TCDD

TOXICOLOGIE

NUMMER 2
MEI 2021

SPECIAL THEME

FOOD & FEED TOXICOLOGY

- LEMONGRASS TO REDUCE METHANE IN COWS
- PLANT HEME “MEAT”, IS IT SAFE?
- ARE MEAT REPLACEMENTS SAFE?

LikeMeat Like Schnitzel (Crispy Chicken) - Soya based,
Photographer & cook: Line Tscherning

NEDERLANDSE VERENIGING VOOR TOXICOLOGIE

Colofon

Toxicologische Communicatie, Data en Documentatie

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Bestuursvergaderingen 2021
17 Mei, 20 September, 15 November

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Het lidmaatschap wordt automatisch verlengd tenzij de NVT-ledenadministratie vóór 1 december van het lopende jaar schriftelijk of per e-mail een opzegging heeft ontvangen. Hiervan ontvangt u een bevestiging.

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TCDD
TOXICOLOGIE

Inhoud

TERUG NAAR INHOUD

Editorial

It looks as though we are entering the last couple of months of a long locked down period where working from home has been the standard for most of us. I hope you have handled it better than I did. “Home alone” was bad enough as a series of awful movies, but the added meaning of sitting in a small attic room behind a computer screen doesn’t help. I’m sure that something good will hatch from an egg that looks pretty hideous from the outside, though; many of us will appreciate our colleagues (even) more than we did before and an office will suddenly be a much more appealing working environment than it seemed before the virus hit. And we have learned to be efficient and effective while working from home; toxicology did not come to a standstill, but our community kept pushing it forward. I anticipate a wave of inspiration and positive energy in the toxicology community in the time to come.

We hope the current TCDD issue will provide something for the reader to enjoy. The theme for this issue is “Food and feed toxicology”; for instance, we have an article on feed supplementation to reduce methane production by cows. There are indications that this can be quite effective. Reducing their methane output is not as effective as switching to other protein sources, of course; my colleague Martje has written a piece about meat replacements, from the safety perspective especially. At some point humanity will need to start eating something else than animal meat because of the associated impact on the climate. It is interesting to see what hurdles, from a toxicology perspective, need to be overcome to make this transition a reality. Adding to this topic, Barae has written an article about the addition of plant hemoglobin to meat replacement products, to for instance create what is called the “Impossible Burger”, that can even be ordered medium-rare or “bloody”! But what about safety?

Of great interest to many will be the overview of the re-registration procedure for toxicologists which is given by Martijn van Veldhoven: after working so hard to become ERT, of course we want to keep that status. Because being a registered toxicologist carries substantial weight and responsibility, a procedure is needed to be able to prolong registration, however. Read all about it in the current issue.

Finally, this TCDD issue contains interviews with Erik Houthoff and Josje Arts, who both work for Nouryon. Many of our colleagues work for the chemical industry, their perspective is interesting and we should keep in mind that chemical innovation leading to cheaper and better products is something that society desires. People also desire safe products, a long and healthy life and a clean environment free of pollutants, so personally I am happy that toxicologists are employed by chemical companies to provide this perspective.

We wish you all the best of luck in what we hope will be the last months where COVID massively impacts society. Eyes on the prize: it will be worth it. For now, enjoy the TCDD issue.

On behalf of the editorial team,

Damiën van Berlo

Photo by Jess Bailey on Unsplash

News from the board

Welcome to the latest issue of TCDD! With a main theme like 'Food and Feed Toxicology,' I'm sure you're as interested as I am to find out what the TCDD editorial board has in store for us this time!

We look forward to seeing you all at our virtual NVT Annual Meeting which will be held on **June 9-10 2021, at the Reehorst, Ede.** We will hold our **members business meeting on June 10 at 13:00-14:00**. The annual meeting promises to be very exciting, with as main theme "*The (r) evolution of toxicological models – how to address safety in target species."* As in previous years, the first day will be specially tailored to our students and PhD candidates, while the second day (June 10) will be for all members of our society. Don't miss this great meeting! https://toxicologie.nl/meeting2021

Let me also take this opportunity to promote **ICT2022, the International Congress of Toxicology, a joint meeting of IUTOX and EUROTOX, organised in cooperation with NVT** (www.ict2022.com). The theme of the ICT2022 is "Uniting in Toxicology" and reflects the desire to address issues related to different disciplines. By working together, these disciplines, with their diverse perspectives, help to promote and encourage the use of new and innovative techniques and strategies that will continue to reduce risks to human health and the environment. The NVT Board encourages members to actively contribute. This can be done until June 1, 2021 by submitting a proposal for a session. After that, there are still opportunities to register for individual presentations, mostly via poster. See Call for session proposals – ICT 2022

On another note, we'd like to remind you all that since 2010, the board maintains a list of members who have consented to be contacted by journalists for questions about toxicological topics. On the NVT website, the secretary of the board is the contact person for the association and can therefore be called by journalists looking for a toxicologist. Our list of members who can be contacted by journalists needs updating! **If you would like to be included in the list, please contact the secretariat for more information**.

Finally, for any of you interested in **joining the NVT board** and playing a role in the important work of our Society in the coming years, we are still looking for volunteers! We have a position available for general board member, preferably for someone working in academia. Please contact me for more information.

Kind regards,

Juliette Legler,
president NVT

NVT 2021 ANNOUNCEMENT

The (r)evolution of toxicological models – how to address safety in target species

As toxicologists we aim to assess the safety of substances in the species we wish to protect, *i.e.* the target species. However, often we cannot simply conduct toxicity testing in the target species itself. This forces us to use model systems, traditionally animal models and nowadays also *in silico* and *in vitro* models. Obviously, these models are a proxy of the truth and have their own pros and cons. While animal models have many advantages, there is an urgent need for replacements. The Dutch government strongly promotes the reduction of animal testing. In addition, the U.S. EPA announced that they will stop conducting or funding mammalian animal studies by 2035. During the upcoming NVT Annual Meeting, we will talk about safety assessment and toxicity testing in target species and discuss the challenges that we face in applying alternative models into (regulatory) practice. What are the best models for the target species of interest? Can we measure within the target species itself? When do we have sufficient information to predict safety in target species? How do we implement innovative and predictive models in regulatory risk assessment? Join our discussion on these important issues during the hybrid NVT Annual Meeting on **June 9-10th**, **2021**, in the Reehorst, Ede and online.

SECTIE GENTOX EN DART

Application of innovative stem cell technologies in genetic toxicology, teratology and reproductive toxicology

In the last decade, stem cells have been the subject of increasing scientific interest because of their utility in numerous applications. Recent progresses in the field of Induced Pluripotent Stem Cells (iPSCs) have opened up many fantastic opportunities for research into new therapeutic possibilities but also in toxicology. iPSCs are the cells which are reprogrammed from somatic cells using different transcription factors. Stem cells, including iPSCs possess unique properties of self renewal, they can be continuously cultured in an undifferentiated state. In addition, they can be differentiated giving rise to more specialized cells of the human body such as heart, liver, bone marrow, blood vessel and nerve cells. Therefore, stem cells are an important new tool for developing unique, *in vitro* model systems to test drugs and chemicals and a potential to predict or anticipate toxicity in humans.

In genetic toxicology, stem cells have been used for a long time in mutagenesis and genome stability research. Due to their stable diploid genome and high replication rate, stem cells have been applied extensively for mutation analysis and mutation fingerprinting, gene targeting, CRISPR-mediated gene modifications and genome stability research. More recently, mouse embryonic stem cells have been in various toxicogenomics studies as well as in the ToxTracker reporter assay for genotoxicity testing.

In the last years, a great deal of research has revolved around the implementation of stem cells for developmental toxicity testing. Differentiation of mouse embryonic stem cells has been used to test the developmental toxicity. The mEST was validated by the European Center for the Validation of Alternative Methods and was able to correctly categorize 78% of tested teratogens. The availability of human induced pluripotent stem cells has spurred the development of assays for developmental toxicity testing. Examples of some of the assays that have been developed using iPSCs are emerging.

This fall, the genetic toxicology and teratology and reproductive toxicology sections of the NVT will organize a joint symposium about the state-of-the-art developments and applications of stem cells in toxicology.

Date: Thursday November 18, 2021
Time: Afternoon

More information about the program and registration will follow soon.

SAVE THE DATE

NVT sectie Risicobeoordeling

Online - Najaarsbijeenkomst:

'Mengseltoxiciteit'

5 oktober 2021 (13.00 – 17.00 uur)

SAVE THE DATE

NVT Risk Assessment Section

Online - Autumn symposium:

'Mixture toxicity'

October 5th 2021 (13.00 – 17.00 h)

SECTIE
GENEESMIDDELENTOXICOLOGIE

Update Section Pharmaceutical Toxicology of the NVT

On the second of March 2021, the Section Pharmaceutical Toxicology of the NVT held its first online Symposium entitled “Challenges in vaccines development during the COVID-19 pandemic”. The exciting 2.5-hour program was led by national experts providing an overview on the history and strategies of the development of (COVID) vaccines. The experts gave insights in the assessment of COVID vaccines, including the usefulness and necessity of animal studies, clinical trial design and endpoints, (long term) side effects and vaccination during pregnancy. Further, the audience gained insights in the importance and challenges of COVID related communication. This was followed by an interesting Q&A session. Sixty-six persons, 86% NVT members from which 40% were Pharmaceutical Section members were registered. Presentations can be found in the members-only section of the NVT website.

Lemongrass to reduce methane in cows

By *Héloïse Proquin*

Methane is a potent greenhouse gas that is released by ruminants such as cows as a byproduct of digestion. As we try to fight climate change, more and more solutions are sought to reduce methane production of cows. One example, publicized by companies like Burger King, is the use of lemongrass in feed. This choice was based on a publication from Vázquez-Carrillo et al.[1] It describes the effects of lemongrass on the methane emission of cows, which, according to this paper, could be reduced on average at 33%. Lemongrass contains essential oils and tannins, both of which have been shown to reduce methane emissions by modifying the gut environment and inhibiting the microbes responsible for methane production.

Other methane-reducing ingredients are researched like seaweed that contains bioactive ingredients that can reduce methane production[2]. Red seaweed was found to reduce enteric methane by more than 80%[3]. Another product, called 3-nitrooxypropanol (3-NOP), is an enzyme disrupter that promises to reduce methane emissions in cattle by 30%[4]. It raises questions regarding the safety of the milk produced by the cows, therefore it is not currently approved by the FDA as a feed additive[5]. Reducing the production of methane in cows is in line with reducing green-house effects, however, the potential toxicity for the bovines and the humans should be thoroughly studied.

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Plant Heme “Meat”, Is It Safe?

By *Barae Jomaa*

Plant hemoglobin promises to bridge the taste gap between meat and meat replacements but this new ingredient has left some worried about potential safety issues.

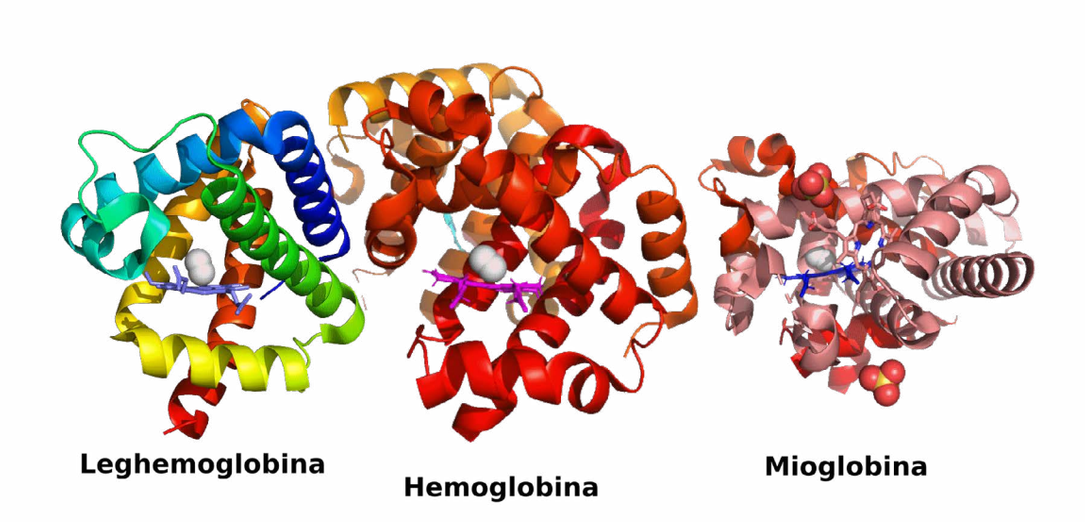
With “bleeding” red burger patties, Impossible Foods have revolutionized the way we think about vegetarian meat. When most of us think of vegetables we think of the green leafy vegetables and carrots that our parents forced us to eat in order to stay healthy. This is all changing as a growing number of people are concerned about their carbon footprint and animal welfare. The development of meat replacements has been nothing short of a modern gold rush with many companies heavily investing in research and development with the hopes of creating the most realistic meat substitutes. But how did Impossible Foods do the impossible and develop “bleeding” vegetarian meat?

An Impossible Burger with fries and ketchup at Gott’s Roadside in Napa, California. (image credit: Missvain)

One of the key components of meat is hemoglobin which gives it a red colour and a slightly metallic flavour. Even more, it is thought that hemoglobin catalyses certain chemical reactions during cooking that release hundreds of odorant molecules[1]. Even though plants do not produce blood, nitrogen-fixing leguminous plants do contain plant hemoglobin, also known as leghemoglobin. This fascinating protein was discovered in Japan back in 1939 by Dr. Kubo Hideo who isolated a red pigment from soybean nodules and identified it as a heme protein[2]. Fast forward to 2011 and Stanford biochemistry professor Patrick O. Brown establishes Impossible Foods which, in 2014, and under US Food and Drug Administration (FDA) oversight, declares leghemoglobin as “generally recognized as safe”.

Impossible Foods has its eyes on Europe and an application was filed in October 2019, with the European Food Safety Authority (EFSA), for the approval to market soy leghemoglobin. The US company produces this protein using genetically modified yeast and as of the latest reports, EFSA is still conducting its risk assessment[3]. →

Though nature-identical, the fact that this vegetable protein



Cartoons of three oxygenated globin molecules- leghemoglobin, hemoglobin, and myoglobin. Oxygen molecules are shown in gray. (image credit: Veronica Stafford)

is being produced in genetically engineered organisms has raised concerns that more needs to be known about its health effects. The Center for Food Safety has filed, on the 28th of January, a lawsuit challenging the FDA's approval of leghemoglobin. They claim that long term carcinogenicity and reproductive toxicity testing should have been carried out and highlight that besides leghemoglobin, the additive product being market also contains yeast proteins[4].

The main safety study published in 2018 and supported by Impossible Foods, evaluated leghemoglobin based on a range of in vitro and in vivo tests. These included the bacterial reverse mutation assay (Ames), *in vitro* chromosomal aberration test and a repeated dose 90-day oral toxicity study in rodents. As a result, a no observed adverse effect level (NOAEL) of 750 mg/kg/d was established and which was deemed as a hundred times greater than the 90th percentile estimated daily intake (EDI)[5]. The authors concluded that there is no reason for "toxicological concern" with regards to leghemoglobin.

Though initial findings are promising, the jury is still out as EFSA reviews additional data being provided by Impossible Foods.

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Tox in het nieuws / Tox in the Media

Zaterdag 24 april was het Wereldproefdierdag waarop aandacht werd gevraagd voor dierenleed in laboratoria. Universiteit Utrecht en UMC Utrecht organiseren later in het jaar debatten over wetenschap zonder proefdieren: Wereldproefdierendag: in gesprek over dierproeven - Nieuws - Universiteit Utrecht (uu.nl).

Meepraten?
Dat kan op de volgende evenementen:
Pint of Science Festival: Can medical science do without animal experiments? (Engelstalig), woensdag 19 mei, 20:00-21:30 uur, online
Debat over dierproeven: Betere wetenschap zonder proefdieren, hoe dan? (Nederlandstalig), dinsdag 8 juni, 15:30-17:30 uur, in Utrecht en online
Helpathon (Engelstalig), dinsdag 13 juli, 10:00-17:00 uur, online, info via tpi@uu.nl

Re-registration as toxicologist (ERT) in The Netherlands – objective, transparent and quantifiable

By *Martijn van Velthoven, MSc, ERT, chair of the NVT Registration Committee*

Re-registration as European Registered Toxicologist (ERT) in The Netherlands can only be done via the online registration tool 'PE Online' as of 1 January 2024. Until that date, re-registration via the re-registration form (available online at NVT) will also be accepted.

On behalf of the Registration Committee, I would like to thank the TCDD editorial board for this opportunity to explain the changes related to re-registration using the PE Online system. Before going into more details, I would like to express my gratitude to Dr. Paul Scheepers and Dr. Josje Arts, my predecessors in the Registration Committee who dedicated a lot of efforts, thoughts and time to develop the online registration system, including development of explanatory documentation and guidance.

It should be emphasized that the re-registration requirements as described here are nothing new. However, by using this new approach that is being implemented, the harmonized professional requirements that have to be met to remain registered as a EUROTOX Registered Toxicologist will become more objective, transparent and quantifiable.[1] The Registration Committee of the NVT is working on a more elaborate guidance on how to re-register via PE Online.

The requirements to qualify for the 5-year re-registration as a EUROTOX Registered Toxicologist (via the NVT national registration) contain the following elements:

One should have been actively working as (eco) toxicologist in the last 5 years:

To assess this, the applicant should provide the following information:

- The number of hours actively working as (eco)toxicologist, which should be at least 16 h per week (or an equivalent number of days per year);
- A description of his/her (eco)toxicological working activities (recommendation: 300-400 words).

The Registration Committee of the NVT advises applicants to be as specific as possible in the description of their toxicological working activities. This enables the Registration Committee to review what activities related to (eco) toxicology have been carried out during the past five years, including an estimate of the number of hours per week spent on these activities.
Example: *'working on health risks of hazardous chemicals at 30%' will need to be specified regarding what activities have been done.*

Specification of activities is also of importance in case one contributes to working groups, scientific panels, scientific reports, etc. in order to judge the toxicological content of that work.

This implies that someone originally trained as (eco) toxicologist, but currently full-time working as manager of a group of (eco)toxicologists, cannot be considered as an active (eco)toxicologist anymore and does not qualify for ERT re-registration any longer!

One should show that sufficient Continued Education has been followed in the past 5 years ('receiving activities'):

With regard to Continued Education (CE), also known as Continued Professional Development (CPD) an applicant for re-registration should indicate what activities have been done in the field of (eco)toxicology during the past 5 years.

General courses on personal and/or professional development not related to (eco)toxicology (e.g. a project management course, leadership training) are not considered as continuing education for the ERT re-registration purpose!

One should show that one has sufficiently contributed to the field of toxicology in the past 5 years ('sending activities'):

With regard to Active Contribution, an applicant for re-registration should indicate what activities have been →

done to actively contribute to the field of toxicology (such as presentations, (co)authoring publications, acting as reviewer, being an expert during a court case, contribution to a PET course, being a member of one of the NVT committees, etc.). These activities should be related to the field of (eco)toxicology during the past 5 years.

The credit point system for the Continued Education and Contribution to the field of toxicology

In the past couple of years, the Registration Committee noted that there was a large discrepancy in the applications for re-registration:

- some applicants provide a very detailed description of continued education and contributions to the field of toxicology (including all additional documentation to justify these requirements for review by the Registration Committee members); whereas
- other applicants made a very concise 1-page summary (e.g. referring to their working environment for active contributions and summarizing continued education as: 'reading scientific articles' and 'attending all NVT annual meetings').

Therefore, the Registration Committee decided that a system needed to be developed that facilitates an objective review of the applications for ERT registration renewal. Such a system should allow a more transparent way of evaluating whether applicants fulfill the requirements with regard to continued education and contributions to the field of toxicology.

For this a credit system was developed: activities considered to be active contributions to the field of toxicology ('sending') or continued education ('receiving') should be listed and are eligible for credit points (where 1 credit point reflect 4 working hours (i.e. half of working day)). On average, each year 10 credits need to be obtained (50 credits for the 5-year period), of which 4 credits need to be obtained for continuing education and 4 credits on contribution to the field of toxicology. The additional 2 credits can be obtained for either 'receiving' or 'sending' activities or both. Credits can be transferred from one year to the other within each 5-year period (thus e.g. a total of 8 credits in 2021 and a total of 12 credits in 2022). It is, however, not permitted to transfer credits used for a previous registration period to the next 5-year period.

In the table below the minimum credit point requirements for re-registration are presented.

Period	Receiving	Sending	Receiving or Sending	Total
Per year	4	4	2	10
Full 5-year registration period	20	20	10	50

This credit system was already presented during the EUROTOX 2018 in Brussels, Belgium[2] and published[3]. These details have been implemented in the PE Online system to facilitate the application of re-registrants.

The expectations of the Registration Committee

Continuing education ('receiving' activities)

For professional development as (eco)toxicologist, an applicant for re-registration should indicate what activities have been done related to continuous education in the field of (eco)toxicology during the past 5 years.
The Registration Committee encourages applicants to follow training courses on (eco)toxicological topics outside their current field of expertise to broaden their view on (eco) toxicology and to take notice of recent developments.

For (eco)toxicology-related receiving activities one could think of attending courses, conferences, workshops, trainings, NVT (subsection) meetings, etc. For each 'receiving' activity you need to provide evidence: e.g. program of the course or workshop in combination with a certificate of attendance and/or proof of payment.

Contribution to the field of toxicology ('sending' activities)
An applicant for re-registration should also indicate what activities have been done to actively contribute to the field of toxicology ('sending' activities). These activities should be related to the field of (eco)toxicology and include a.o. presentations, (co)authoring publications, acting as reviewer, being an expert during a court case, contribution to a PET course, being a member of one of the NVT committees, etc.

Also, for each 'sending' activity evidence is needed. This can consist of proof of e.g. a program/ agenda of the meeting with the name of the presenter, an abstract of the publication of which one is (co)author, or an acknowledgement for a reviewing activity for a scientific journal.

Many common toxicology-related journals are included (and can be selected) in the online system. In case a publication is issued in a journal that is not listed in the online system, the link to the website of the journal should be provided by the applicant.

Portfolio assignment of credit points ('sending' or 'receiving')
Although the Registration Committee has tried to cover as many sending and receiving activities as possible, not all activities for which credit points can be obtained will have been listed. Such remaining activities can be described in the portfolio section. →

Also, the portfolio assignment can be used to include a (long) list of publications, which may be more convenient than adding each publication as a single entry.

These (eco)toxicology-related activities need to be well described and supported by evidence.

Some (non-limited) examples are:

- Toxicological advice
- Membership of a toxicology-based committee/working group/expert group/task force in industry organizations (e.g. VNCI, AISE, Cosmetics Europe, CEFIC, CEPE, etc.
- Attendance of a committee/working group of national or international organizations such as ECHA, EFSA, Gezondheidsraad, IARC, OECD, etc.
- (Co-)author of a textbook (or chapter) for (education in) toxicology
- Toxicological reports prepared as Study Director (these should be summarized in a list with titles, for example as follows: Report of a 90-day oral toxicity study with Substance X in rats, according to OECD guideline …).

Please also note that applicants may claim confidentiality. However, in order to apply for credits, it is expected that the applicant will provide the required information in a general way to ensure the Registration Committee members can evaluate what has been done without having to use confidential details (such as name of substance).

For each of these activities in the portfolio assignment, the applicant can propose the number of credit points (as long as it can be justified by the applicant to be in line with the 4 working hours per credit point).

Please note the portfolio assignment is meant for obtaining the last missing credits to arrive at the minimum because preparation of reports of (eco)toxicity studies, attendance of certain committees, or providing (eco)toxicological advice may be part of daily work activities. It is therefore required that the majority of the credits is earned at sending and receiving as indicated in the Tables in the Annex.

Assessing re-registration by the Registration Committee

Credits allocated by the applicant will be checked by the Registration Committee. For each dossier, two members will review independently before discussing their findings in the Registration Committee meeting.

Please note that the Registration Committee members assigned to your dossier will also have to approve the activities eligible for credit points. This evaluation will be based on supporting documents as indicated above. These supporting documents should be uploaded in the PE Online system.

To avoid delays in your re-registration, please make sure that your dossier is kept up to date on a regular basis. This will avoid that you miss out on credit points necessary for your re-registration and it will allow the Registration Committee to keep a 'running review' of your dossier and to update your dossier 'dashboard' in PE Online. This dashboard shows the progress of your dossier and will make it easier for the applicant to see what activities to focus on before the 5-year period has passed.

COVID-19 considerations (based on the EUROTOX Executive Committee letter from December 2020)

The COVID-19 pandemic has severely affected normal Continued Education opportunities with the cancellation or postponement of many congresses and meetings combined with local measures and travel restrictions. On the other hand, many organizations have quickly moved to provide alternatives such as virtual meetings, webinars and other online offers, providing ample possibilities for continuing education.

The EUROTOX Executive Committee and the Registration Subcommittee are mindful of the challenges that this poses to all toxicology professionals and have offered some guidance to the National Registers. First and foremost, Continued Education remains the cornerstone of maintaining professional competence for ERTs. However, for the year 2020, the requirement for CE will be handled flexibly and according to national and individual circumstances. The educational hours spent participating in online offers should count in full, provided that there is a clear link to toxicology.

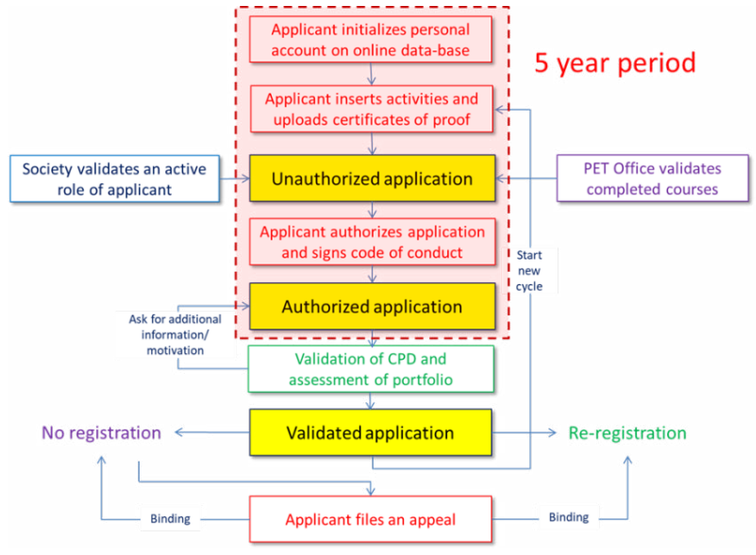

Figure 2. Flow diagram of the re-registration process. Different colors indicate the various roles: applicant (red), Society (blue), PET-Office (purple), RT (green).

AIO toxafette - Warner van Kersen

1. Can you introduce yourself?

Warner van Kersen is my name and I have been working on my PhD at the Institute for Risk Assessment Sciences since April 2018. I grew up in Hardinxveld-Giessendam, the south-western appendix of the bible belt close to Rotterdam. I have a bachelor in biomedical research from the Rotterdam University of applied science and a master in biology from Leiden University. In my free time I volunteer as a coach and instructor on a program by and for people who stutter. Besides that I do quite a lot of sports and when there is no ongoing pandemic, I also practice longsword fencing. Currently, I'm confined to virtual reality which, while it saves me a few bruises, isn't as fun as the real deal.

2. How would you explain the subject of your research to a layperson?

I'm investigating the effects of livestock farms on the respiratory health of people living nearby. We know for instance that the airway disease COPD is less common in people who live close to livestock farms but, and this is where it gets interesting, if people with COPD live close to livestock farms their disease is worse compared to COPD patients without farms close to their home. This implies that something from these farms is affecting people with COPD. One of the suspects we've been looking at is livestock-related air pollution.

Ammonia is a gas typically emitted by livestock farms so we decided to use ammonia levels in the air as a marker for the intensity of livestock-related air pollution at a given day. In the same area where we measured the ammonia levels, we also had COPD patients measuring their lung function. It turned out that on days with higher ammonia levels, the COPD patients had an increased risk of experiencing a substantial drop in lung function.

So we know that livestock farms have an effect on respiratory health, we are just not sure exactly what is causing the effect. It's unlikely that it's the ammonia itself as livestock farms emit a complex mixture of particles and gasses. One of the possible pathways through which livestock-related air pollution could affect the airways is by influencing the community of bacteria, called a microbiome, living there. Currently I'm are looking at the microbiomes of COPD patients near livestock farms to see if these differ from those of healthy people.

3. What was your motivation to start a PhD program?

As someone who stutters I used to think that job happiness wasn't for me. It took some time to realize that I was just holding myself back, making choices out of the fear of having to speak and showing my stutter. Having a job revolving around communicating your research, both in written but especially with the spoken word, is highly beneficial to me as it constantly stimulates me to improve speech. You can see the PhD as a way for me to find out what I'm capable off.

4. Why did you choose a subject in toxicology?

I think my project is more epidemiology than toxicology but I especially like to work a topic that involves environmental health. I'm a bit of a jack of all trades which suits the multidisciplinary approach. Having this complex puzzle of which the solution could advance what we know about how our environment affects our health appealed to me.

5. How do you see the future of your research topic (follow-up research / social impact)? What do you hope for?

Currently the livestock sector is at a bit of an impasse and the way forward is unclear for all parties involved. I hope that providing answers helps to cool down the public debate and furthers the transition to a more responsible food supply.

6. What is the biggest challenge for you in doing PhD research?

I'm currently working on a microbiome dataset which is multidimensional, think of it as a rubics cube were every square is a puzzle of its own. Maintaining a clear overview of this multidimensional project is the biggest challenge I've encountered so far. Especially in this pandemic situation where you can't just pop into your colleague's office for some pointers on something you're struggling with. →

7. What is the best advice that you have received as a PhD student or would like to give to another PhD student?
Number one would be “remember that a PhD is a four year educational track, not a four year exam”. Followed closely by “Ask for help when you’re stuck”.

8. Is there an experience that you had that you would like to share with other PhD student in case it happens to them?
I had the opportunity to give a PechaKucha presentation at a congress once. PechaKucha is a presentation format where you have 15 or 20 slides which automatically progress after 20 seconds. This forces you to make your talk crisp, concise and well-rehearsed. It forces the presenter to focus on what’s really important and it saves the audience of having to listen to those talks that just keep going. If you ever get the opportunity to do a presentation this way, I can’t recommend it enough.

9. How do you combine your PhD project with your personal life? Are there choices you have to make?
Like many I often feel torn between my personal life and my PhD project. It’s important to notice that they are two sides of the same coin. Taking care of your other needs helps you to keep performing on your project and vice versa. I keep a rather simple journal to see how balanced my efforts are. It helps me decide if I need to put in a few more hours into a problem or call it day and go do something silly.

10. What goals do you have regarding your career after your PhD? Would this be inside or outside academia, and why? Would you consider going abroad?
I haven’t really thought about my career after my PhD that much yet. Mainly because I’m focused at getting the job done, not so much what happens after. I enjoy research as well as teaching so I just might stay in academia. Going abroad was always an ambition so that is definitely an option if the opportunity presents itself.

11. Please answer the question from the last toxafette PhD-candidate: “Name one opportunity and challenge you face when researching the microbiome?”
On a personal level, an opportunity of working with multidimensional data is that its sure to get your coding skills to a higher level. To illustrate, I had to dive into a couple of dedicated tutorials for R just to be able to clean up the data and get into a format I could analyze. Scientifically, there is the opportunity to gain a better understanding of how the bacteria we live with affect our health.

A challenge of working on the microbiome, which uses DNA sequencing data, is that every step from sampling to the final dataset has an enormous influence on your data. Therefore, it’s extremely important to standardize everything from the sampling procedure to the laboratory procedures and to check for anything that might have introduced bias. For example, if the sample collection or the lab work was done by several people you need to check to which extent that introduced variation in your data.

12. Could you suggest the next PhD-candidate for the Toxafette, and propose a question? (please include e-mail address, it will not be published)
Ceder Raben might be interested (c.r.raben@uu.nl) What do you expect from your PhD on a personal level, how will the experience change you?

TiO’s:

Voorletters	Achternaam	Opleider	Datum inschrijving
J.	Chen	Prof.dr.ir. I.M.C.M. Rietjens	03-03-2021

Registrants:

Voorletters	Achternaam	Datum inschrijving	Datum afloop registratie
J.J.M.	Freriksen	03-03-2021	03-03-2026
A.M.	Tukker	03-03-2021	03-03-2026
P.N.H.	Wassenaar	03-03-2021	03-03-2026

Working in the chemical industry

I (Josje) was asked to present my view on 8 April, with the announcement of a deadline of 16 April. But in industry we are used to short deadlines. After having worked for more than 20 years at TNO, I started at AkzoNobel (now Nouryon Chemicals) in 2008. This may seem a big step (from a research organization to industry) but at TNO I used to work for all kinds of sponsors including ministries and industry. That's where I learnt it is the data that do matter, and not feelings or belief.

By *Josje Arts,*
Senior Toxicologist, Nouryon

However, I soon learnt that when authorities have a concern, that industry has to prove the concern is real, limited or not existing (although the latter is impossible). And if we want to do this by using alternative test methods, these studies are generally not accepted, only if these are accompanied by the required OECD testing guideline studies which require huge numbers of vertebrate animals. Also, in case of grouping or read across it is accepted to use positive test results (thus negative outcome); however, negative studies are not accepted for such purposes because authorities do not like to take any risk.

Regarding risk, risk and hazard are not the same as all (eco)toxicologist have learnt (see also the article that follows). Yet, currently, everything seems to be based on hazard, and hazard testing seems to be mainly used for CLP classification only. For many endpoints this is not a real issue as classification is based on potency (acute toxicity, skin irritation and sensitization, eye irritation and STOT 1/2); thus the ability to cause more severe effects at the same or lower dose/concentration is indicated by a more severe category paired with an appropriate warning pictogram and stronger warning language in the hazard (H) statement. However, this does not apply to CMR classification which is based on level of evidence only (evidence in humans cat 1A, clear evidence in animals cat 1B, limited evidence in animals cat. 2; all irrespective of dose or concentration). In fact, it means that chemicals such as formaldehyde and ethanol are both considered carcinogens (cat. 1) in NL whereas their official Dutch 8-h OELs differ by a factor of ca. 2000! Which would mean that risk management measures - which was the ultimate goal of classification at the start, viz providing crucial information on which risk management measures to be advised - would need to be quite different.

Speaking about ethanol, Greece has proposed to have ethanol classified as Repro cat. 2, but based on available human data I think there is no other conclusion other than that ethanol can cause cancer and is harmful to the unborn child. But although this may be the result of excessive alcohol intake, based on the current CLP regulation it can only lead to classification as CMR cat. 1A. But rather than concluding there is something wrong with the CLP system, people have been arguing that ethanol should not be classified as CMR substance because, if classified, people would not have any confidence in the classification system anymore…..

I often asked myself how it is possible to have two different systems (potency versus evidence) into one classification system but it seems to date back more than 50 years ago when people thought that one molecule could cause cancer, and thus there would be no threshold. And at that time, they apparently thought the same for reproductive toxicity as well. Although we have gained extensive more knowledge since, still everyone is trying to adapt to this system. But regardless whether a substance is of low or high potency, a CMR cat. 1A/B is not allowed in consumer products (except ethanol, even in NL). →

So, within industry we are working hard together with test labs such as CRL on hazard assessment of all our chemicals, and combined with exposure assessment, to assess the risk(s) for man and environment. Industry is often seen as the bad guy only aiming at making profit. However, many consumers would not like to miss their mobile phone or other equipment, and my colleagues and I are striving for a sustainable future to work and live in.

And indeed, to do so, we are performing hazard testing, not safety testing, as chemicals from the chemical industry are not medicines or food. If you wish to see how we are doing safety testing, you should come to our Safety Lab in Deventer where we test flammability and explosivity of our peroxides.

Overall, working in industry is challenging because of tight deadlines and the wide variety of (eco) toxicological issues while maintaining a high quality and integrity in the work. Never a dull moment!

What is risk?

By *Erik Houthoff,*
Senior Toxicologist, Nouryon

Living is taking risks. With everything we do, risks are involved. Participating in traffic can kill you, drinking the wrong herbal tea can lead to kidney disease, and even breathing oxygen is thought to be the cause of background cancer incidences. Also, there is chance and bad luck: if you are allergic to nuts a peanut can kill you. And even when risks are regulated, fraudulent activity cannot be excluded (example from own experience: our product choline chloride in feed of chickens resulted in unacceptable high dioxin levels in eggs, because someone used saw-dust from pentachlorophenol treated wood as carrier, instead of intended clean kernels from maize corns).

Picture of Erik that is "symbolic for how industry is kept under pressure"

Point is, risks are always involved and in the evaluation of potential hazards from chemicals there is always a level of uncertainty. But we cannot deal with uncertainty. Hazard testing needs to be performed at the highest possible dose level - in rats. And because a rat is not a human, at least a factor 100 is added to reduce any uncertainty. This procedure has not changed the last 50 years; however, it may be adapted soon by adding an additional factor of 10 because people are thought to be unintentionally exposed to several chemicals at the same time.

What *has* changed though is our knowledge on possible adverse effects and on modes of action. Currently, there is much focus on endocrine disruption and neuro-developmental disorders. This has resulted in a substantial number of additional parameters to be included in the rat studies, making these studies more and more complex. As such, it is almost inevitable that finding(s) will be noted, not only because of the high dose levels (Paracelsus!) or physiological adaption, but also by chance. In case of many independent parameters, evaluated at a 5% statistical significance level, 1 out of 20 parameters can be expected to show significance just by chance. Adding all these parameters in standard testing basically leads to bad science: data dredging without a clear, up-front, hypothesis. Thus, almost always something pops up and the possibilities to address concerns are limited. A repeat of animal studies is not at all desirable and not allowed, and a conservative regulatory approach will be taken. As a consequence, the perception of hazards from industrial chemicals is increasing, aiding to the notion that we seem to live in a chemical environment getting more and more hazardous, whereas risks may be very low or even absent. But it also has happened that when nothing was found in a study, even at a high dose, regulators concluded the study must not have been performed well enough.

Chemicals: you don't want them, but you can't live without them.

Are meat replacements safe?

By *Martje de Groot*

One of the hottest topics in food research is meat replacements, or alternatives for meat (or even animal protein in general). Although already studied for over a few decades, the search for meat replacements has intensified over the last years. This is mainly driven by the increasing concern about the environmental and health effects of the increasing meat consumption.

Over the last two decades, the global demand for meat has increased by 58%, due to the increase in global population and rapid economic development (Whitnall & Pitts, 2019). The market is predicted to expand even further with 15% by 2027 (OECD/FAO, 2018). The concerns of this large meat production include -but are not limited to- the pressure that the production and consumption of meat has put on land and water requirements, the inefficiencies of meat production versus crop harvesting, pollution and greenhouse emissions, the loss of biodiversity and public health (e.g. the relationship between processed meat and colon cancer) (van der Weele et al, 2019). Moreover, animal welfare is an ever-increasing concern. As such, a logical solution to many of the environmental and -possibly even- health concerns would be to dramatically reduce meat consumption.

Conventional proteins comprise animal-based protein sources such as chicken, beef, fish, seafood and dairy, whereas alternative proteins are from non-animal based sources. These comprise four main categories: plant-based foods, edible algae (e.g. seaweed) and cultured meat (in vitro meat or lab-grown meats) (Tso, 2021). Due to these increasing concerns mentioned above, food industries have been looking for ways to introduce meat alternatives, using non animal-based protein sources, such as the highly processed plant-based meat proxies, cultured meats (also clean meat or in vitro meat), fungi-based alternatives (e.g. QuornTM products) and insect-based meat products (He et al. 2020).

Plant-based meat alternatives (PBMAs)

The current article will focus on the plant-based meat alternatives, or PBMAs, as plant proteins can be utilized directly to construct meat-like alternatives and hence represent a primary sector in the meat replacement industry (Sha and Xiong, 2020).

PBMAs are highly processed and have complicated formulations, using soy protein, pea protein and wheat gluten as the most commonly used plant protein alternatives. In addition, other plant proteins are often included to provide structural and nutritional properties. As the sensation of meat is crucial for the willingness of the consumer to replace meat, various functional ingredients are required to create and mimic the type of texture, appearance, flavour, and mouthfeel of animal protein-based products. The number of ingredients in PBMAs usually exceeds 20 and can even go up to 40 ingredients. Common additives include preservatives, stabilizers, and colorants that are not commonly added in regular meat products (for a more comprehensive overview, the reader is referred to Sha and Xiong, 2020, Santo et al. 2020, or He et al., 2020).

However, despite the increasing production and marketing of PBMAs, research concerning safety, shelf life, and long-term nutritional and health effects of PBMAs is limited (Santo et al. 2020; Tso et al., 2021). Moreover, the available research is often funded by the companies developing these products and organizations promoting them (Santo et al. 2020). Since meat alternatives are primarily based on food sources that are generally recognized as safe (GRAS) for human consumption, developers generally believe their new products are safe. But are they? And what are potential risks?

Microbial count

PBMAs have a neutral pH as well as a high protein and moisture content (de Wild et al., 2014; Sha et al., 2020). As

such, they are in theory highly susceptible to spoilage. Of this spoilage, microbiological activity is the most important cause, whereas microbial enzymes and metabolites play a secondary role. Data describing the microflora of high-moisture meat analogues produced from plant proteins are not widely available in the scientific literature, but some studies have been performed to identify and characterize relevant spoiling and potentially hazardous microorganisms.

In a study from de Wild et al. (2014) the microbial load of a plant-based meat alternatives (both ingredients and end products) was evaluated. The authors hypothesized that powdered plant-based proteins - the main ingredient of PBMAs - are not likely to promote the growth of microorganisms due to its low water activity. Endospore-forming bacteria however, might survive the extrusion process by which these powdered proteins are produced. Depending on the microbial load of the selected raw materials, relevant concentrations of microorganisms could occur in the pre-mixtures and recipes of meat analogues, which may include pathogenic and toxicogenic microbial species. Within the EU-funded LikeMeat project the microflora in a broad spectrum of potential protein ingredients and extruded products were characterised for quantitative microbial level and species composition. The results showed that the microbiological quality of raw ingredients for PBMAs was satisfactory. However, the results of the microbial analysis of the refined products demonstrated the need for post-process heat treatment or freezing of the intermediate product in order to ensure a shelf-life of several weeks under chilled storage conditions. They state that the shelf-life of intermediate LikeMeat products without preservatives and without postprocess heat treatment is comparable to fresh meat products (de Wild et al. 2014). As such, the authors highly recommend that the system for the storage and handling of PBMAs should be similar to that of raw meats.

Allergens

Depending on the susceptibility of the consumer population, allergenicity of some plant proteins, such as soy protein is considered a health risk (Sha and Xiong, 2020). Similarly, for PBMAs containing wheat proteins, glutensensitivity, intolerance and allergy are potential risk factors that must be carefully monitored amongst susceptible populations (Miller, 2018). Moreover, next to these “known” allergens, novel plant-based protein products are prepared using new production methods which come with risks of introducing unknown allergens and contaminants that are currently poorly understood (Tso, 2021).

Contaminant

Other contaminants in PBMAs such as heavy metals, aflotoxins or pesticides from ingredients or surrounding environment, are of concern, but have not been widely studied. There are a limited number of small studies that have investigated the safety of insects and algae which highlight that indeed products derived from these sources may contain pesticides (Saeed et al. 1993), heavy metals (Green et al., 2001; Banjo et al., 2010) or allergens, or act as hosts for parasites (Chai et al., 2009).

Additives

Some concerns have also been raised about the safety of new additives – and as you may recall from abovementioned, there are many – present in some plant-based substitutes, such as liquid smoke used as flavouring agent (Hossain et al., 2013), mycoprotein used in Quorn products and soy leghemoglobin used in Impossible Foods products (see elsewhere in this issue).

Carcinogens

High-temperature processing of protein foods could generate toxicants and carcinogens, such as heterocyclic aromatic amines. This has been reported for meat subjected to high-temperature cooking, including grilling, roasting, frying, and baking. The high protein content of PBMAs, which are often prepared in a similar way as the meat they aim to replace (i.e. at high temperatures), predisposes them to similar toxicant formation (Sha and Xiong, 2020). Although carcinogen production by high-temperature processing has not been studied in plant-based meat alternatives, based on how these toxicants are formed, it is hypothesized that PBMA products may also possess these compounds. However, scientific literature lacks sufficient data to come to a conclusion (He et al. 2020).

Taken together, although there are several points of concern that -at this point in time- have been merely touched upon, the combination of: 1)the use of high-quality ingredients for PBMA production that are GRAS, 2) the highly controlled production process together with 3) appropriate packaging and storing of raw ingredients and end products, in combination with 4) cooking at the right temperature for sufficient time, should serve as effective strategies to ensure the safety of PBMAs. However, when going through the literature on meat replacements, it becomes clear that supplementary scientific data are needed to support these assumptions. Food for thought, I would say… Or for study!

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Call for Proposals for the International Congress of Toxicology 2022 Scientific Program

Maastricht, **September 18-22, 2022**. The International Congress of Toxicology is a joined meeting of **IUTOX and EUROTOX** (www.ict2022.com) organized by the **Netherlands Society of Toxicology**. The theme of the 2022 ICT **"Uniting in Toxicology"**, reflects our desire to address topics dealing with different disciplines.

GENERAL GUIDELINES FOR SCIENTIFIC PROPOSALS

- Submit your scientific proposals via the **submission platform** making sure to include the organiser details, the recommended speakers, and a brief presentation for the proposal.
- Proposals will be evaluated based on the following criteria:

a. Scientific excellence of the proposed individual talks and the session overall.
b. Relevance of the topic of the Symposium to the overall theme of ICT XVI.
c. Balance of the scientific content with respect to the utility and relevance of the information presented for improving human and environmental health in countries with both robust and developing research and regulatory enterprises.
d. Speaker diversity with respect to the geographical area represented, gender, and sector (government, industry, academia, other).
e. Availability of support for speaker travel to ICT XVI. [This criterion is not a requirement, but a strong encouragement.]

- The Congress Organization strongly encourages interaction between Academia-Industry-Regulators and interdisciplinary approaches (basic research, clinical/ epidemiological, regulation) therefore we ask that you make every effort to include speakers from all mentioned organizations.

SPECIFIC GUIDELINES FOR SCIENTIFIC PROPOSALS

SCIENTIFIC SESSIONS:

Aims to be topical or cutting-edge scientific knowledge exchange with experts in the area presenting data from their research and answering questions from the audience. A Chair and a Co-Chair should be included.

- Should last a total of 120 minutes and have a maximum of 4 speakers setup as a workshop or symposium.
- The first speaker should set the scene for the symposium by explaining briefly the background to the topic before presenting his/her data.
- Presentations should be 25 minutes with 5 minutes for questions for a total of 30 minutes per speaker.
- Proposal for 60 minute round table sessions will also be considered.

CONTINUING EDUCATION COURSE (CEC):

An educational session with 3-4 experts on a particular topic presenting a balanced view of established principles. Proposers/Chairs should ensure they work with individual speakers to achieve balance and prevent topic overlap. Scheduled Sunday, September 18 from either 9h00 to 12h00 or from 13h – 16h00. A full day format including coffee and lunch breaks may be proposed.

Please note: All fields of toxicology are welcome as topics for a CEC, except Regulatory Toxicology and Immunotoxicology.

TERUG NAAR INHOUD

TCDD is de nieuwsbrief van de Nederlandse Vereniging voor Toxicologie (NVT).