffering and dying from blood poisoning due to an infected catheter. Further welfare concerns were seen when staff were incompetent in administering pain relief to sick pigs and medicine was also seen to be three years out of date. Critical video footage was also obtained showing the brutal decapitation of piglets, which is not a permitted method of killing according to national and EU regulations (only being permitted for birds up to a certain size and rodents only when other methods are not possible).

Rabbits

Rabbits, like all animals have the basic rights to suitable food, water, bedding and quiet space to sleep and nurse their young. As they are particularly social, playful and inquisitive animals, they benefit hugely from being in companion relationships with one or more other rabbits and easily and quickly display signs of stress and upset from isolation and fear, loud noises or the threat of harm. Stereotypical behaviour in rabbits includes chewing cage bars, over grooming and grooming at speed, moving in circles and sitting in a hunched position, showing anxiety.

Given the sensitivity and specific welfare needs of rabbits, the findings of those housed at VUB were particularly alarming. The rabbits seen had suffered in cardiac experiments over a year ago.

These poor animals had been found still caged in the laboratory and had their hearts damaged to such an extent that they were in a fragile and highly stressed state, so much so that transporting them or even just moving or cleaning their cages could kill them from stress/panic – tragically, our investigator was informed that animals had already died like this.

There are key species differences in rabbit and human hearts; rabbits hearts are smaller, beat higher and faster and are particularly prone to stress. Rabbits are often the experimental 'model' of choice used in desperate attempts to try and improve on poor results in other animals, e.g mice and rats.^{ix}

Animal experiments at VUB

Some key examples of recent animal experiments carried out at VUB are summarised below. In the analysis, the details of how the animals were used (including procedures, numbers of animals) are provided wherever available, as well as an analysis of the levels of suffering the animals would have experienced, taking into account both publicly available information and links to evidence revealed by our investigation.

1. A recent, highly experimental MRI imaging study, co-authored by the **Chief Veterinary Officer of the VUB Animalarium** involved Gottingen mini pigs, to investigate whether using a higher magnetic field strength during imaging (known as '3T') caused a significant temperature increase in the unborn animals, on the basis that temperature rises during imaging are associated with a risk of birth defects (teratogeny).^x Six pregnant mini pigs (at various stages of pregnancy) were anaesthetised and operated on to insert catheters into the brain, bladder and abdomen of their unborn foetuses, as well as the amniotic fluid sacs. Following this, temperature sensors were inserted via the catheters. They were also injected with birth-delaying hormones. The animals then underwent a series of MRI scans lasting 15 minutes each, during which the foetal temperatures were monitored with varying strengths (including those used in human MRI) totalling to 1 hour of scans per parent animal. Following the imaging process, the mini pigs were injected with an agent to induce labour, after which the delivered foetuses were killed and dissected for post-mortem examination. It is unclear from the paper whether the mother animals were used in further experiments, as they were apparently not killed on this occasion.

The setbacks and difficulties of using animals in this experiment are clear. Firstly, the mini pigs were registered on the MR machine with false bodyweights (approximately twice their own weight) to mimic human children ' *otherwise they cannot be reasonably expected to be representative of the human population*'. Secondly, the authors also state that '*it is clear that any extrapolation from the animal model to the human situation needs extreme care and a straightforward easy and accurate model for this is currently unknown*'.^{xi}

The authors concluded that in pregnant miniature pigs, the use of 3-T magnets during MRI did not lead to significant temperature increases above 1°C, if imaging time was kept below 30 minutes, but that from this study, human predictions cannot be made. '*It therefore seems essential that we remain cautious about the use of 3T magnets in pregnant women....of longer than 30 minutes until further research is conducted*'.^{xii}

The study also shows a clear failure to recognise alternative approaches to animals. This is explained further in the 'Alternatives' section later in this report.

Mini pigs arriving at the facility

As noted in investigational logs for April 2016, some mini pigs arrived at the Animalarium from Denmark. This may be for work similar to that published by the CVO and the team previously, with more mini pigs intended for use in NMR imaging experiments.

Suffering in Pigs

The use of catheters in the mini pigs in the above experiment relates to suffering observed by our investigator in a pig used for cancer experiments. The pig suffered greatly due to an infection in the catheter and subsequent blood poisoning and had to be killed.

2. Many of the researchers encountered during the investigation are linked to the **Laboratory of Molecular and Cellular Therapy (LMCT)** ^{xiii} ^{xiv} who carry out extensive in-vivo experiments in animals involving cancer growth and vaccine testing. A number of senior members of the group recently published an HPV vaccine study in mice. ^{xv} A total of 68 mice were purchased from Charles River in France (there is on-going, routine purchase and transport of mice to VUB from various CR sites worldwide). The mice were females of 6-12 weeks old, to be used for testing of new HPV/cervical cancer vaccines; in particular in this experiment, how cancer develops when induced in three different areas, subcutaneously (under the skin); in the lungs or in the genital tract of the animals.

For the subcutaneous experiments, the mice were injected in the right flank with tumour cells. Once the tumours had grown to a certain size to test either early (day 6) or late (day 21) tumour development, they were injected with vaccine three times with 5 day intervals. For the lung tumour experiments, further mice were injected with tumour cells directly into the nerves underlying the ribs (intercostal nerves) and further mice had their genital tracts destroyed with hormones and injected with tumour cells before being repeatedly vaccinated. Further animals also were injected with chemotherapy agents as well as the vaccine to test this as a form of combination therapy. All mice were killed and dissected for further examination.

The paper states that '*data collection was stopped when one of the humane endpoints dictated by Ethical Committee of Vrije Universiteit Brussel was met. In this regard mice were sacrificed when tumour size exceeded 2000 mm³ or the body weight loss was greater than 20%*'. This information was supported by observations made during our investigation of mice with large tumours, allowed to grow to maximum permitted before the mice are killed.

This publication is key as it is one of the most recently available and involves a number of key authors named in the investigational diary logs, responsible for dead and injured mice.

3. The LMCT also carry out extensive experimental research in mice to investigate **mRNA based anti-cancer vaccines**. In a 2014 study carrying a high level of severity and suffering, xvi, co-authored by several researchers encountered during the investigation, female genetically modified (GM) mice of 6-12 weeks old were purchased and transported from Charles River USA to VUB. Other GM mice (with a deficient immune system) were also supplied by the University of Ghent for harvesting and supply of cells. The aim of the experiment was to investigate the injection of a particular mixture of fundamental genetic material (known as mRNA) cells and proteins (the mixture being termed a 'fusokine') to stimulate the immune system into suppressing tumour growth.

Firstly, the mice were inoculated with several types of mRNA containing cancer cells to induce deliberate tumour growth of up to 500mm³. When the mice had developed tumours of this size, they were split into groups to receive either a single injection of the test protein mixture or a control. The test mixture was found to delay tumour growth for 3 days, so the researchers continued to inject the mice every 3 days, increasing to a 5-fold higher dose of the test mixture to see if this affected tumour growth further, again versus mice injected with a control . The experiment found that the test mixture delayed tumour enlargement in the mice, by continuing for 30-40 days, with the animals dying as they succumbed to the effects of the tumours which, according to graphical presentation, were allowed to grow up to 2000-3000mm³ in size. (Note; other recorded conversations confirm how mice would be killed once tumour size reached between 1500-2500mm³ depending on the experiment¹). There is no reference to any humane evaluation of the condition or on-going suffering of the mice , merely that the mice were '*housed and handled according to the regulations of the Animal Care Committee of the VUB*'.

This type of cancer experiment corresponds with several pieces of conversational and visual evidence on the mice with large tumours observed during our investigation. **Of the most grave concern is that one of the authors of this experiment, with the highest levels of severity and capacity for suffering, serves as a member of the VUB Ethics Committee on animal experiments and according to conversations with staff , appears to routinely disregard welfare and care of the animals.** More detail on this is provided later in this report.

4. '**eTheRNA**' is a biotech company, set up as a spin-off from the VUB in 2013. xvii This was due to the development of the 'TriMix' technology by the LMCT, headed by lead researcher Kris Thielemans. It is called 'TriMix' due to the three types of mRNA in the product, designed to activate cells of the immune system into fighting cancer growth. The company website states how the technology has so far been tested 'ex-vivo' both '*validated in mouse models*' and '*four clinical trials in humans*'; two phase I xviii xix and two phase II studies, the latest published in March 2016 xx Ultimately, the company intends to make its final product 'in-vivo' for humans i.e. direct injection without the use of cells as carriers.

Both of the animal experiments described in sections b) and c) above relate to the TriMiX/eTheRNA research team. Further animal research carried out by the team for its commercial products includes yet more experiments to inject mice with tumours xxi and as can be seen from the investigational evidence, extensive animal use continues, despite this technology now being widely tested, far more relevantly in humans.

Karine Breckpot manages approval and use of the Virus Production Unit in the animal facility at VUB. As well as co-authoring on mouse studies as above, she has recently co-authored a number of papers, including cancer experiments in mice which were '*further validated in cancer patients in comparison with healthy donors*'.^{xxii} This demonstrates a common and very retrograde step i.e. the use of human studies to validate animal tests for further use.

Conversational evidence recorded by our investigator with staff explained how Breckpot disregarded overcrowding by putting six mice in cages only meant for four and apparently failed to check or supervise other staff in appropriate monitoring and care of animals, for example with severe tumour growth.

This is even more concerning given that Breckpot, along with Chief Veterinary Officer Sigrid Van Laere is a member of the VUB Ethics Committee on Animal Experiments. Breckpot's actions are highlighted in several of the conversational diary logs from the investigation. A number of recent anti-tumour research publications by key team members described at the LMCT demonstrate the routine use of mice observed during our investigation. xxiii xxiv xxv. The fact that researchers routinely carrying out such invasive and severely painful experiments are permitted to serve on the ethics committee demonstrates the high level of bias and severe lack of independent monitoring of animal research that carries on daily at VUB.

The CVO has very recently co-published new animal experiments, involving experimental dissection of the front legs of rats to cause severe swelling and inflammation, to investigate risk factors of breast cancer from comparing types of surgical techniques. xxvi Again, this is a highly experimental study causing pain and suffering in rats, when far more humane and scientifically relevant clinical studies are carried out on identical techniques in humans, to directly help breast cancer patients. xxvii

5. The Haematology and Immunology Group (HEIM) at VUB works under three main areas which are detailed on their website^{xxviii} xxix; they are Multiple Myeloma (bone marrow cancer); Stem cell research (for blood stem cell therapy) and Thrombophilia.

As noted by our investigator, the group's research involves extensive bone marrow experiments in mice. This research spans across the first two areas described above. One of the 'models' most commonly used is the 5TMM (multiple myeloma) mouse model *which they are using along side human MM patients and healthy volunteers.*

Negligence was observed during our investigation with regard to sick and injured mice as a result of these experiments. Corresponding publications by the team include severely inhumane tests^{xxx xxxi} involving the mice being injected with melanoma cells to induce tumours to grow under their skin, before being force fed a test, anti-cancer drug *already in human use and provided for free to the researchers by Pfizer* and finally, subjected to stereotaxic surgery to inject cancer cells directly into their brains. Little regard is given for animal welfare in this publication, simply stating that '*All animals were bred, housed and handled according to the European guidelines for animal experimentation. All experiments were reviewed and approved by the ethical committee for use of laboratory animals of the Vrije Universiteit Brussel.*'

The HEIM group is in some cases sponsored by major names such as Janssen Pharmaceuticals or AXA Research^{xxxii} for example injecting them with multiple myeloma on a daily basis (2014) but also using readily available human cell lines.^{xxxiii} This highly inhumane study split the mice into two groups. The first group were injected with cancer cells six days a week, then all killed after three weeks and examined. The pain and distress they experienced included hind limb paralysis. This links to many observations of paralysis in the mice used across the research teams at VUB during our investigation, for example a conversation was logged on how the hind legs of the mice were becoming paralysed and drilling holes was a technique used. This observation is supported by descriptions of procedures from the HEIM team which show how tumour and bone marrow levels are assessed by isolation from the hind leg.^{xxxiv}

In an additional, severely cruel and inhumane second set of procedures in this experiment, a further group of mice suffered in a so-called 'survival study', basically meaning that the animals continued to be injected with cancer cells until each one died. These experiments use a 'well established' and very commonly used series of mouse 'models' known as '5TMM', bred and used over and over again at VUB for years.^{xxxv}

Findings during the investigation reveal the suffering and lack of concern for animal welfare in the HEIM group, with technician staff responsible for the animals used by the team *stating how it is not ethical to let animals suffer this long and if inspectors were present, a warning would be issued due to the unethical state the animals had been left in for too long*, with very enlarged spleens from

injection of cells to create bone marrow tumours. This information cross references with publications co-authored by the HEIM team, mentioned also in diary logs and in the HEIM group research profile.^{xxxvi}

As stated above, mice used in these projects become paralysed . Stereotypical behaviour observed in mice used in these projects also includes jumping up and down and a senior researcher was recorded even laughing at this on one occasion. Video footage recorded also shows the mice behaving like this. Another recent bone marrow experiment in mice by the HEIM group gives little away on the suffering shown in the diary logs, but appears to be directly relevant and explains how mice (purchased from Harlan in Holland) are injected with bone marrow tumour (multiple myeloma) cells to become diseased, before being killed and their cells extracted for injection into further, healthy 'naïve' mice.^{xxxvii}

6. The BENE (Beta Cell Neogenesis) Group^{xxxviii xxxix} focus on generation of Beta cells to regulate insulin production and find potential diabetes treatments. They make extensive use of animals. Publications co-authored by the BENE team include deliberately injuring the pancreas in mice, by tying off and blocking blood vessels , a well used technique known as 'partial duct ligation', so well known it is abbreviated to 'PDL'.^{xl xli}

Several investigational diary logs of conversations, as well as video footage throughout the March - April 2016 period reveal how BENE staff routinely left many animals in overcrowded cages with no water or food (in one case, 17 mice and further pups were left in a cage meant for 5 animals- some mice died as a result of this) ; how staff failed to check on animals and comments by technicians and caretakers on a need for the situation to change. Lack of care and appropriate hygiene/cleaning for the animals was also found to be particularly poor by the BENE team on repeated occasions. One particular researcher was mentioned as being negligent in not checking on mice injured for over a week.

In turn, the closely linked Beta Cell Group at VUB, also involved in diabetes research, focus on animal 'models' of pancreatic injury and regeneration. Betacell^{xlii} are a company located very close to the VUB campus, led by Lab Director Sofie Notebaert. They state that they work closely with the diabetes research department at VUB. Their current headline product is 'BetaGraft', an anti-diabetic preparation containing insulin-producing cells harvested from piglets, which they state on the website has been tested in mice and preclinical testing is still being 'accelerated' with the aim of human trials soon- the cells are 'humanised' pig cells being trialled in volunteers. This corresponds with initial findings and conversations in our investigation on how the piglets are being used and killed for their cells.

7. The Liver Cell Biology Lab (LIVR) is another department on the medical campus at VUB who routinely conduct animal experiments . Recent examples include deliberately damaging the livers of mice by surgically tying off their bile ducts (known as bile duct ligation or BDL) then feeding them chilli pepper extract to see if it had a therapeutic effect on their injuries^{xlii}. Not only is this a highly experimental and curiosity driven study, but far more directly relevant results would be obtained in alternative, human *in-vitro* tests or ethically controlled clinical trials in human volunteers and patients, as the extract used (capsaicin) is already widely available for human use.

Disturbing diary accounts during our investigation reveal yet more cruelty and negligence to animals at the hands of the LIVR team. In one account , *a sick and distressed mouse failed to be checked properly and was being eaten by other mice*. Two PhD students responsible for this and other mice behaved irresponsibly, as it became clear that they knew this particular animal was suffering but still left it in the euthanasia station for several days. In other cases, very sick and suffering mice used by the LIVR team, seen to need urgently euthanasing by several research staff and technicians, were also left for several days later until after the weekend as the CVO could not be reached. Some animals died in the meantime.

A further, gravely concerning conversation with a researcher from the LIVR team reveals how they

sometimes forget to give water to the mice that they use and also how the mice cannibalise each other. Additional disturbing conversations recorded with this researcher exposed that they do not know how to see signs of pain and distress in the animals and they do not know what pain level they are working to (Mild/Moderate/Severe as per animal experiment regulations).

8. The Dept of Immunology & Physiology (FYSP) includes several senior researchers and

has been carrying out various experimental immunology, cancer and HIV testing in mice for years, For example back in 1998 to infect mice with cancer cells to develop tumours to test vaccines^{xliii} and many older experiments, as well as more recently published research in 2014 to implant tumours into mice to test rejection of grafts.^{xliv} Further recently published experiments on naïve, knockout mice include a highly experimental, curiosity driven study by the immunology and diabetes teams, involving surgically inducing pancreatic injury in the animals (as above, by a commonly used and highly inhumane surgical procedure known as partial duct ligation (PDL)) to examine specific immune responses.^{xlv}

The team co-author many papers on a variety of research areas, for example from HIV to cancer, diabetes to arthritis, many appear to be experimental and repetitive research (e.g collagen induced arthritis in animals has been repeated over and over again in thousands of experiments for decades and inflicts some of the most severe levels of suffering and pain on the animals.^{xlvi}

A critical conversation with a researcher from the FYSP team revealed a major failure of animal welfare at VUB some months ago, with a lack of supervision in the most severe cancer-inducing studies in mice. The researcher (then a junior under the supervision of Karine Breckpot) explained how he was allowing mice to develop tumours of 4000mm³ in size, when they would normally be killed at a size of 2000mm³, (other publications by the team also state how mice are killed once tumours reach 2500mm³)^{xlix} basically watching them die in agony as he had been given no guidance.

9. The FASC group (Dept of Pharmaceutical Chemistry and Drug Analysis)^{xlvii} at VUB perform some of the most severe and stress-inducing animal experiments, including invasive brain surgery to induce epilepsy and fits in mice and try to mimic the symptoms of human Parkinson's disease in rats; as well as depression studies in aging mice, using cruel and outdated testing methods like the forced swim test, where rodents are forced to keep swimming in a cylinder or platform full of water they cannot escape from, to induce a state of 'learned helplessness' which researchers attempt to link to human anxiety and depression. Other animal suffering at the hands of the FASC team includes a severe, invasive and extremely cruel brain experiment published in August 2015 to investigate response to anti-epilepsy drugs xlviii during which rats were mounted in a stereotaxic frame and their brains injected with a chemical (to allow radiolabelling for clarity in visual examination later) before being killed by lethal injection and their brains removed for dissection. Additional 18 day old rat embryos were dissected for brain structure examination in separate procedures within this study. With no regard to ethics, the authors merely state that '*any distress caused by the procedures is in accordance with various EU and VUB guidelines*'. In other words, claiming that the animals suffering is legally permitted. In further, highly invasive and inhumane procedures, more rats underwent stereotaxic surgery, to insert electrodes into their brains which were connected to electrotransmitters implanted under their skin and microtubing to deliver fluids, before also administering drugs to deliberately induce seizures and measured according to a series of effects described as stereotypical mouth and facial movements, 'wet dog shakes'; head nodding and head on one side, moving in circles, tremors, staring; limb shaking, rearing and falling. In accordance with the diary logs recorded during the investigation, there appear to be numerous, somewhat dismissive comments from the researchers about this being 'expected' or 'stereotypical behaviour'. It states that the numbers of animals are '*kept to a minimum*'. Many similar tests are quoted as already having been performed in different animals. Some of the same researchers, plus others have published further severe seizure experiments in mice.^{xlix}

Further recent experiments co-authored by the FASC group include cruelly invasive stereotaxic

Parkinson's disease experiments in rats¹. The animals were anaesthetised and killed and it is very likely they were decapitated to remove the brains for analysis, as this is a routine procedure. This corresponds to some of the observations noted in the diary logs during our investigation. Other recent publications include highly stressful experiments to induce epileptic seizures in mice and drug administration via the tail.ⁱⁱ This is well known to be not only a difficult procedure, but very painful for the animals and corresponds to tail injuries observed, as well as neglect by the researchers in not checking on the mice adequately, especially according to the logs during April 2016 when our investigator noted great concerns.

Investigational findings also expose how mice were used in aging projects on anxiety and depression, involving being caged in isolation (in itself very distressing for mice who are sociable animals) in preparation for their brains to be removed for analysis. According to paragraph 3.3.1 in annex 4 of the Royal Decree of 2013, "all animals, except solitary ones, must be kept in stable social groups of compatible individuals. In case individual housing is granted for exceptional scientific purposes, or to ensure their welfare, which needs to be confirmed by a positive, ethical evaluation, the length of their confinement must be limited to a minimum required period. Visual, auditory, olfactory and tactile contact must be maintained with the other animals. In one particularly sad case, a researcher revealed how an aged mouse was isolated for this purpose, but forgotten about until it was 'too late to take out the brain', therefore the animal was subjected to these procedures for nothing. This emphasises once again the complacent and negligent attitudes of research staff. Recently published experiments at VUB relating to this include inducing mood disorders in adult vs aged mice, involving several pointless and inhumane tests repeated over and over again by many different researchers over decades, to induce anxiety, fear and depression in the poor animals. Tests include such cruel and archaic methods as forced swimming, tail suspension, maze analysis and light and dark exposure.ⁱⁱⁱ Other relevant experiments here include comparing increased depression in isolated vs socially housed animals.ⁱⁱⁱⁱ

Conversations recorded reveal anxiety research staff explaining use of the 'fear conditioning box' during brain experiments in mice. The animals are placed in the box and hear a tone, as well as receive a shock. Testing also often involves shocking the mice as they eat food or applying a shock to the toes. The aim of these barbaric, pointless and cruel tests is to then reduce the fear in the animals by removing the shock and only playing the tone. The mice suffer substances being injected often directly into the brain. Other experiments include hanging mice by their tails and injecting hunger inducing substances into the brain to attempt to make links between fear, trauma and obesity in humans.

Further, on-going breaches of guidelines were revealed in the FASC group. For example, one female mouse became accidentally pregnant under the responsibility of one researcher and placed in a cage with three other females- this is against regulations since only one pregnant female per cage is permitted. When questioned on this, deputy veterinary staff stated how protocols to deal with this were still yet to be written.

Our investigator found a particularly concerning lack of communication and animal monitoring within the FASC department, with the same researcher as mentioned above going on holiday and leaving very, very sick and distressed animals uncared for, with no system in place to ensure other staff monitor the animals or were available when needed, although it was observed that another researcher who was not responsible for the animals did check on them. One mouse was in such a poor state and clearly in pain that the investigator insisted that euthanasia be carried out immediately, after receiving a nonchalant and indifferent response from staff.

10. The Pharmacology Department (FARC) carries out extensive animal experiments. Video and conversational footage obtained with one staff member from FARC shows him using the guillotine to decapitate rats and then they are 'bled out' (exsanguinated) and their livers removed for experiments.

11. The **EMGE (Embryology and Genetics)** group^{liv} are part of the Faculty of Medicine and Pharmacy at VUB. They conduct fertility experiments on animals. Examples include senior investigators in the department injecting cells from the testes of mice killed for their cells, into human testes (donated from cadavers)^{lv} One researcher in the department currently studying for a PhD carries out transplantation studies in mice to investigate sperm cell fertility and the mice are subjected to radiation in some tests. When asked, the researcher did not know what pain level (Mild/Moderate/Severe- Levels 1-3) he was working on. Investigational footage showed mice in this researcher's care to be very unwell, with hunched posture, poor fur condition and in urgent need of overdue medical care and in some cases, immediate euthanasia.

Alternatives to animal testing

There are a wealth of human-derived research approaches which can and should always be investigated as an alternative to animals. Generally speaking these include lab based human *in-vitro* methods, *in-silico* (computer based) approaches and most fundamentally, making use of the vast and ever-increasing amount of human clinical and epidemiology (population) data available from research, in both volunteers and patients. Failure to genuinely and comprehensively investigate alternative methods places any Belgian animal research establishment in breach of national animal welfare legislation^{lvi} which states that '*experiments can be performed only if the objectives cannot be achieved by other methods*' (Art. 24(2)) and EU directive 2010/63 which '*seeks to facilitate and promote the advancement of alternative approaches. It also seeks to ensure a high level of protection for animals that still need to be used in procedures. in light of evolving science and animal-protection measures*'.

We would also like to mention article 29 of the Royal Decree of 2013 which stipulates that “in order to avoid the repetition of experiments to meet national or European legal requirements, it is forbidden to conduct animal testing, if data were generated in another member state, obtained from experiments approved by EU legislation, unless it is absolutely necessary to conduct additional experiments based on the existing data in order to protect public health, safeguard security or the environment.”

Yet, despite such measures in national legislation, as well as other member states, it would be naïve to ignore the well-known reality that many research departments, entrenched in long-term animal use, simply continue such use without ever considering any other methods.

The routine use, neglect and suffering of animals at VUB demonstrates an apparent disregard (deliberate or otherwise) for alternative, non-animal methods, particularly when far more directly relevant, human-based approaches are available. This is unsurprisingly common in a research department heavily focussed on use of animals on a day to day basis, where seeking the use of alternative approaches is either ignored, discouraged or simply not even considered, especially as many or all of the staff may have become accustomed to using animals throughout their education and careers. This is a much wider problem within the animal research community generally. There is arrogance, complacency and failure to acknowledge more relevant studies in humans as well as disregard for data from previous animal experiments, which would at least spare further animals from suffering. For example, with regard to the invasive uterine study co-authored by the Chief Veterinary Officer (see section a above) , this was a highly experimental study in mini pigs, especially given the significant amount of publication evidence of 3T imaging already being performed in human pregnancy, including a series of clinical studies in Spain.^{lvii lviii lix lx} and recent 3T imaging during post mortem studies at KU Leuven.^{lxi} Others include human foetal studies cited by the authors.^{lxii lxiii} and specific comparison of temperature changes when comparing different strengths of MRI (1.5 T and 3T magnets).^{lxiv} The mini pig paper also outlines that '*to date, no hazardous effects of MR imaging on human beings have been reported and the potential prevalence of delayed sequelae seems to be low or even non existent.*' ('sequelae' being after-effects or side

effects).^{xvii}

With regard to other animal experiments at VUB, for example the research into HPV/cervical cancer vaccines by the Laboratory of Molecular and Cellular Therapy, the failure of animal testing to deliver accurate prediction in humans is widely recognised, but used to justify further attempts using animals. A very recently published study by the LMCT team^{lxv} highlights the '*lack of appropriate mouse models*' and failure of animal tested (preclinical) vaccines to translate into clinical trials and how '*It is therefore critical to improve the translational value of animal experiments by utilizing models that more accurately reflect the human pathology and allow for better assessment of new therapies*'. Although the papilloma virus exists in other species, by its very nature HPV (Human Papilloma Virus) occurs only in humans. therefore human based research is the only model that is appropriate, Examples of human based research into HPV include *in-silico* (computer based) analysis of the genetic structure of the HPV vaccine, to identify and extend protection against several different types of the virus, where currently available vaccines fail, having been tested and considered adequate in animals.^{lxvi}

In some cases of experiments, animals continue to be used experimentally, even after testing in humans has commenced. In the case of the eTheRNA spin-off company research, mice continue to be used, even though the technology has been trialled in phase 1 and II studies in patients.

Conclusions and References

Summary of findings and concerns

Our investigation, via extensive visual and recorded conversational footage, exposes a situation of grave concern at VUB, with a catalogue of pain, cruelty and suffering in the animals housed and handled, not only as a result of their intentional use in procedures, but from incompetent care, neglect and clear, routine disregard for their welfare and after-care, for example of animals in a fragile post-surgery condition, or with injuries requiring attention.

Our findings, coupled with the severity of the nature of the procedures the VUB teams are carrying out, as confirmed in available publications (e.g.severe brain surgery and stereotaxy, deliberately inducing large tumour growth and allowing extra suffering) suggest a concerning lack of regard for animal welfare and desensitisation among staff, supported by evidence of failure to respond to investigator/caretakers concern over animals, and disregard of alarm over injury, disease state and infighting. One senior researcher is also the President of the Ethical Committee for animal experiments at VUB and has been seen to be somewhat indifferent to concerns at overcrowding in animal cages. It is especially useful that some of the animal technician staff appear to reveal neglect and disregard by some of the researchers. Violations of the existing law add to these problems.

This exposure of malpractice at VUB should warrant an official investigation by the relevant authorities. GAIA urges the Brussels Animal Welfare Secretary of State, Bianca Debaets, at least to suspend and eventually revoke the VUB's licence to conduct experiments on animals until the routinely, daily cruelty towards and suffering and neglect of laboratory animals as witnessed at the VUB is brought to an end.

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