Master 'Cancer, Stem Cells and Developmental Biology'
Writing Assignment

Accelerating the Transition from Animal Studies to Next Generation Risk Assessment for Pharmaceuticals

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Abstract

New approach methodologies (NAMs) are techniques that can be used for the safety assessment of pharmaceuticals and other chemicals. These techniques exclude the use of animals. The regulatory acceptance of the usage of those NAMs in next generation risk assessment (NGRA) proceeds very slow. This writing assignment shows by a literature study, in the form of a literature mapping review, which workflows are currently available for pharmaceuticals (and also for other chemicals and cosmetics), what are the regulations and which stakeholders are involved when performing risk assessment with NAMs in NGRA. Moreover, opportunities are summarized how to accelerate the transition, in this case the regulatory acceptance, from animal studies towards NAMs in NGRA for risk assessment of pharmaceuticals.

Laymans summary

In the pharmaceutical world still a lot of animals are used in safety assessment of drugs. During safety assessment the aim is to clarify whether a compound is or is not safe for human and animal health. Animal studies are seen as the 'gold standard' for this aim, that is why those are required for safety assessment by law in the EU, US and elsewhere. But for pharmaceuticals often the translation from animals to humans goes wrong during clinical testing. Therefore, the use of NAMs in NGRA are on the rise. These methods do not require the use of animals during safety assessment and can be more accurate in protecting humans from harm of chemicals, because e.g. human tissue can be used during *in vitro* testing, eliminating species differences. Even though this seems promising, still a lot of resistance is observed in the regulatory acceptance of those NAMs.

In this writing assignment the workflows available for NAMs in NGRA for safety assessment of pharmaceuticals (and also other chemicals and cosmetics) are summarized to see whether NGRA could be a good method. Also the regulatory rules are summarized to see where the opportunities lie in changing regulations, and the stakeholders involved are summarized to see which parties are involved in this whole process.

In conclusion, there are good workflows available for NAMs in NGRA for safety assessment of pharmaceuticals, but more should be invested in the translation into practice with more examples of case studies. These can build more confidence in the usage of those NAMs. Also this offers opportunities in changing the regulations on safety assessment, but first more communication between regulators and other stakeholders involved is needed for accelerated regulatory acceptance.

Introduction

Importance animal free testing for regulatory safety assessment of pharmaceuticals – current situation

Animal testing is widely used worldwide in the pharmaceutical industry for regulatory safety assessment of pharmaceuticals. Testing on animals is believed to be the 'gold standard' for safety assessment of compounds which we come in contact with (Mangipudy et al., 2014). This contributes to the belief that animal testing leads to more safety and this will contribute to the high quality of our health care for (veterinary) medicine to improve the well-being of our society. This causes animals to still be used according to regulations to confirm pharmaceuticals are safe (Swaters et al., 2022).

However, more and more friction is observed in society because of the large number of animals used in (bio)medical research, because of animal welfare and sustainability reasons.

Moreover, the costs that come with using animals for research are high and the development time for pharmaceuticals is a lengthy process. Also investments in R&D for pharmaceuticals has declined because of the high costs (*Seize the Digital Momentum Measuring the Return from Pharmaceutical Innovation 2022 Contents*, 2023). This leads to the desire for methods to shrink the number of animals used in laboratories. One of those methods is the 3Rs: replacement, reduction and refinement. This method originated from *The principles of Humane Experimental Techniques* from 1959 by Russell and Burch's, where it is described how animal welfare of laboratory animals should be improved by using the 3Rs. The goal was to improve animal welfare and reduce the number of animals used, with the same high quality of research. With replacement the number of animals is minimized and replaced by other methods (*in vitro* or *in silico* methods) that can give at least the same or even better (translatable) information. Reduction states that the number of animals is minimalized (Tannenbaum & Bennet, 2015). Refinement causes deduction of distress and improvement of welfare of the animals that are used for research (Tannenbaum & Bennet, 2015). These 3Rs are also implemented in legal documents, for example the EU directive 2010/63/EU (*Directive 2010/63/EU*, 2010).

Challenges

Even though with the 3R-method an active goal of decreasing the number of animals is pursued, still there is a low acceptance rate of animal-free testing for pharmaceuticals. The low acceptance rate is inconsistent with flaws in animal testing which has been demonstrated earlier (Dirven et al., 2021; Hooijmans et al., 2012). Even if animal trials show promising results, 86-90% of the pharmaceuticals fail in human clinical testing. Here the translation from animal to human goes wrong (*Accelerating the Growth of Human Relevant Life Sciences in the United Kingdom (White Paper)*, n.d.). Examples of translational failures are the use of probiotics when patients are suffering from severe acute pancreatitis and more broad drug-induced liver injury which is caused by adverse effects of pharmaceuticals (Dirven et al., 2021; Hooijmans et al., 2012). This can be explained by inter-species and even inter-individual variability in animals when comparing the response to different compounds (drugs, environmental pollutants and food/flavour/fragrance compounds). This shows that animals are not always the 'gold standard', even though this is believed (Burnett et al., 2021). However, even though this information is available, this does not lead to the usage of fewer animals.

In the European Union a ban on animal testing was established for cosmetics in 2013, which resulted in more animal-free methods to be used (*Regulation (EC) No 1223/2009*, 2009). However, this has not resulted directly in the desirable result towards fewer animals used in studies, because other regulations like the Registration, Evaluation, Authorization and restriction of Chemicals (REACH) state that *in vivo* testing is still required for safety assessment (Knight et al., 2021). However, the cosmetics ban had induced major efforts towards replacing animal studies for safety testing of chemicals, building a good basis towards an animal-testing free future.

Even though there are a lot of hurdles, a more recent case study example showed that during the COVID-19 pandemic, alternatives for animal testing were used more because of the pressure of the crisis. In this crises the production of new vaccines needed to be accelerated to protect human health. After only 12 months, new mRNA vaccines were conditionally approved on the market. Normally, this approval process takes around 10 years. Fewer animal studies were used in this process, non-animal alternatives were accepted faster, historical data from earlier vaccine studies was used and human studies began earlier. This shows we are capable as a society to use fewer animals for safety testing for pharmaceuticals, but still this is not applied directly in a broader perspective (Ritskes-Hoitinga et al., 2022).

Regulatory safety assessment

How safety assessment is regulated for pharmaceuticals will be briefly explained here. First the principles of safety assessment itself will be explained. Safety assessment for compounds usually consists of four main components: hazard identification, hazard characterization, exposure assessment and risk characterization. Before the hazard is identified, the problem has to be formulated. Then the hazard is identified and it is examined if there is a potential hazard for humans or animals. Second, more in depth research is performed on hazard characterization to show what properties of the compound could cause a harmful effect and if there are already certain guidelines from agencies which describe the rules about using the compound. Third, exposure assessment leads to research of how the compound comes in contact with an organism and how much and for how long exposure would occur. The last step involves risk characterization where conclusions are drawn in case of effects of the compound on the organism (*WHO Human Health Risk Assessment Toolkit: Chemical Hazards*, 2010). While performing safety assessment for pharmaceuticals, the main goals of safety evaluation are identifying a safe starting dose, identifying whether there are potential target organs for toxicity and if this is reversible, and identifying safety parameters for clinical monitoring (Vichare et al., 2021). This preclinical safety assessment is performed before or next to human clinical trials to determine whether there are off-target effects. Moreover, besides the safety, the efficacy of the compound is tested as well (Turner et al., 2023). Efficacy testing is beyond the scope of this paper.

How the performance of safety assessment is regulated, differs around the globe. In this writing assignment, the focus will be on the EU and the US. In Europe the European Medicines Agency (EMA) is responsible for the safety assessment of pharmaceuticals according to EU law. Here more information can be found about authorisation, manufacturing and distribution of medicine (*Directive 2001/83/EC*, 2001; *Regulation (EC) No 726/2004*, 2004; *Regulation (EU) 2019/6*, 2018). In the US the Food and Drug Administration (FDA) is responsible. This is performed according to US law. In the Federal Food, Drug and Cosmetic Act (FFDCA) the safety of chemicals in food, drugs and cosmetics is discussed more broadly (*Website United State Codes: Law US Title 21, Chapter 9*, n.d.). Both the EU and US support the use of more non-animal alternatives in safety assessment. In the EU there is a specific law for the protection of animals used for scientific purposes (*Directive 2010/63/EU*, 2010) and the EMA has a 3Rs-working party to promote implementing the 3Rs in research (*Website EMA: 3Rs Working Party*, n.d.). In the US recently the Modernization Act 2.0 of the FDA has been approved and added to the FFDCA. This law focuses on animal testing alternatives (FDA Modernization Act 2.0 (H.R. 2565), n.d.). So, in all there is an ongoing shift towards an animal-free testing regulatory domain. The question now is how this could be applied more into practice.

Non-animal alternatives in next generation risk assessment – replacing animal studies for regulatory safety assessment

More and more suggestions how to use non-animal alternatives for safety assessment of compounds are made by professionals in the form of workflows. In those workflows different kind of *in vitro* and *in silico* tests are implemented together to develop a method which can be used for safety assessment. Those methods are referred to as NAMs when non-animal alternatives are used for safety assessment. Methods used as NAMs are e.g. *in vitro* systems cells that grow in culture, with a range of different possibilities: from cells in a primary cell culture to 3D cell cultures. A wide variety of cells can be grown in laboratories, such as cell lines from stem cells or cells with a specific mutation. Also fresh cells from patients can be grown. Another advantage is that human cells can be cultured, which causes interspecies variation to disappear and can also be used for personalised medicine for the individual. However, it can be ethically difficult to obtain human cells and simple cell systems don’t give

information about how a whole organism would react to a compound. More complex *in vitro* models can help here, think about co-culture systems and organoids (Bhogal et al., 2005).

Risk assessment in *in vitro* systems can give information about the effect that a compound has on specific molecular cell characteristics (Bhogal et al., 2005). Also *in silico* methods can be used as a non-animal method. Those methods are computational based. The data of already performed research can be used to predict the toxicity of a compound on a higher level (Turner et al., 2023). Think about exposure to the whole organism which can be predicted. *In vitro e*xperiments which result in transcriptomics, proteomic and metabolomic high throughput data, can be researched with *in silico* methods (Bhogal et al., 2005; Carmichael et al., 2022). It could be that the *in vitro* data cannot answer the same research question as with *in vivo* research, but the question can be answered in a different way. Moreover, by combining different *in vitro* techniques with advanced *in silico* techniques, the *in vitro* data can be put into context (Chapman et al., 2013; Turner et al., 2023).

These workflows with NAMs can be implemented into a workflow called NGRA. The definition given by Carmichael et al., 2022 explains that NGRA is: “an exposure-led, hypothesis-driven risk assessment approach that integrates NAMs to assure safety without the use of animal testing”. Especially, whether exposure to the chemicals is expected to occur or not is important to check. When animal test are performed, this method aims to predict whether the compound could cause harm for humans. With NGRA it is important not to predict, but to protect humans from harm, which can be established by taking different approaches. This include asking important questions first (will humans be exposed at all?) and performing the suitable NAMs with human material. If there is no expected human exposure, you don’t need to test any further. This is a new way of a stepwise approach in safety assessment towards establishing whether a compound can be harmful (Carmichael et al., 2022; M. P. Dent et al., 2021).

For chemicals and cosmetics already frameworks towards the use of NGRA workflows have been developed, but for pharmaceuticals there is not yet a clear source of information available about which workflows only use NAMs and which can be applied into (regulatory) practice.

Research questions

This leads to the following research question: How can the transition to animal-free next generation risk assessment (NGRA) for pharmaceuticals be accelerated? This question was addressed by answering the subsequent subquestions through literature study, in the form of a literature mapping review:

1. Is NGRA a good method for safety assessment of pharmaceuticals?
2. How is NGRA regulated for pharmaceuticals?
3. Which stakeholders are involved in the acceptance of using NGRA for safety assessment of pharmaceuticals?

This writing assignment gives an overview of the information available on safety assessment of pharmaceuticals and shows the opportunities for accelerating the transition (to regulatory acceptance) to animal-free NGRA for pharmaceuticals. In subquestion 1 workflows of NGRA for safety assessment of pharmaceuticals are addressed, with some recommendations for the implementation. In subquestion 2 the regulatory rules and in subquestion 3 the stakeholders involved are summarized. The focus of this writing assignment lies on the challenges and opportunities in regulatory acceptance of NAMs in NGRA for pharmaceuticals.

1. Methodologies

1.1 Searching strategies

A plan for searching literature was discussed with librarian Felix Weijdema during a meeting on the 30th of January 2023. It was concluded that PudMed, Embase and Overton were the right searching machines for this Writing Assignment. In PudMed and Embase a search for scientific papers was performed, and in Overton a search for legal documents on NGRA and safety assessment for pharmaceuticals was executed. The specific search queries for those searching machines can be found in the appendix (A1). Moreover, because the search for legal documents in Overton was not as straightforward as predicted, the search for legal documents was continued on Google on the specific sites of the agencies. The search terms and results can also be found in the appendix (A2). The selected resources from all search methods were categorised in a writing plan which can be found in the appendix (A3).

1.2 Experts

A number of experts in the field of NAMs, NGRA and safety assessment were contacted by email. They were asked whether they knew articles about the acceleration of the transition from animal experiments to NAMs or NGRA from their point of view. Which experts were emailed and what information they sent can be found in de appendix (A4). Not every expert contacted sent a reply.

1.3 eLearnings and seminars

For my own understanding eLearnings were selected and seminars were watched. Those can be found in the appendix (A5).

2. Subquestion 1: Is NGRA a good method for safety assessment of pharmaceuticals?

Back in 2014 it was already stated that opportunities of improving the efficiency of safety assessment of drugs lie in improving technology and obtaining more suitable information about a compound in the early drug discovery phase. This was stated because the productivity of drug development was decreasing. The biggest failures in predicting human safety can occur in the early stages of drug development which are often performed in animals, before the drugs are tested in humans. In the 2014 article, tests for better safety assessment are depicted for *in vitro, in silico*, but also for *in vivo* methods (Ahuja & Sharma, 2014). Nowadays, there is a shift towards more usage of NAMs in NGRA to eliminate the usage of animals. Moreover, the right use of NAMs can make safety testing more efficient and the research more translatable to humans (Chapman et al., 2013).

2.1 Workflows for safety assessment of pharmaceuticals

Different NAMs are nowadays summarized into workflows. For drug development of pharmaceuticals it is now still unknown how to implement NAMs for safety assessment. Some research is conducted which resulted in workflows of NAMs which could be implemented for safety assessment and this research proposed suggestions on what needs to change to accelerate the transition to NAMs for safety assessment. One of those workflows, presents a 3 step workflow, which focusses on the evaluation of usage of pharmaceuticals and other chemicals in the pharmaceutical industry. The 3 steps are depicted in figure 1. Before going through the 3 steps, at first problem formulation is a very important initial step in risk analysis. Here it becomes clear what question needs to be addressed and what regulatory determination should be made (Parish et al., 2020; *WHO Human Health Risk Assessment Toolkit: Chemical Hazards*, 2010). After this, step 1 is initiated where the context of use is determined. Here three aims can be chosen: prioritization, hazard screening or risk assessment. This is important to choose from to know what criteria need to be set and which NAMs are suitable to use. Step 2 is addressing the core principles (in no particular order): accuracy, transparency, understanding limitations and domain of applicability. Those principles help to design good research. Step 3 is analysing fit-for-purpose criteria, where the list of criteria is used to evaluate the suitability of a NAM. All the criteria are depicted in figure 1. In this article four case studies were performed to test the workflow. Here the opportunities can be observed that the workflow gives. However, this workflow does not focus on the implementation of the NAMs in the pharmaceutical industry and/or regulatory decision making, but it is focussed on identifying and documenting the information that is needed to get more confidence in the usage of NAMs, so that the implementation into practice would eventually go faster. Eventually the authors suggest that it is key to reach a consensus about a workflow. It is not mentioned how and with whom this consensus needs to be reached. Moreover, there are still challenges, like evaluation and interpretation of the data by different regulatory agencies (Parish et al., 2020).

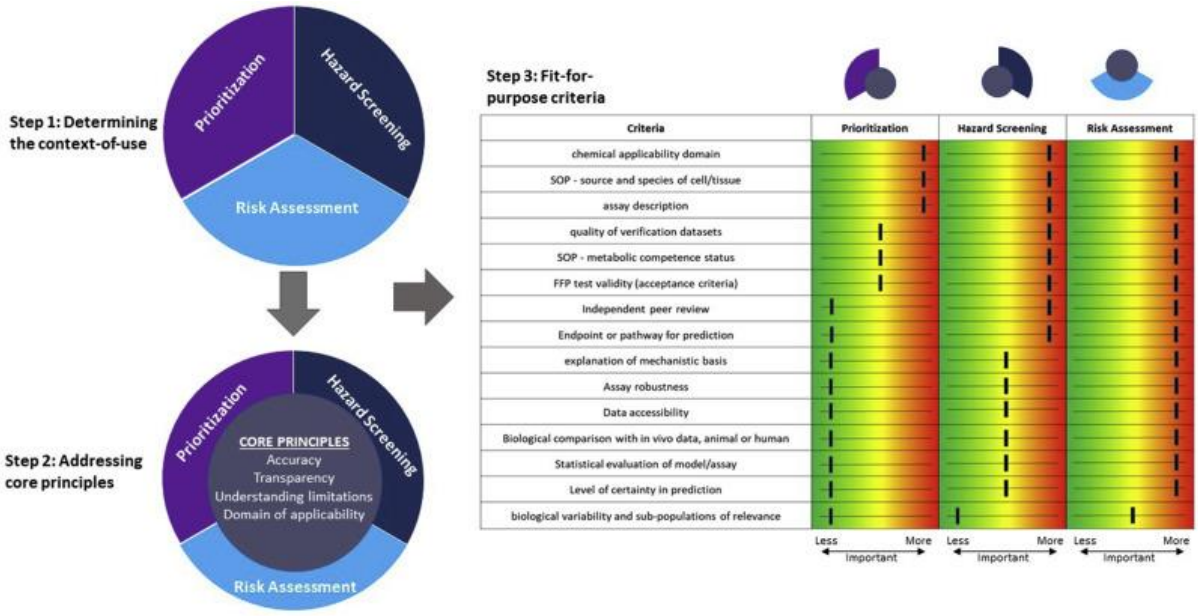

Figure 1: The 3 step workflow for NAM evaluation for pharmaceuticals is depicted above. These steps are initiated after the problem formulation and these steps help to choose which NAMs are suitable for each step individually in safety assessment (Parish et al., 2020).

Besides the evaluation of the NAMs for safety assessment, the following workshop report went further into detail by summarizing specific NAMs that could be used for the safety assessment of nonclinical pharmaceuticals. Nonclinical refers to research not related directly to living patients (Turner et al., 2023). The NAMs could target the off target effects of compounds in drugs. In this research the focus was on *in vitro* NAMs. It is still unknown how NAMs could be used during drug development for safety assessment, so maps were developed with NAMs for specific organs: cardiovascular system, respiratory system, central nervous system and the liver. Moreover, a workflow for safety assessment of pharmaceuticals was designed, where the exposure of the chemical was taken into account, with e.g. the PBK-models (figure 2). In this figure the NAMs per organ are as well implemented in the safety evaluation by NAMs (on the left in figure 2). The maps were produced in workshops organized with 13 individuals (two preclinical scientists, five NAM developers, five persons who performed both of the previous jobs and one regulator) and it was defined what outcomes were measured which could be used to identify changes in key processes of the organ, what NAMs were used and gaps which need to be filled with further research on NAMs. It is mentioned that NAMs have an advantage, because there is a direct translation to humans possible, but there are also some factors which discourage the uptake of NAMs. Some experts in the workshop still hesitated whether NAMs are able to generate data about a whole organism. Moreover, they hesitated whether the quality of the cells was high enough, because of possibilities *in vitro* that the cells undergo genetic drift, that there are batch-to-batch differences and media problems. A reaction to this was that computational models, like PBK-models, are capable to address these challenges. Still some things need to be addressed before the implementation of NAMs can occur into practice. It is recommended that there should be more communication and collaboration between different stakeholders, there should be a discussion about the usage of NAMs with decision making agencies before the NAMs are developed/used. It is mentioned that not that much representation of regulators was present during the workshop. So more regulatory input would have caused more insights if the NAMs in the workflows would address the needs of regulatory agencies. Also pharmaceutical companies need to be more transparent about how NAMs are used for safety assessment. The advantages, factors discouraging the uptake and

factors likely to increase adoption of NAMs were summarized by the authors in a table (figure 3) (Turner et al., 2023).

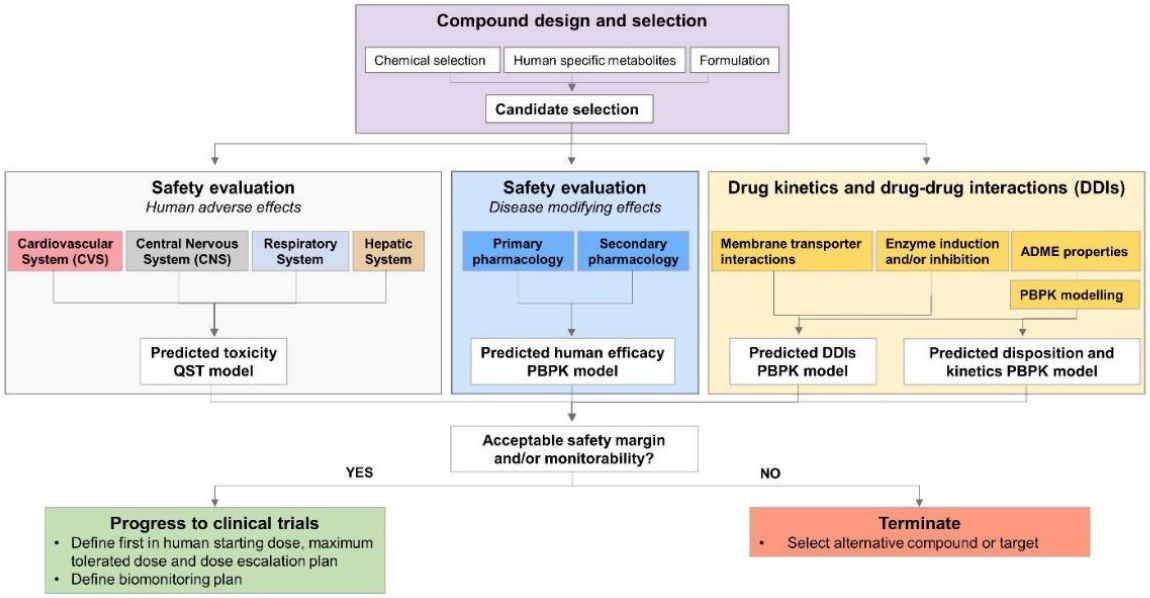

Figure 2: The workflow design with NAMs for the safety assessment of pharmaceuticals is depicted above. The different organ systems are depicted here as well (Turner et al., 2023).

Overarching category	Sub-categories
Advantages of NAMs	Predictive
	Regulatory confidence in some NAMs
	Insight into mechanisms
	Representation of human variability
	Human-relevant disease models
	Ability to conduct long-term studies
	Ability to do high throughput screening
Factors discouraging uptake of NAMs	Cell quality and standardisation issues
	Inability to represent whole organism or measure higher level endpoints
	Not tailored to regulatory needs
	Lack of clarity from regulators
	Some companies not yet sufficiently confident to jettison animal tests
	Benefits of NAMs not publicised
	Lack of reference data
	Cost
Factors likely to increase adoption of NAMs	Collaboration, especially with regulators
	Learn from other regulated sectors
	Use NAMs to assess developmental and reproductive toxicology
	Describe NAMs conducted in-house when making submission to regulators
	Rewording of ICH guidelines
	Use battery of tests
	Make NAMs commercially available
	Understand purpose of *in vivo* studies
	Bridging studies from animal to human

Figure 3: In this table the advantages, factors discouraging the uptake and factors likely to increase adoption of NAMs for pharmaceutical safety assessment are summarized (Turner et al., 2023).

The maps in the article above were focussed on *in vitro* NAMs. Besides *in vitro* NAMs, *in silico* NAMs are described as well in the article of Ford, 2016. These techniques have advantages:

they are less expensive than animal studies, can generate and combine a lot of high throughput data, can be standardized, are less time consuming, are safer (because some chemicals are hazardous to work with in laboratories) and have more possibilities (because some experiments are not possible to perform in laboratories). Three main approaches are explained to predict toxicological off- and on-targets and endpoints of pharmaceuticals for risk assessment: (1) grouping approaches (which include read-across (RAX) sources), (2) structure-activity relationships (SARs) and quantitative SAR (QSAR), and (3) experts rules based systems. Endpoint prediction is possible with already available software programs, such as: Organization for Economic Co-operation and Development QSAR Toolbox, eTox, Toxmatch and toxRead. With these programs the effect of a compound can be predicted with using an endpoint as for example mutagenicity, hepatoxicity, eye irritation, skin sensitization, rat oral acute toxicity (LD_{50}) and carcinogenicity. Moreover, in the case of aiming to reduce the number of animals in animal experiments, *in silico* tests can also be used to better plan *in vivo* tests. Besides the promising opportunities, critical points about *in silico* testing are that the data is complex and it could be that the algorithm misses metabolites of the test compounds or other parameters. This can be mended by spending more time and money on *in silico* testing (Ford, 2016).

2.2 Workflows for safety assessment of chemicals

There are more (detailed) workflows available for implementing NAMs in NGRA for chemicals and/or cosmetics in safety assessment, than for pharmaceuticals. Learning about those workflows can help with translating those to risk assessment of pharmaceuticals. In this writing assignment a few of those workflows are addressed.

The first workflow can be applied on chemicals, but also more broadly to other compounds (including drugs, industrial chemicals, food and cosmetics) (figure 4). Certain *in silico* methods can be implemented into a NGRA workflow which produces and interprets big data: SARs, RAX and QSARs. A more specific *in silico* model mentioned is a physiologically based pharmacokinetic (PBK) model, which is a mathematical technique where *in vitro* and *in silico* models can be combined to mimic different organs to predict the effect of a chemical on the body. PBK models are interesting to use to extract data from already available data to come up with a dose for pharmaceuticals, and are promising in NGRA regulatory decision making (Paini et al., 2019; Ram et al., 2022). More promising (than just looking to the methods alone) is to combine *in vitro*, *in silico* and artificial intelligence methods in a workflow based on adverse outcome pathways (AOP). Those are models which can identify the order of events that are required to decide whether a chemical has a toxic effect in an organism when exposed to this chemical. Besides already existing opportunities, recommendations are that it would be better to have more open access to data (sharing) to assess for the interpretation of this big data. Moreover, with these *in silico* techniques a big amount of big data is produced. Because of the amount available, a critical attitude against the quality vs quantity is needed. Other challenges are getting funding for *in silico* projects in case of aiming for regulatory acceptance of compounds (Ram et al., 2022).

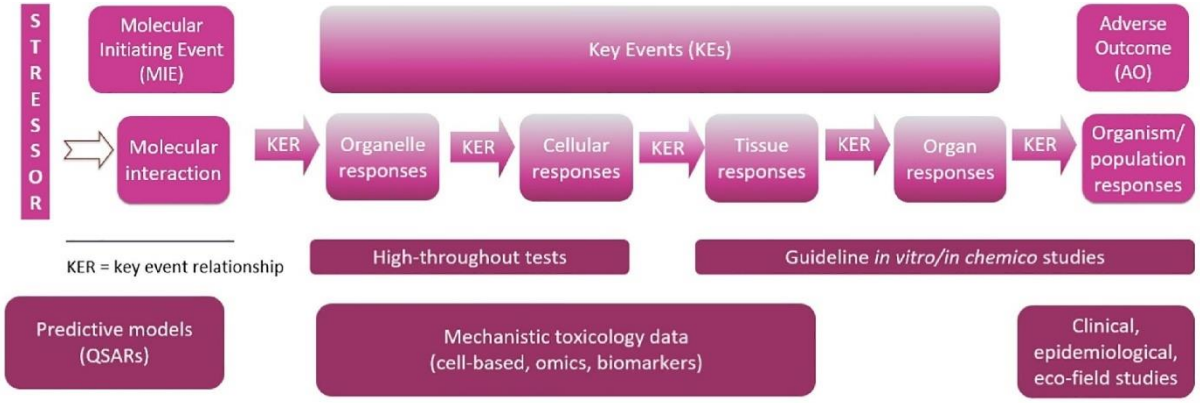

Figure 4: Due to a specific order of events by exposure to a chemical, this can lead to an adverse effect. This is described in an AOP which can be used for safety assessment of chemicals (and more broad on other compounds such as drugs, industrial chemicals, food and cosmetics) (Ram et al., 2022).

The other workflows described in this subquestion are specifically developed for chemicals. The European Horizon 2020 risk assessment of chemicals integrating human-centric next-generation testing strategies promoting the 3Rs (RISK-HUNT3R) project designed one of the workflows. The goal of the project is to design a workflow which combines computational, *in vitro* toxicology and systems biology. The workflow was created with specific NGRA characteristics: it is focused on safety, exposure-led, hypothesis-driven, and uses a tiered and iterative approach. The goal is that different subpopulations and vulnerable groups are also protected with this kind of safety assessment. The three main topics are tiered steps: exposure, hazard and risk. Those can be observed in figure 5. First the problem formulation occurs and from there the iterative process starts. In the exposure step there are two modules. Module 1 is ‘from external to internal exposure’ and module 2 is ‘metabolism/toxicokinetics’. In the first two modules it is identified how exposure occurs when exposed to the chemical. In the ‘exposure’ step experiments are conducted whether the chemical can pass the first barriers and after that data is integrated in PBK models where the systematic behaviour of the chemicals can be researched. Then it goes more into detail how the chemical is processed and metabolized. In the ‘hazard’ step there are also two modules. Module 3 is ‘effect identification’ and module 4 is ‘adversity/quantification’. In module 3 and 4 the hazard of the chemical will be characterized. Follow-up assays are conducted by *in vitro* and *in silico* testing to identify specific AOP networks. In the AOP chemical interactions and biological responses become clear. The last step can be observed in module 5 ‘integrated NGRA’ in the ‘risk’ step, where the overall risk characterization is performed and the data is evaluated. Moreover, uncertainties become clear here. Because of this, transparent reporting and decision making is important. The biggest challenge that is proposed for NGRA is the translation of *in vitro* work to human systems and to AOPs. But with advances *in silico* methods and modelling this problem will be assessed in the best possible way (Pallocca et al., 2022).

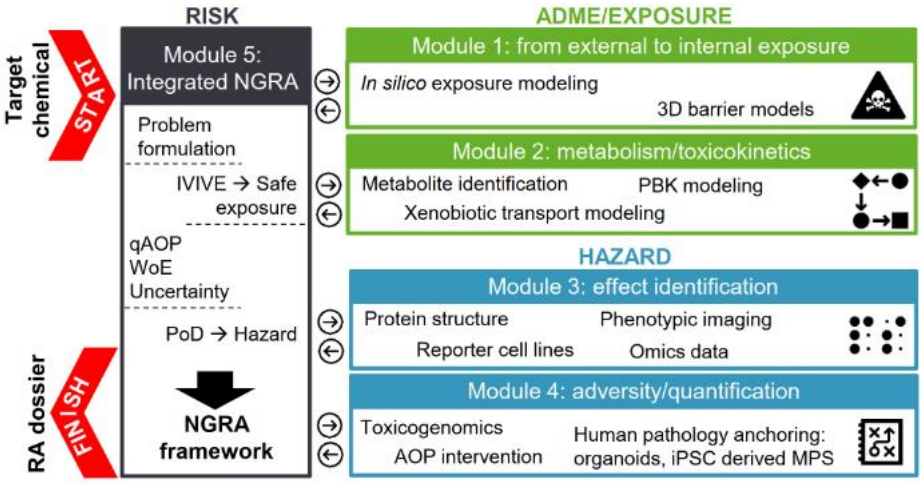

Figure 5: The 3 main steps (exposure, hazard and risk) with the 5 modules of the proposed workflow for the risk assessment of chemicals are depicted above in the RISK-HUNT3R project (Pallocca et al., 2022).

Another new article which focussed on implementing NAMs in the NGRA approach was the article of Middleton et al., 2022. The workflow presented here, performs systemic safety assessment of chemicals for adult consumers by the use of *in vitro* and *in silico* NAMs. Besides this an approach, to estimate the bioactivity exposure ratio (BER) from the points of departure (POD), was implemented. This had not yet been included in a workflow yet. The workflow was inspired by the Baltazar et al. 2020 study that is hypothesis driven and tiered. There are 3 modules in this workflow: C_{max}, POD and BER estimation, shown in figure 6. In the 'C_{max} estimation' module the internal exposure will be estimated by using already existing *in vitro, in vivo* and *in silico* data which is implemented in PBK models. In the 'POD estimation' module the POD will be estimated by using already existing *in vitro* data which focussed on bioactivity of the chemical. Here high throughput transcriptomics, a cell stress panel and *in vitro* pharmacological profiling takes place. Lastly, in the 'BER estimation' module all data comes together and is combined to reach an estimation of the systemic toxicity of the chemical in adults. After this, a Bayesian model can be used to identify the uncertainty of the C_{max}. What is interesting is that the workflow was evaluated by performing safety assessment on ten chemicals. Concluded was that 69% of the low risk and 100% of the high risk chemicals were identified correctly. This demonstrates that this risk assessment can be performed with NAMs, but a critical attitude is needed, because not all hazard was detected (Middleton et al., 2022).

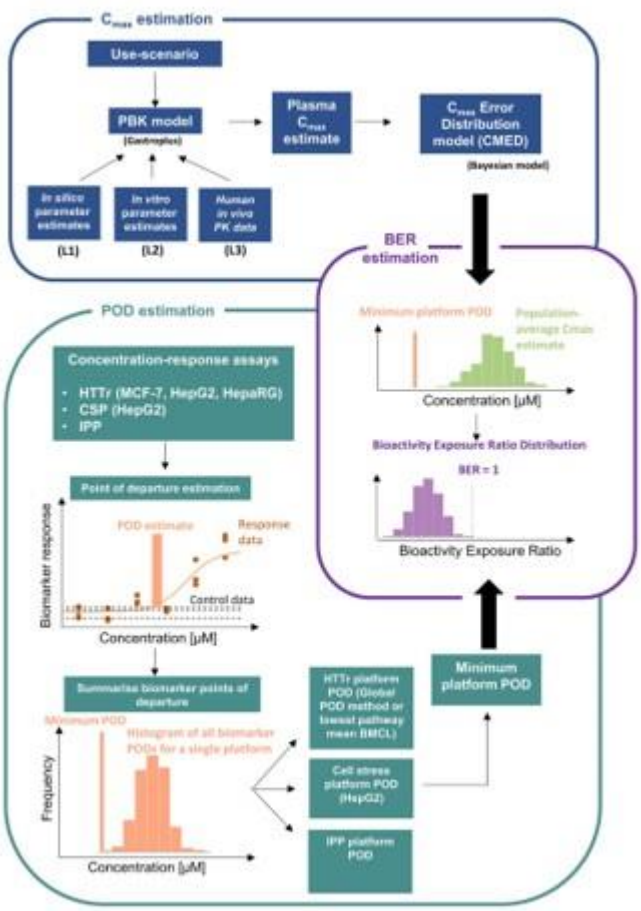

Figure 6: The 3 main steps (C_{max}, POD and BER estimation) of the proposed workflow for the risk assessment of chemicals are depicted above (Middleton et al., 2022).

In the articles above, chemicals specific workflows were presented. In another recent publication, the usage of NAMs in NGRA for safety testing was discussed (Carmichael et al., 2022). The main conclusion is that the use of NAMs in NGRA is ready for chemical safety assessment. NGRA is a promising approach, because it will not try to predict toxicity that a human would not be exposed to. This results in protection of human health rather than prediction of the toxicity of a chemical. It is summarized that with *in vitro* research, PODs can be set with NAMs (based on the bioactivity of a chemical), and research can be performed with specific internal exposure levels (determined by systemic PBK of a chemical), to perform sufficient risk assessment. Moreover, when performing PBK modelling, the activity of the chemical or metabolites can be implemented as well. From here, together with the POD, decisions about safety are drawn from the estimated BER. A small BER is less likely to give adverse high effects. In the article it supports to work with higher tiered levels, which are AOP-driven, when safety is not demonstrated on lower tier levels. Examples of workflows are explained in this review and already known information is summarized. The promising characteristics of those workflows are specified for the specific research areas. Besides this, people are still uncertain about using NAMs for safety assessment, because of the long history of using animals. On the other hand NAMs have proven to be good or even better for the protection of human health. A recommendation is to build confidence and experience in NAMs. Another recommendation is to invest money in the translation of the available workflows into practice, together with new regulations (Carmichael et al., 2022).

Carmichael et al., 2022 expressed the need for more case studies, and Fragki et al., 2023 answered to this. In the research of Fragki et al., 2023 a case study for specifically the hazard characterisation of per- and polyfluoroalkyl substances (PFAS) was proposed. With NAMs, the oral dose which caused adverse effects was measured in cell systems for a few specific PFASs. This was performed by researching concentration-response data which was produced by PBK modelling and the biokinetics which was studied in the cell systems. The calculated dose which caused adverse effects overlapped with the current known dietary exposure to PFASs. It is stated that therefore this methods which uses *in vitro* and *in silico* data can be applied for more PFASs in hazard characterization (Fragki et al., 2023).

2.3 Workflows for safety assessment of cosmetics

The research of the Safety Evaluation Ultimately Replacing Animal Test (SEURAT-1) and Long Range Science Strategy (LRSS) programs are based on cosmetics, but can also be translated to other chemicals. One of the workflows was developed within the SEURAT-1 programme (2011-2015), which was financed by the European Commission (Berggren et al., 2017; Desprez et al., 2018). The goal of the workflow was to predict no-adverse health effects for the safety assessment of cosmetics and other chemicals (plant protection products, biocides and pharmaceuticals), by formulating a hypothesis on existing data and *in silico* modelling. This tells you more about the AOP of a specific compound. The used approach is a tiered approach: tier 0, tier 1 and tier 2, shown in figure 7. At tier 0 level it is identified what the exposure level is to the chemical and additional information is collected by for example using *in silico* methods, such as QSAR models. The Threshold of Toxicological Concern (TTC) approach can be used for low-risk chemicals to determine if there is exposure which is lower than the threshold where there is a low noticeable risk to human health. This approach does not require a lot of toxicological data. When this approach is applied, it can be determined whether further toxicological testing is required. No further refined risk assessment is needed when this risk is quantified as low. At tier 1 level the hypothesis is formulated by modelling. For cosmetics it is convenient if the PBK model can predict whether the compound can cross certain barriers and after this gives more information about the systematic behaviour of the compound. After that the hypothesis can be generated by using the mode-of-action (MoA). This is used to describe how a compound can affect the health of humans. At tier 2 level there is the application of the *ab initio* approach. This assessment is hypothesis-driven based on new *in vitro* mathematical modelling data and combining it with *in silico* data. Here it will become clear what kind of NAMs will be used to perform risk assessment. When performing one case study it became clear that the workflow can cover a lot of different chemicals, endpoints and exposure scenarios, which leads to reliable risk assessment (Berggren et al., 2017).

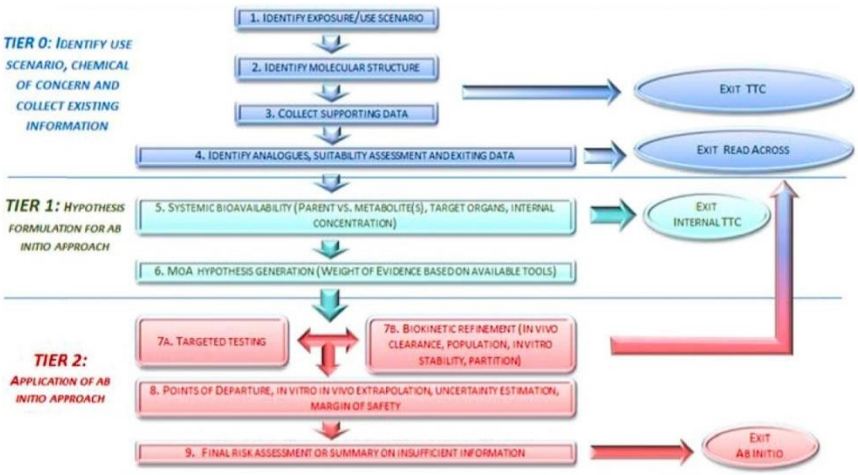

Figure 7: The tiered approach in the proposed workflow for the risk assessment of cosmetics (but also other chemicals such as plant protection products, biocides and pharmaceuticals) is depicted above (Berggren et al., 2017).

Based on the outcomes of the SEURAT-1 programme workflow, the LRSS programme was launched by Cosmetics Europe from 2016 to 2020. The project was prolonged to 2022 (Desprez et al., 2018; *Website LRSS: The Long Range Science Strategy: Our Main Research and Science Programme*, n.d.). As well as the SEURAT-1 programme, the LRSS programme focussed on cosmetics, however it is applicable to other chemicals as well. The focus of the LRSS lies on specific projects: the toxicokinetic and toxicodynamic projects. The overall approach used is adapted from SEURAT-1, based on alternative approaches and is tiered. The same 3 levels of tiers are observed here: tier 0, 1 and 2 (shown in figure 8) (Desprez et al., 2018). This approach is also included by the Organisation for Economic Co-operation and Development (OECD) for chemicals more broadly (*Chemical Safety Assessment Workflow Based on Exposure Considerations and Non-Animal Methods*, 2017). The tools for safety assessment in the workflow of the LRSS are internal TTC, read across, toxicokinetic and toxicodynamic tools. All the tiers lead eventually to the integration within the safety assessment paradigm (the 4 basic steps of safety assessment which are hazard identification, hazard characterization (also called dose-response relationship), exposure assessment and risk characterization), which can be observed in detail in figure 9, 10 and 11. After uncertainty characterisation and prediction the safety assessment can take place. These workflows were made to eventually use this in a regulatory context and to promote the shift from theory to practice. The LRSS also wants to perform case studies just like the SEURAT-1 project to prove their concept will work (Desprez et al., 2018).

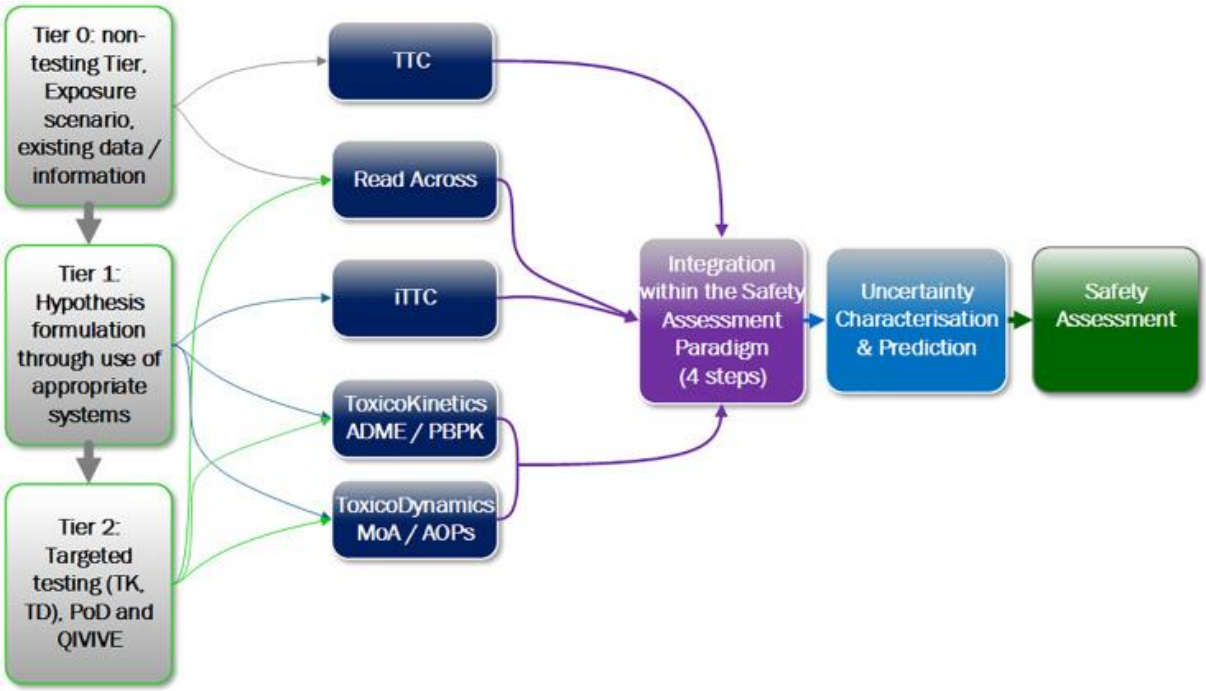

Figure 8: The tiered approach in the proposed workflow for the risk assessment of cosmetics is depicted above and is based on the SEURAT-1 project (Desprez et al., 2018).

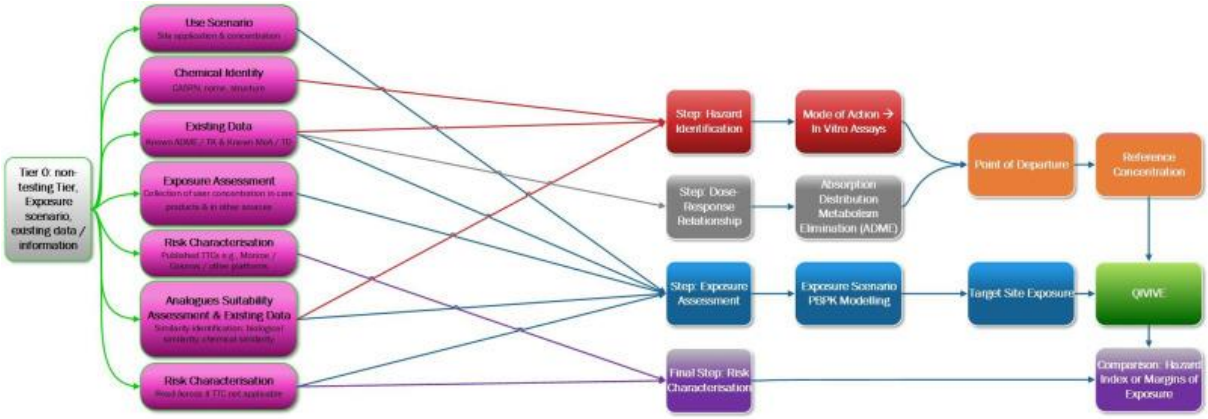

Figure 9: Tier level 0 was elaborated per step taken and connected to the 4 steps of the safety assessment paradigm (Desprez et al., 2018).

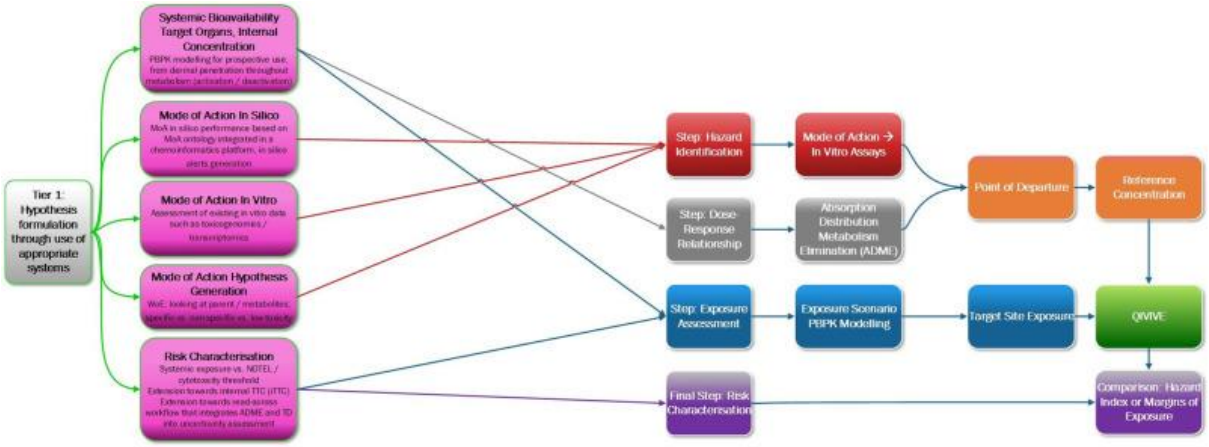

Figure 10: Tier level 1 was elaborated per step taken and connected to the 4 steps of the safety assessment paradigm (Desprez et al., 2018).

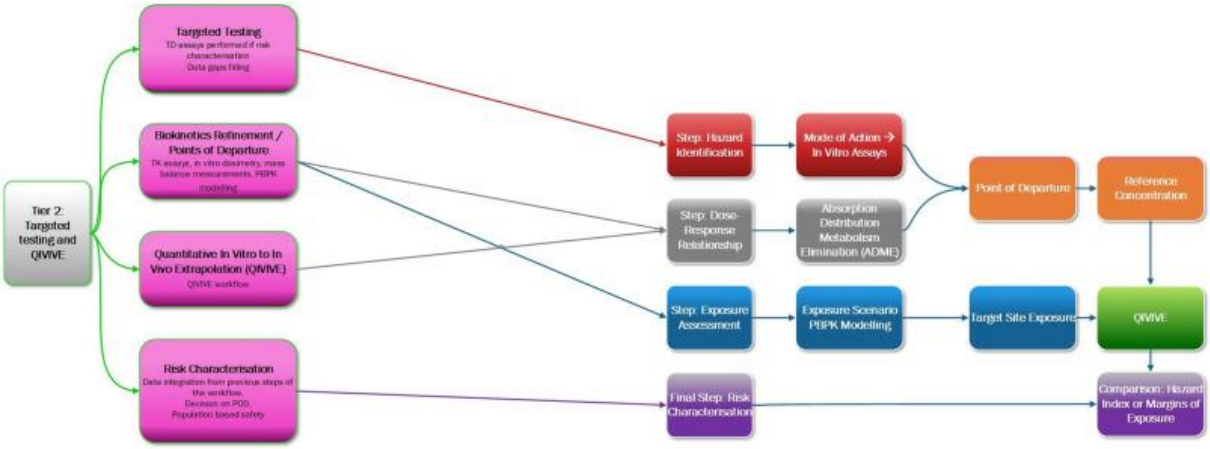

Figure 11: Tier level 2 was elaborated per step taken and connected to the 4 steps of the safety assessment paradigm (Desprez et al., 2018).

Another workshop was organized by the Methodology Working Group of the Scientific Committee of Consumer Safety (SCCS) in February 2019. The article summarized the outcomes of this meeting of the possibilities of using NAMs in NGRA for cosmetics in the EU. During the workshop an overview of NAMs and strategies in a workflow for cosmetic ingredients was provided, based on the data from Berggren et al., 2017 and Dent et al., 2018 about NGRA for cosmetics. In this article it is mentioned that NGRA is more specific than traditional safety assessment. In NGRA there is more attention brought to the breakdown of processes to ensure that the right things are tested *in vitro* and those results can be compared in the final safety assessment. Hereby the tested cases will cover a broad spectrum of relevant information. For cosmetics the emphasis so far has been on dermal exposure. Recommendations are that there is a need to build confidence in the usage of NGRA for cosmetic safety assessment for all stakeholders and a need for more concrete examples also beyond skin. The proposed workflow can be observed in figure 12. Here it is proposed to work with the concept of internal TTC (based on the plasma concentration) to provide exposure limits which can be used in safety assessment. This internal TTC can be derived after multiple steps: first ADME data will be obtained of a specific cosmetic by existing literature data and *in silico* techniques. After that with PBK modelling, the internal TTC can be identified. Other *in silico* methods are discussed as well, such as read-across and (Q)SAR models for the safety assessment of cosmetic ingredients. Positive is that those models can run a lot of data for substances. Two read-across case studies with already available data were discussed and it was concluded that those are promising to obtain reliable information about the cosmetic. But, it is suggested that NAMs lack the more complex toxicological endpoints, so more should be invested in case studies and examples of the usage of NGRA for cosmetics. Moreover, NGRA is promising, but more close interactions are needed by the different stakeholders in the field (multidisciplinary), so that a complete workflow for NGRA for cosmetics could be made, which also could give insight into the knowledge gaps (Rogiers et al., 2020).

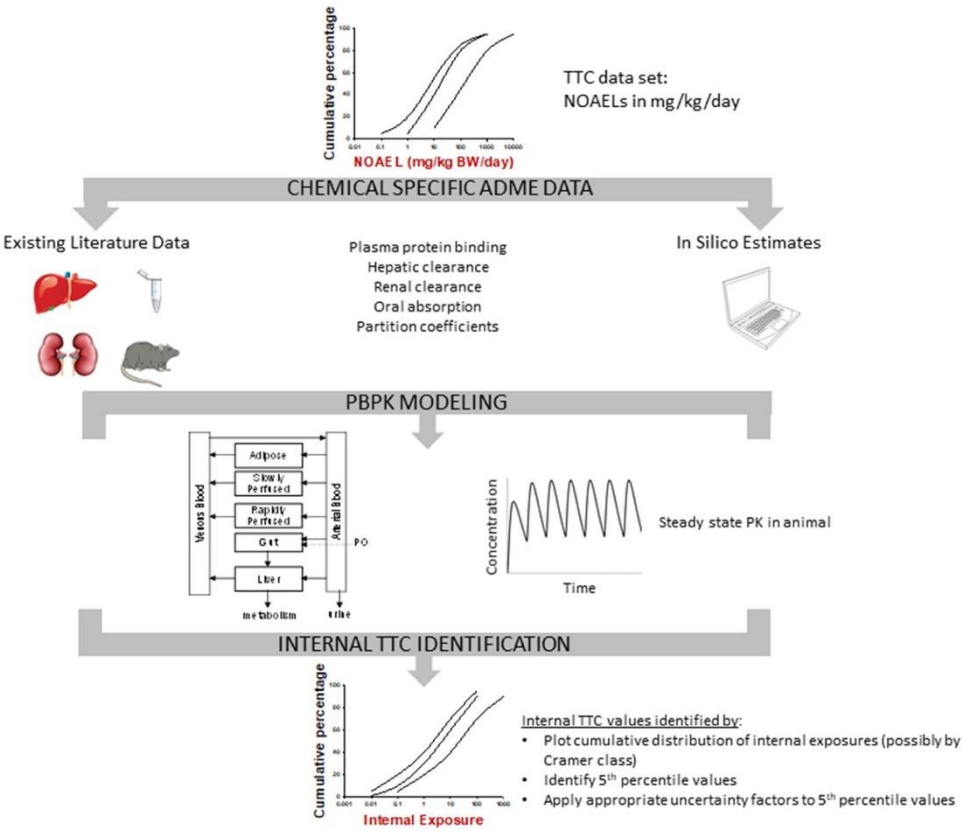

Figure 12: The identification of the internal TTC of the proposed workflow for the risk assessment of cosmetics is depicted above (Rogiers et al., 2020).

More recently, an example of a NGRA case study has been published by Baltazar et al., 2020. Here the focus is on the hypothetical safety assessment of 0.1% coumarin which can be present in cosmetic products (face cream and body lotion). This case study integrates and interprets already existing animal and human data with new *in silico* data of coumarin for safety assessment. The workflow that was used in this example can be observed in figure 13. Here the internal concentrations of coumarin were estimated by PBK when applied dermally and systemic toxicity was assessed by identifying multiple PODs. The conclusion was that coumarin was not toxic. So combining exposure science, computational modelling and *in vitro* bioactivity data seems promising for safety assessment without the usage of animals. The authors have high confidence in their workflow. It is recommended that there should be continued development and application of NAMs, especially more specific case studies should be performed, because more confidence is needed (Baltazar et al., 2020).

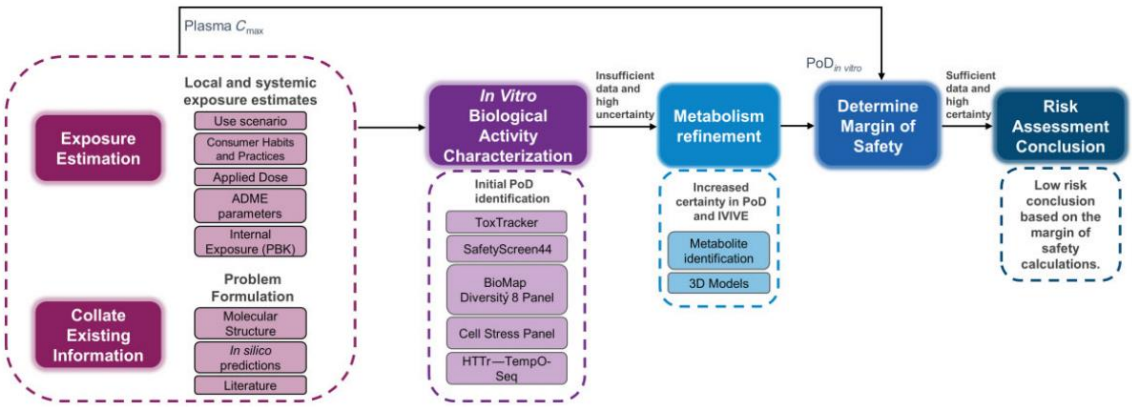

Figure 13: The proposed workflow above was used for the safety assessment of 0.1% coumarin in cosmetic products (Baltazar et al., 2020).

Besides that, a review by Dent et al., 2021 summarized which NAMs in NGRA can be used and how those can be made useful for the safety assessment of cosmetic ingredients. This is a continuation of the article by Dent et al., 2018, which was more about the principles of NGRA for cosmetics. In the article in 2021 a workshop is summarized that was held in Montreal in 2019. In the workshop all the basics of NGRA in relationship to safety assessment for cosmetics and if NGRA can be protective for human health were discussed. It became clear that the application of NGRA for regulatory safety assessment needs to be developed further. Here 7 key areas were proposed to make NGRA more useful for cosmetics and to build more confidence (Dent et al., 2021):

1. Make sure the toxicokinetic and metabolite predictions by PBK modelling are rigid;
2. Make sure the different non-animal experiments cover a broad enough spectrum;
3. Be clear about the level of confidence (including uncertainty) per method and results;
4. Make standards for using techniques and reporting data;
5. Make a distinction between an adaptative and adverse effect;
6. Update the workflows that are already made when new information becomes available;
7. Invest in more case studies as examples.

The information above about safety assessment with NAMs in NGRA for pharmaceuticals, chemicals and cosmetics has been summarized in table 1.

Article	*Compounds*	*Product for safety assessment*
Ford 2016	Pharmaceuticals	Examples *in silico* NAMs
Parish et al. 2020	Pharmaceuticals	Evaluation usage of NAMs in which context
Turner et al. 2023	Pharmaceuticals	Workflow + examples *in vitro* NAMs for 4 organs
Ram et al. 2022	Chemicals, can also be used for pharmaceuticals, industrial chemicals, food, cosmetics	Workflow for *in silico* NAMs
Palloca et al. 2022	Chemicals	Workflow for *in vitro* and *in silico* NAMs with NGRA
Middleton et al. 2022	Chemicals	Workflow for *in vitro* and *in silico* NAMs with NGRA
Carmichael et al. 2022	Chemicals	Summary of workflows for NAMs with NGRA
Fragki et al. 2023	Chemicals	Case study on a chemical (PFASs)
Berggren et al. 2017	Cosmetics, can also be used for plant protection products, biocides, pharmaceuticals	Tiered workflow for *in vitro* and *in silico* NAMs
Desprez et al. 2018	Cosmetics, can also be used for other chemicals	The steps in the different tiers are distinguished and elaborated more and into the general safety assessment paradigm, based on the workflow of SEURAT-1
Rogiers et al. 2020	Cosmetics	Workflow for existing literature data and *in silico* NAMs with NGRA
Baltazar et al. 2020	Cosmetics	Hypothetical case study on a cosmetic (0,1% coumarin)
Dent et al. 2021	Cosmetics	Summary of how NAMs can be used with NGRA with 7 key areas to focus on

Article	*Example of usage of the workflow?*	*Project*
Ford 2016	Yes, there are examples of cited papers how toxicological endpoints are decited in specific f	Research group
Parish et al. 2020	Yes, 4 case studies	Research group
Turner et al. 2023	No	5 workshops with 13 experts (2 preclinical scientists, 5 NAM developers, 5 persons who performed both of the previous jobs and 1 regulator)
Ram et al. 2022	No	Research group
Palloca et al. 2022	No	RISK-HUNT3R
Middleton et al. 2022	Yes, 24 exposure scenarios from 10 chemicals	Research group
Carmichael et al. 2022	Yes, recalls on examples from other articles	Research group
Fragki et al. 2023	Yes, this study itself is a case study	Research group
Berggren et al. 2017	Yes, 1 case study	SEURAT-1 by the European Commission
Desprez et al. 2018	No, but they want to perform case studies	LRSS
Rogiers et al. 2020	Yes, during the workshop 2 examples of case studies were discussed	Workshop by the SCCS by the EU
Baltazar et al. 2020	Yes, this study itself is a case study	Research group
Dent et al. 2021	Yes, examples of performed case studies are summarized	Workshop

Article	*Recommendations*
Ford 2016	Computational toxicology tools are useful for the 3Rs in toxicity testing.
Parish et al. 2020	The case-studies show that the workflow can be used in various stages of development, but they recommend that a consensus about a workflow should be reached for faster implementation to practice.
Turner et al. 2023	A recommendation that was really specific was that they recommend that there should be more transparancy about the use of NAMs in pharmaceutical companies, more communication with regulators and more case studies.
Ram et al. 2022	The combination of *in vitro, in silico* and artificial intellegence would be promising in a workflow where there is open access to + data sharing of big data.
Palloca et al. 2022	The goal with this kind of safety assessment is that different subpopulation and vulnarable groups will be protected.
Middleton et al. 2022	The workflow with NAMs can be used to provide the BER based on the 69% succes rate for low-risk chemicals and 100% for high-risk chemicals.
Carmichael et al. 2022	NAMs and NGRA for chemicals are ready for regulatory use, so confidence in the usage of NAMs should be build and more should be invested in the translation of workflows to practice with new regulatory frameworks.
Fragki et al. 2023	*In silico-in vitro* methodology can be used for other PFASs in hazard characterization.
Berggren et al. 2017	The workflows covers a lot of different chemicals, endpoints and exposure scenarios, but with their case study it became clear that the uncertainty should be defined.
Desprez et al. 2018	Because eventually case studies will be performed, there will be more a shift from theory to practice.
Rogiers et al. 2020	It is recommended that more should be invested into NAMs with more complex endpoint and there should be better communication between the different stakeholders in the field.
Baltazar et al. 2020	It is recommended that there should be continued development and application of NAMs, especially more specific case studies should be performed, because more confidence is needed.
Dent et al. 2021	The focus areas that are called upon specifically in the abstract are performing more case studies and the development of frameworks where you will be guided to document the performance and reporting

Table 1: The information in the table above summarizes the articles described in subquestion 1.

In conclusion, there are specific NAM/NGRA workflows and software programs which seem good methods for the safety assessment of pharmaceuticals, or workflows for chemicals/cosmetics which can also be translated to pharmaceuticals. The given workflows change the basic safety assessment paradigm. There is an overlap observed in the different workflows. The overlap can be observed in the fact that the workflows give examples of NAMs which can be used during safety assessment. Also they focus on different tiered levels by hypothesis and exposure driven research. There are also some differences which can be observed. For pharmaceuticals and chemicals the 4 steps of the safety assessment paradigm (hazard identification, hazard characterization, exposure assessment and risk characterization) are used to build up the workflows, but for cosmetics the backbone of the workflow is build up from the different tiered-levels. In the different tiered-levels the 4 steps of the paradigm are as well implemented (figure 9, 10 and 11), but the main message of the workflow for cosmetics is that it should be tiered. The workflows are summarized in figure 14, 15 and 16 for pharmaceuticals, chemicals and cosmetics separately. Because of the observed overlap (the steps in all workflows can be implemented in the safety assessment paradigm, which is for NGRA hypothesis-driven and tiered), it could be possible to combine the workflows in the future according to the tiered approach which is already observed in cosmetics, with keeping the specific purpose of the safety assessment in mind. In this writing assignment the information available is summarized for a tiered workflow in figure 17.

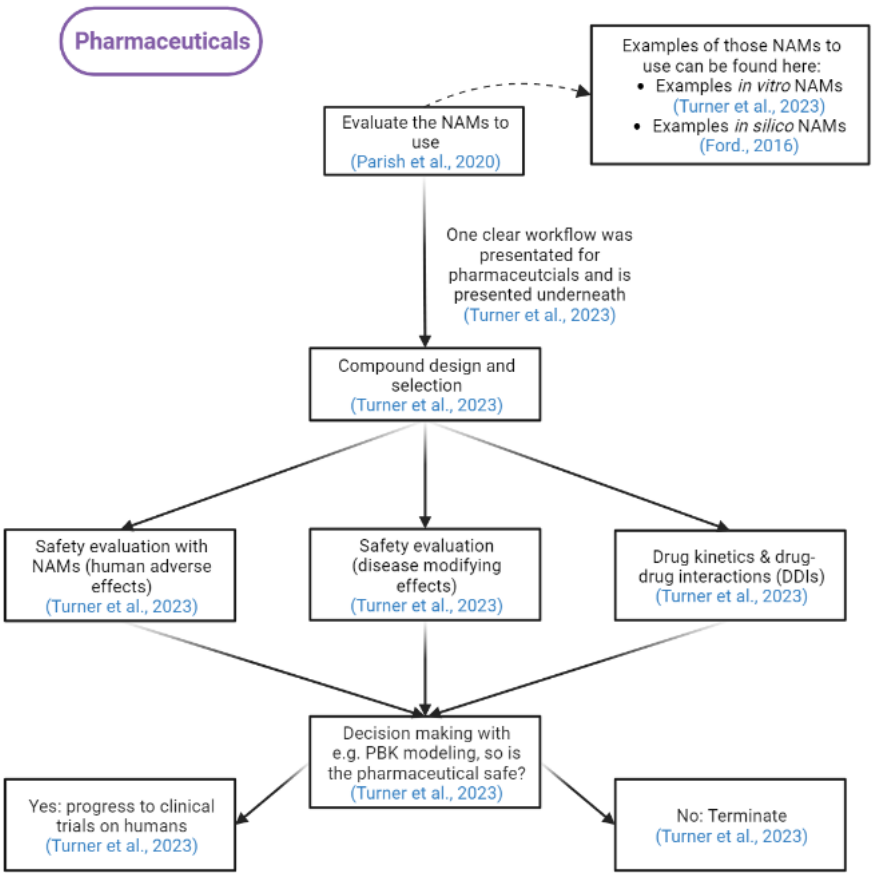

Figure 14: The information in subquestion 1 about safety assessment with NAMs in NGRA for pharmaceuticals is summarized in the figure above. With the arrows the workflow can be read step by step. The lined arrows indicate extra explanation about a step in the workflow. This figure is made with Biorender.

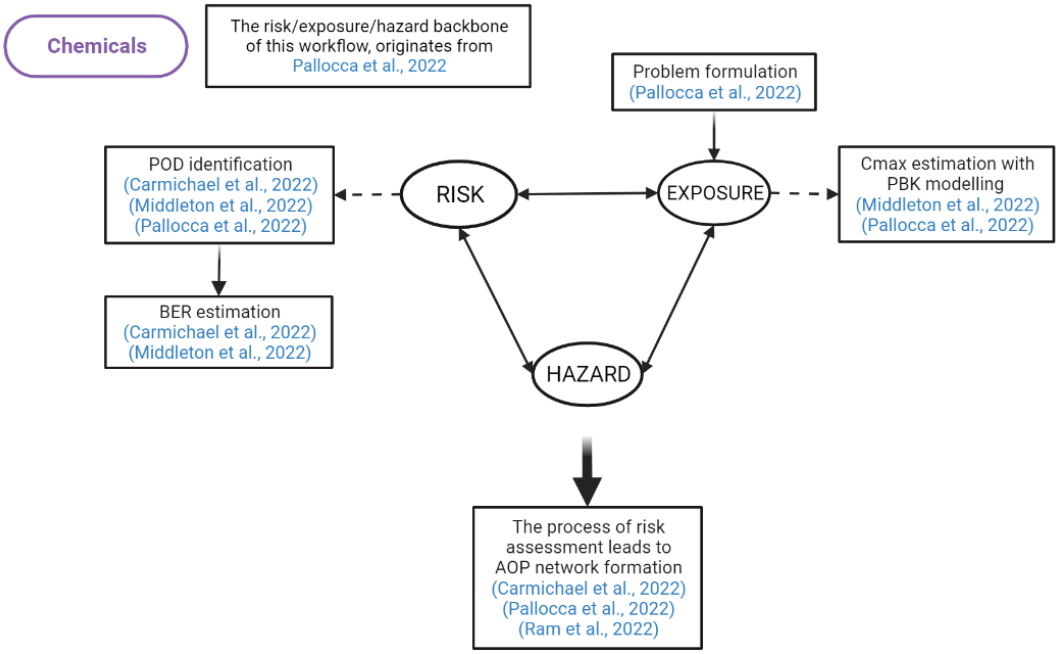

Figure 15: The information in subquestion 1 about safety assessment with NAMs in NGRA for chemicals is summarized in the figure above. With the arrows the workflow can be read step by step. The lined arrows indicate extra explanation about a step in the workflow. This figure is made with Biorender.

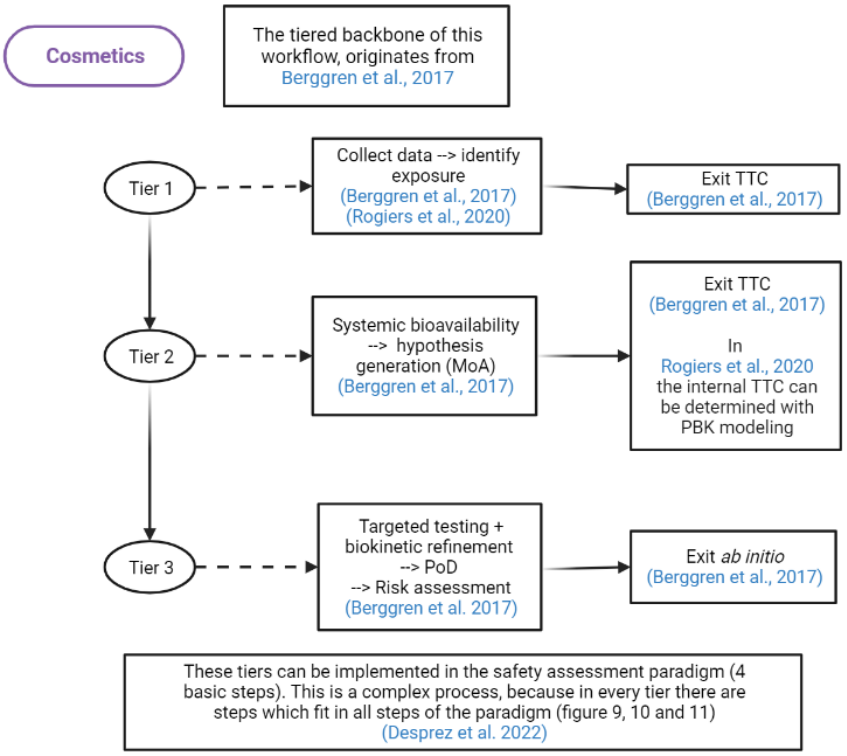

Figure 16: The information in subquestion 1 about safety assessment with NAMs in NGRA for cosmetics is summarized in the figure above. With the arrows the workflow can be read step by step. The lined arrows indicate extra explanation about a step in the workflow. This figure is made with Biorender.

The tiered backbone of this workflow for cosmetics, originates from Berggren et al., 2017 and Desprez et al., 2018	Cosmetics (all information cited from Desprez et al., 2018)	Pharmaceuticals	Chemicals
Tier 0 = non-testing tier, exposure scenario, existing data/ information	"Non-testing information on the type of chemical & exposure scenarios are collected. The safety assessment approaches used are TTC and read across" (figure 9).	• The read across *in silico* method is mentioned in Ford 2016	• The problem formulation is discussed in Pallocca et al., 2022, which is important before even starting with the 4 steps of risk assessment • The read across *in silico* method is mentioned in Ram et al., 2022
Tier 1 = hypothesis formulation through use of appropriate systems	"Other tools are used if Tier 0 was not conclusive, and this allows hypothesis formation that may serve as the basis for further (experimental) work. The hypothesis is formed by using toxicokinetic tools (ADME parameters, PBK modelling) and toxicodynamic tools (broad MoA considerations, AOPs) and can lead to exposure based waiving based on internal thresholds of toxicity concern *i.e.* internal TTC"(figure 10).	• *In silico methods* mentioned in Ford 2016 could help with the PBK modelling • The *in vitro* techniques in Turner et al., 2023 can be used to search for already existing information	• *In silico* methods mentioned in Ram et al., 2022 could help with the PBK modelling • PBK modeling for researching the systemic behaviour of chemicals is mentioned in Middleton et al., 2022 and Pallocca et al., 2022. ◦ Extra: in these articles the Cmax is extimated with the PBK modelling. • AOP pathways are discussed in Carmicheal et al., 2022, Pallocca et al., 2022 and Ram et al., 2022
Tier 2 = targeted testing and quantitative *in vitro-in vivo* extrapolation	"Testing is conducted if Tier 1 was not conclusive. This encompasses targeted TD assays, TK assays & PBPK modelling." This gives more information about the POD. "This should allow Quantitative *In Vitro* to *In Vivo* Extrapolation (QIVIVE) and risk characterisation. At Tier 2, the data generated may be towards an *ab initio* assessment but may also allow performance of read across" (figure 11).	• *In silico methods* mentioned in Ford 2016 could help with the PBK modelling • Examples *in vitro* NAMs to perform are mentioned in Turner et al., 2023 • To evaluate which NAMs would be good to use, the workflow from Parish et al., 2023 can be used	• *In silico* methods mentioned in Ram et al., 2022 could help with the PBK modelling • The POD is discussed in Carmicheal et al., 2022, Middleton et al., 2022 and Pallocca et al., 2022 ◦ Extra: after POD identification, the BER can be estimated in Carmicheal et al., 2022 and Middleton et al., 2022 • IVIVE is discussed in Pallocca et al., 2022

Figure 17: The tiered workflow of cosmetics is used in the figure above as a backbone for a summary for the information available on both pharmaceuticals and chemicals. The information that can be found about cosmetics is information directly from the article of Berggren et al., 2017 and Desprez et al., 2018. The information that can be found about pharmaceuticals and chemicals is obtained from the knowledge in subquestion 1 and sorted out in a tiered workflow backbone. This figure is made with Biorender.

By understanding more and more about workflows with NAMs in NGRA, it will become more clear how to fulfil a reliable risk assessment for pharmaceuticals. The NAMs that support NGRA need to be developed even further. Interesting is investing in higher tier models as NAMs, such as 3D cell culture models (e.g. organoids and organ-on-a-chip systems), which have good potential for testing drug safety and efficacy (Wang et al., 2021).

Besides the theory and ongoing research, it is also important to implement the workflows into practice. This is where opportunities lie in performing case studies based on the workflows. For chemicals and cosmetics there are some examples, but for pharmaceuticals not at all. Moreover, there is still a lack of trust and confidence in these NAMs. This is for example the case in the regulatory realm caused by e.g. risk aversion. How safety assessment is regulated for pharmaceuticals, will be discussed in the next chapter.

3. Subquestion 2: How is NGRA regulated for pharmaceuticals?

In this chapter the regulations in the EU and the US regarding safety assessment for pharmaceuticals and the usage of animal testing will be discussed. The goal is to summarize the regulatory rules involved for pharmaceuticals for safety testing with animals and NAMs.

3.1 Regulation of animal testing in safety assessment in the EU

In the EU the EMA is responsible for safety assessment of pharmaceuticals according to EU law (*Directive 2001/83/EC*, 2001; *Regulation (EC) No 726/2004*, 2004; *Regulation (EU) 2019/6*, 2018). The EMA has provided clinical efficacy and safety guidelines for different kind of specializations of pharmaceuticals, think about the different organs in the body like the cardiovascular and nervous system or products from those organs, like blood products. More categories can be found on the EMA website. This helps the applicants to prepare marketing authorisation applications (*Website EMA: Clinical Efficacy and Safety Guidelines*, n.d.). Rules relating to animal testing can be found in the Directive 2001/83/EC. Here it is stated that *in vivo* experiments should be performed for safety assessment. In module 4 of the directive it is presented how non-clinical reports should be built up. Here it can be observed that animal experiments are obligatory to be performed. Even though, animal testing is obligatory, there is a specific law for the protection of animals used for scientific purposes (*Directive 2010/63/EU*, 2010). Moreover, the EMA has a 3Rs-working party. This working party focusses on the 3Rs while giving advice on cases which involve animal testing in medicine. Moreover, the focus lies on actively cooperating in giving workshops and training and discussing with different stakeholders how to implement the 3Rs (*Website EMA: 3Rs Working Party*, n.d.). The focus of the EU and EMA lies on the reduction and refinement, because animal studies are still required. A lot of animal testing is still performed for safety assessment of pharmaceuticals.

This problem is also observed with safety assessment of chemicals and cosmetics in the EU. For chemicals more broadly the REACH regulation by the European Chemicals Agency (ECHA) is applicable for safety assessment (*Regulation (EC) No 1907/2006*, 2006). In the REACH regulation safety assessment testing for chemicals is focussed on non-animal alternatives, however it is stated that animal *in vivo* test can be required to ensure good human health. This is in contrast with the animal ban for cosmetics in 2013 (Knight et al., 2021). So for cosmetics, as well as for pharmaceuticals, there are requirements observed about the need of animals in safety assessment. This results in the fact that still a lot of animals are used.

3.2 Regulation of animal testing in safety assessment in the US

In the US the FDA, more specifically the Center for Drug Evaluation and Research (CDER), is responsible for safety assessment of pharmaceuticals according to US law (the FFDCA) (*Website CDER: Center for Drug Evaluation and Research*, n.d.; *Website United State Codes: Law US Title 21, Chapter 9*, n.d.). The FDA has, like the EMA, published guidelines available for new drug applications in the US. Here more broad information can be found about approval of drugs, but also manufacturing and production for example (*Website FDA: New Drug Application (NDA)*, n.d.).

Very recently in the US more support for more non-animal alternatives in safety assessment has arisen. The Modernization Act 2.0 of the FDA has been approved and added to the FFDCA. This law focuses on animal testing alternatives. Because of this last change by law, non-animal testing *in vitro* and *in silico* is now allowed to be performed to test the effectiveness of pharmaceuticals by the FDA. Besides this, the requirement to use animals is removed for products that are biosimilar or interchangeable with other biological products (*FDA Modernization Act 2.0 (H.R. 2565)*, n.d.) However, animal testing is not banned and still

required in other cases of safety assessment for pharmaceuticals (*Website United State Codes: Law US Title 21, Chapter 9*, n.d.). But, it is promising that NAMs can be used as an alternative to decrease the number of animals used.

3.3 Moving forward towards regulatory acceptance of NAMs

Besides the efforts by the EMA and FDA, also other parties are involved in the field of developing alternative animal methods and regulatory acceptance, A few examples in the EU are given here. The European Partnership for Alternative Approaches to Animal Testing (EPAA), which is an initiative of the European Commission which focuses on the 3Rs to ensure safety of substances (*Website EPAA: European Partnership for Alternative Approaches to Animal Testing*, n.d.). Then the EU Reference Laboratory for alternatives to animal testing (EURL ECVAM), is also an initiative by the EC, which promotes NAMs in research (*Website EURL ECVAM: EU Reference Laboratory for Alternatives to Animal Testing*, n.d.). The EURL ECVAM consists of a Scientific Advisory Committee (ESAC) which gives validation for NAMs for example (*Website ESAC: EURL ECVAM Scientific Advisory Committee*, n.d.). The EURL ECVAM has made a system which tracks the progress of the non-animal alternatives which can be used for testing of chemicals or other compounds towards regulatory acceptance. This system is called Tracking System for Alternative methods towards Regulatory acceptance (TSAR) and it documents NAMs not only considered acceptable within the EU, but also for example within the US and the OECD. This system can be used to check whether a NAM is regulatory acceptable for safety assessment. The steps in the progress are submission, validation, peer-review, recommendation and regulatory acceptance/standards. During the validation step the test methods are rigorously evaluated whether they are science-based (*Website TSAR: Tracking System for Alternative Methods towards Regulatory Acceptance*, n.d.). Then there are also project groups, like EU-ToxRisk which is funded by the EU. Their drive is to drive the paradigm shift from animal testing in toxicological testing (*Website EU-ToxRisk: About EU-ToxRisk*, n.d.). Another project group is called Animal-free Innovations (TPI; Transitie Proefdiervrije Innovaties) in the Netherlands, where the Dutch government stimulates development and application of non-animal applications (*Website TPI: Home Animal Free Innovations*, n.d.). The last example is the VAC2VAC project group which aims to develop and validate vaccines using non-animal methods (*Website VAC2VAC: VAC2VAC Home*, n.d.).

In the US there is an initiative that is called the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) which has developed a strategic roadmap (by input of 16 federal agencies, multiple interagency working groups and the public) that serves as a guideline to implement NAMs for safety assessment of substances such as pesticides, consumer products, cosmetics, pharmaceuticals, medical devices, workplace chemicals and chemicals in transportation. The three main points to ensure implementation of NAMs are (according to the ICCVAM): a connection with the end users (federal agencies and regulated industries) and with the developers of NAMs; making sure the practices are flexible and robust to cause confidence in NAMs; and encouraging the adoption of the NAMs by federal agencies and regulatory industries (*A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States*, 2018).

Moreover, global initiatives encourage the use of NAMs, for example the Organisation for Economic Co-operation and Development (OECD) (*Website OECD: OECD Encourages the Development of Non-Animal Test Methods for the Detection of Thyroid Disrupters*, n.d.). Another example is the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) who made e.g. a document for the detection of

reproductive and developmental toxicity for human pharmaceuticals. Here the focus lies on the usage of less animals, but animals are still permitted to be used (*ICH Harmonised Guideline: Detection of Reproductive and Developmental Toxicity for Human Pharmaceuticals S5(R3)*, 2020). Besides this, they made a guideline for the validation of analytical procedures. Guidance is given on how to obtain and evaluate validation tests (*ICH Harmonised Guideline: Validation of Analytical Procedures Q2(R2)*, 2022).

In conclusion, there are many promising initiatives which promote the use of NAMs in NGRA instead of animal studies for risk assessment of pharmaceuticals, but the rules of the law still instigate the use of animals, which make it hard to let go of this so-called ‘gold standard’. More should be invested to changing those laws, which included, communication with regulators.

4. Subquestion 3: Which stakeholders are involved in the acceptance of using NGRA for safety assessment of pharmaceuticals?

The goal of this chapter is to summarize which stakeholders are involved in the safety assessment of pharmaceuticals and the implementation of NAMs. In different research papers the groups of stakeholders involved in this process have been summarized.

In 2013 the challenge of delay between the validation of scientific and technological advances and the acceptance by in companies and regulators was already recognised. Here experts came to the conclusion that there was a need to increase communication and therefore data sharing between pharmaceutical organizations, contract research organizations and regulators (Chapman et al., 2013). A year later an article pointed out which stakeholders are involved in the regulatory acceptance for pharmaceuticals and other chemicals. It is mentioned that (1) regulatory authorities, legislators and policy makers; (2) academia and research organisations; and (3) industry are three big stakeholder groups. In this article also actions for implementation of the 3R model are pointed out. It is mentioned that drivers for implementation could be policy goals, and catalysts are commitment, communication, cooperation and coordination (Schiffelers et al., 2014). Interesting is that society, so also consumers and patients, only play a small role in the transition to NAMs at this moment. With better interdisciplinary education more knowledge can be obtained, which can also increase the societal pressure. Also investors play a big role, without the targeted funding for NAMs, the implementation and validation of NAMs goes a lot slower (Abarkan et al., 2022).

In conclusion, which stakeholders play a role in the regulatory acceptance of NAMs in NGRA for pharmaceuticals becomes more and more clear, but better communication between those stakeholders is key to the actual implementation of NAMs.

5. Discussion and conclusion

Like mentioned before, still limited information is available about how NAMs in NGRA could be used during drug development for safety assessment of pharmaceuticals (Turner et al., 2023). To answer the research question, in this writing assignment in subquestion 1 different workflows for this process have been presented for pharmaceuticals. Besides this, these workflows were also presented for chemicals/cosmetics more broadly. Still a lot of hesitance and fear is present if NAMs can be used to obtain data which covers the reaction of the whole system of an organism to a compound (Turner et al., 2023), However, ideas of how to use NAMs in a sufficient way for risk assessment for pharmaceuticals and other chemicals was proposed in a these workflows. These workflows can help in the acceleration of the transition to NAMs in NGRA for pharmaceuticals as well. However, the implementation of the NGRA approach still faces difficulties. For example, implementation of NGRA in the regulations still has a long way to go. In subquestion 2 it is made clear how the usage of animals and NAMs for safety assessment of pharmaceuticals and other chemicals in the EU and the US have been regulated. Which stakeholder groups are involved in this process are summarized under subquestion 3.

Opportunities for accelerated regulatory acceptance of animal-free NGRA for pharmaceuticals have been given in the form of recommendations throughout this writing assignment. For pharmaceuticals specifically there should be more transparency about the usage of NAMs in pharmaceutical companies and there should be better communication with regulators. A recommendation to come to regulatory acceptance for pharmaceuticals into practice is to get more confidence in the usage of NAMs in NGRA with more examples of case studies based on workflows (Turner et al., 2023). The better communication between the different stakeholders (especially between academia and pharma with the regulators) and more investment in case studies to build more confidence to implement the workflows into practice, are recommendations which are also brought up in articles which focussed on chemicals/cosmetics (Baltazar et al., 2020; Carmichael et al., 2022; Chapman et al., 2013; M. P. Dent et al., 2021; Desprez et al., 2018; Parish et al., 2020; Rogiers et al., 2020; Schiffelers et al., 2014). Even though, more is known about these workflows for chemicals/cosmetics in comparison to workflows for pharmaceuticals, the implementation into practice is also still a challenge. Also coming to a consensus about one workflow per division (pharmaceuticals, chemicals and cosmetics), or even a combined tiered workflow, can help with the implementation (Parish et al., 2020).

Besides the recommendations to accelerate the regulatory acceptance, the current regulations of the EU and the US offer a lot of opportunities for the usage of NAMs instead of animals for safety assessment for pharmaceuticals. It is positive that NAMs are by law allowed to be used for safety assessment of pharmaceuticals in the US (*FDA Modernization Act 2.0 (H.R. 2565)*, n.d.), but it is also still allowed and a custom to use animals in the US and the EU (*Directive 2001/83/EC*, 2001; *Regulation (EC) No 726/2004*, 2004; *Regulation (EU) 2019/6*, 2018; *Website United State Codes: Law US Title 21, Chapter 9*, n.d.).

In conclusion, to accelerate the regulatory acceptance of the usage of NAMs in NGRA, besides investing on making clear workflows for safety assessment of pharmaceuticals, more focus should be given on the implementation into practice. This could be achieved by investing more on performing case studies with NGRA workflows to build confidence, and more communication within the different stakeholder groups involved (especially more communication with regulators).

6. Resources

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7. Appendices

Appendix A1: Search queries for PudMed, Embase and Overton

PudMed (31-01-2023)

Searched on Google for the PudMed site and after that the following query was added in the search bar:

(“New Approach Method*"[Title/Abstract] OR "Novel Approach Method*"[Title/Abstract] OR "Next Generation Risk Assessment*"[Title/Abstract] OR "Animal Testing Alternative*"[Title/Abstract] OR "Animal Use Alternative*"[Title/Abstract] OR "Animal testing alternatives"[MeSH Terms] OR "Animal use alternatives"[MeSH Terms]) AND ("safety assessment*"[Title/Abstract] OR "risk assessment*"[Title/Abstract] OR "risk assessment"[MeSH Terms]) AND ("pharmaceutical*"[Title/Abstract] OR "pharma"[Title/Abstract] OR "drug*"[Title/Abstract] OR "pharmacology"[Title/Abstract] OR "pharmacology"[MeSH Terms] OR "pharmacology, clinical"[MeSH Terms] OR "biological products"[MeSH Terms])

Embase (02-02-2023)

Searched on Google for the Embase site, logged in with my students UU account and after that the following query was added in the search bar:

(‘New Approach Method*’:ti,ab,kw OR ‘Novel Approach Method*’:ti,ab,kw OR ‘Next Generation Risk Assessment*’:ti,ab,kw OR ‘Animal Testing Alternative*’:ti,ab,kw OR ‘Animal Use Alternative*’:ti,ab,kw OR ‘Animal testing alternative’/exp OR ‘Animal use alternatives’/exp) AND (‘safety assessment*’:ti,ab,kw OR ‘risk assessment*’:ti,ab,kw OR ‘risk assessment’/exp) AND (‘pharmaceutical*’:ti,ab,kw OR ‘pharma’:ti,ab,kw OR ‘drug*’:ti,ab,kw OR ‘pharmacology’:ti,ab,kw OR ‘pharmacology’/exp OR ‘clinical pharmacology clinical’/exp OR ‘biological product’/exp)

#1 AND [embase]/lim NOT ([embase]/lim AND [medline]/lim)

Overton (03-02-2023)

Searched on Google for the Overton site, logged in with my students UU account and after that the following query was added in the search bar:

(“New Approach Method*" OR "Novel Approach Method*" OR "Next Generation Risk Assessment*"OR “NRGA” OR "Animal Testing Alternative*" OR "Animal Use Alternative*" OR “Exposure-driven approach*”) AND ("safety assessment*" OR "risk assessment*") AND ("pharmaceutical*" OR "pharma" OR "drug*" OR "pharmacology")

Appendix A2: Google search results subquestion 2 and 3 (because Overton was not as specific as we thought)

- EU
  - ECHA (European Chemicals Agency) /REACH (Registration, Evaluation, Authorisation of Chemicals) (15-02-2023)
    - The ECHA website was reached by searching on Google for “ECHA”:
    - On the ECHA website searched for: “REACH legislation”
      - Found:
        - All legislation documents: Wetgeving - ECHA (europa.eu)
        - Legislation 2006: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1907
    - The European Commission was reached by searching on Google for “European Commission”:
    - On the European Commission website searched for: “REACH revision”
      - Found:
        - Revision - REACH - Chemicals - Environment - European Commission (europa.eu)
    - On the European Commission website searched for: “REACH and animal testing”
      - Found:
        - Animal testing - REACH - Chemicals - Environment - European Commission (europa.eu)
  - EMA (European Medicine Agency) (15-02-2023)
    - The EMA website was reached by searching on Google for “EMA”:
      On the EMA website searched for: “Legal framework”
      - Found:
        - Legal framework | European Medicines Agency (europa.eu)
    - On the EMA website searched for: “3Rs working party”
      - Found:
        - 3Rs Working Party | European Medicines Agency (europa.eu)
    - On the EMA website searched for: “Safety guidelines” (22-02-2022)
      - Found:
        - Clinical efficacy and safety guidelines | European Medicines Agency (europa.eu)
    - On the EMA website searched for: “Protection animals directive” (22-02-2022)
      - Found:
        - EUR-Lex - 02010L0063-20190626 - EN - EUR-Lex (europa.eu)
  - EPAA (European Partnership for Alternative Approaches to Animal Testing) (15-02-2023)
    - The European Commission website was reached by searching on Google for “EPAA”:
    - On the European Commission website searched for: “European Partnership for Alternative Approaches to Animal Testing”
      - Found:
        - European Partnership for Alternative Approaches to Animal Testing (europa.eu)
  - EURL ECVAM (European Union Joint Research Centre for Alternatives to Animal Testing) (15-02-2023)
    - The European Commission website was reached by searching on Google for “European Commission”:

        - On the European Commission website searched for: “EURL ECVAM”
            - Found:
                - EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) (europa.eu)
        - On the European Commission website searched for: “TSAR”
            - Found:
                - Validated test methods - health effects (europa.eu)

        - On Google searched for: “ECVAM status report 2021”
            - Found:
                - https://euroocs.eu/eurl-ecvam-status-report-2021-on-alternative-methods-published/

        - On Google searched for: “ESAC EURL ECVAM” (10-03-2023)
            - Found:
                - ESAC - EURL ECVAM Scientific Advisory Committee (europa.eu)

    - EU-ToxRisk (15-02-2023)
        - On Google searched for: “EU-ToxRisk”
            - Found:
                - EU-ToxRisk - About EU-ToxRisk

    - TPI (transitie proefdiervrij innovatie) (15-02-2023)
        - On Google searched for: “Transitie proefdiervrije innovatie”
            - Found:
                - Home | Transitie Proefdiervrije Innovatie

    - SCCS (Scientific Committee on Consumer Safety) (15-02-2023)
        - The European Commission website was reached by searching on Google for “European Commission”:
        - On the European Commission website searched for: “Scientific Committee on Consumer Safety”
            - Found:
                - Scientific Committee on Consumer Safety (SCCS) (europa.eu)
    - Vac2Vac
        - On Google searched for: “Vac2vac
            - Found:
                - Home | Vac2Vac (europevaccine.wixsite.com)

- US
    - FDA (US Food and Drug Administration) (17-02-2023)
        - The FDA website was reached by searching on Google for “FDA”:
        - On Google searched for: “Federal Food, Drug and Cosmetic act 1938”
            - Found:
                - Part II: 1938, Food, Drug, Cosmetic Act | FDA

        - On the website of the FDA searched for: “Federal Food, Drug, and Cosmetic Act”
            - Found:
                - Federal Food, Drug, and Cosmetic Act (FD&C Act) | FDA
                    - On this site clicked further to:
                    OLRC Home (house.gov)
        - On the website of the FDA searched for: “guideline drugs applicants” (22-02-2023)
            - Found:
                - New Drug Application (NDA) | FDA

        - On Google searched for: “FDA Modernization act 2.0”
            - Found:
                - FDA Modernization Act 2.0 allows for alternatives to animal testing - Han - Artificial Organs - Wiley Online Library
                    - In this article looked to the ‘similar articles’ section, on articles about the revision of the law in 1938 in 1997 (snowballing): https://pubmed.ncbi.nlm.nih.gov/11364915/
                - The FDA Modernization Act 2.0 - What does it mean? - CN Bio (cn-bio.com)
                - Law 2022:
                    - H.R.2565 - 117th Congress (2021-2022): FDA Modernization Act of 2021 | Congress.gov | Library of Congress
                    - S.2952 - 117th Congress (2021-2022): FDA Modernization Act of 2021 | Congress.gov | Library of Congress
                    - S.5002 - 117th Congress (2021-2022): FDA Modernization Act 2.0 | Congress.gov | Library of Congress

        - On Google searched for: “CDER” (10-03-2023)
            - Found:
                - Center for Drug Evaluation and Research | CDER | FDA

    - EPA TSCA (Environment Protection Agency with the Toxic Substances Control Act) (17-02-2023)
        - The EPA website was reached by searching on Google for “EPA”:
        - On the EPA website searched for: “Toxic Substances Control Act”
            - Found:

- The Frank R. Lautenberg Chemical Safety for the 21st Century Act | US EPA

- Global
  - OECD (Organisation for Economic Co-operation and Development (27-02-2023)
    - The OECD website was reached by searching on Google for “OECD”:
    - On Google searched for: “OECD”
      - Found:
        - OECD encourages the development of non-animal test methods for the detection of thyroid disrupters - OECD

  - ICH (The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use) (10-03-2023)
    - On Google searched for: “ICH Q2R1”
      - Found:
        - ICH Q2(R2) Validation of analytical procedures - Scientific guideline | European Medicines Agency (europa.eu)

Appendix A3: Writing plan (the resources used in this Writing Assignment are marked in green)

Introduction

Subjects	Topic	Literature	Conclusions literature	Questions and answers
1	Importance animal-free testing	- The Many Benefits of Using Alternatives to Animal Testing - InVitro Intl - McCANN, T. E. R. R. Y., and CAROL TREASURE. "How do you define truly animal-free testing?." xcell_HPC3_2020.pdf (x-cellr8.com) - deloitte-uk-seize-digital-momentum-rd-roi-2022.pdf - Swaters, Doortje, et al. "A history of regulatory animal testing: What can we learn?." *Alternatives to Laboratory Animals* 50.5 (2022): 322-329. A History of Regulatory Animal Testing: What Can We Learn? (sagepub.com) 3Rs: - Definition of the Three Rs as given by the European Commission. Animals used for scientific purposes - Environment - European Commission (europa.eu) - Vinken, Mathieu. "3Rs toxicity testing and disease modeling projects in the European Horizon 2020 research and innovation program." *EXCLI journal* 19 (2020): 775. 3Rs toxicity testing and disease modeling projects in the European Horizon 2020 research and innovation program - PMC (nih.gov) - Tannenbaum, Jerrold, and B. Taylor Bennett. "Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose." *Journal of the American association for laboratory animal science* 54.2 (2015): 120-132. Russell and Burch's 3Rs Then and Now: The Need for Clarity in Def...: Ingenta Connect - Ritskes-Hoitinga, Merel, and Judith van Luijk. "How can systematic reviews teach us more about the implementation of the 3Rs and animal welfare?." *Animals* 9.12 (2019): 1163. Animals | Free Full-Text | How Can Systematic Reviews Teach Us More about the Implementation of the 3Rs and Animal Welfare? (mdpi.com) - See other resources of the EU-52 eLearning Example refinement: - Chien, Hsiao-Tzu, et al. "Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight of evidence approach." *Regulatory Toxicology and Pharmacology* (2022): 105329. Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight of evidence approach - ScienceDirect - Prior, Helen, et al. "The use of recovery animals in nonclinical safety assessment studies with monoclonal antibodies: further	- Costs are high and investments decline - About mAbs and the long-term safety assessments of those. This was evaluated. (Peter van Meer) - This review summarizes important	- Reduction numbers of animals used + refine the way animals are used (animal welfare, ethics) (for example Directive 2010/63/EU) • 3Rs (replace, reduce, refine) → still a lot of animals are used - Health of people/animals • Animal-free testing can be better than animal testing - Society is less accepting - Animal tests are expensive and investments decline - Sustainability

		3Rs opportunities remain." *Regulatory Toxicology and Pharmacology* (2023): 105339. The use of recovery animals in nonclinical safety assessment studies with monoclonal antibodies: further 3Rs opportunities remain - ScienceDirect - Buckley LA, Chapman K, Burns-Naas LA, Todd MD, Martin PL, Lansita JA. Considerations regarding nonhuman primate use in safety assessment of biopharmaceuticals. Int J Toxicol. 2011 Oct;30(5):583-90. doi: 10.1177/1091581811415875. PMID: 22013138. Considerations regarding nonhuman primate use in safety assessment of biopharmaceuticals - PubMed (nih.gov)	scientific and regulatory perspectives derived from presentations and audience discussions in an educational forum at the 2010 annual American College of Toxicology meeting regarding opportunities for employing alternative approaches to minimize NHP use in mAb drug development.	
2	Problem	- Hooijmans, Carlijn R., et al. "The effects of probiotic supplementation on experimental acute pancreatitis: a systematic review and meta-analysis." *PloS one* 7.11 (2012): e48811. The Effects of Probiotic Supplementation on Experimental Acute Pancreatitis: A Systematic Review and Meta-Analysis | PLOS ONE - Dirven, Hubert, et al. "Performance of preclinical models in predicting drug-induced liver injury in humans: a systematic review." *Scientific reports* 11.1 (2021): 1-19. Performance of preclinical models in predicting drug-induced liver injury in humans: a systematic review | Scientific Reports (nature.com)	- This result was unexpected in light of the results of the animal studies referred to in the trial protocol. - Article about a study they published a couple of years ago useful to start framing my thinking about this problem: (Katya Tsaioun)	- Low acceptance of animal-free testing for chemicals and pharmaceuticals in the medical world • Which does not make sense: Some animal experiments fail to predict toxicity in humans, ○ BUT there are specific NAMs which could predict this
		- Accelerating the Growth of Human Relevant Life Sciences in the United Kingdom https://www.humanrelevantscience.org/wp-content/uploads/Accelerating-the-Growth-of-Human-Relevant-Sciences-in-the-UK_2020-final.pdf	- Suggestion Merel: numbers of experiment that fail to predict toxicity	• Also challenges in clinical trials with animal testing
		- Burnett SD, Karmakar M, Murphy WJ, Chiu WA, Rusyn I. A new approach method for characterizing inter-species toxicodynamic variability. J Toxicol Environ Health A. 2021 Dec 17;84(24):1020-1039. doi: 10.1080/15287394.2021.1966861. Epub 2021 Aug 24. PMID: 34427174; PMCID: PMC8530970. A new approach method for characterizing inter-species toxicodynamic variability - PubMed (nih.gov)	- Characterising inter species variability	
		- van Meer, Peter, et al. "Animal free applications in the development of cell-based therapies." *Authorea Preprints* (2020). Animal-free applications in the development of cell-based therapies - Meer - 2021 - British Journal of Clinical Pharmacology - Wiley Online Library	- We discuss the use and implications of several methods and tools to assess the generalisability of animal data to humans. (Peter van Meer)	○ So to be better at predicting the effect of pharmaceuticals in human, more investment in good tests
		- Singh, Sonal, and Yoon K. Loke. "Drug safety assessment in clinical trials: methodological challenges and opportunities." *Trials* 13.1 (2012): 1-8. Drug safety assessment in clinical trials: methodological challenges and opportunities |	- methodological challenges in the reporting, analysis and interpretation of safety data in clinical trials	

		SpringerLink - Rovida C, Asakura S, Daneshian M, et al. Toxicity testing in the 21st century beyond environmental chemicals. *ALTEX*. 2015;32(3):171-181. doi:10.14573/altex.1506201 Toxicity testing in the 21st century beyond environmental chemicals - PubMed (nih.gov) - Ritskes-Hoitinga, Merel, Yari Barella, and Tineke Kleinhout-Vliek. "The promises of speeding up: Changes in requirements for animal studies and alternatives during COVID-19 vaccine approval–A case study." *Animals* 12.13 (2022): 1735. Animals | Free Full-Text | The Promises of Speeding Up: Changes in Requirements for Animal Studies and Alternatives during COVID-19 Vaccine Approval–A Case Study (mdpi.com) - Ritskes-Hoitinga, Merel. "Medical regulators: look beyond animal tests." *Nature* 604.7907 (2022): 599-599. Medical regulators: look beyond animal tests (repec.org) - Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (europa.eu) - Knight, Jean, et al. "Continuing animal tests on cosmetic ingredients for REACH in the EU." *Alternatives to Animal Experimentation: ALTEX* 38.4 (2021): 653-668. Continuing Animal Tests on Cosmetic Ingredients for REACH in the EU (uni-konstanz.de) - Mangipudy R, Burkhardt J, Kadambi VJ. Use of animals for toxicology testing is necessary	- However, due to the high failure rate of drugs during the clinical phases, a new approach for a more predictive assessment of drugs both in terms of efficacy and adverse effects is getting urgent - Sped up alternatives for animal studies during COVID-19 - In 2009 is was determined that in 2013 there would be a ban in the EU on: *The Commission established timetables of deadlines up to 11 March 2009 for prohibiting the marketing of cosmetic products, the final formulation, ingredients or combinations of ingredients which have been tested on animals, and for prohibiting each test currently carried out using animals. In view, however, of tests concerning repeated-dose toxicity, reproductive toxicity and toxicokinetics, it is appropriate for the final deadline for prohibiting the marketing of cosmetic products for which those tests are used to be 11 March 2013. On the basis of annual reports, the Commission should be authorised to adapt the timetables within the abovementioned maximum time limit.* - In vivo research is often still needed to fulfil REACH, even though there is a ban on in vivo research for testing cosmetics - Use of animals is necessary according to this article	• During COVID-19 alternatives were used quite fast (test with cell culture + computational science) • Moreover, for cosmetics animal tests were banned - Even though, there are still challenges: often animal test are required for safety assessment - Even though: even in science there is a certain belief

		to ensure patient safety in pharmaceutical development. *Regul Toxicol Pharmacol*. 2014;70(2):439-441. doi:10.1016/j.yrtph.2014.07.014 Use of animals for toxicology testing is necessary to ensure patient safety in pharmaceutical development - PubMed (nih.gov)		
3	Safety assessment	About basics safety assessment: introduction thesis Emma Kasteel Safety assessment: - WHO Human Health Risk Assessment Toolkit: Chemical Hazards WHO Human Health Risk Assessment Toolkit: Chemical Hazards Articles with basic information about NAMs/NGRA: - Carmichael, Paul L., et al. "Ready for regulatory use: NAMs and NGRA for chemical safety assurance." *ALTEX-Alternatives to animal experimentation* 39.3 (2022): 359-366. Ready for regulatory use: NAMs and NGRA for chemical safety assurance | ALTEX - Alternatives to animal experimentation - Bhogal N, Grindon C, Combes R, Balls M. Toxicity testing: creating a revolution based on new technologies. *Trends Biotechnol*. 2005;23(6):299-307. doi:10.1016/j.tibtech.2005.04.006 Toxicity testing: creating a revolution based on new technologies - PubMed (nih.gov) - Dent, M. P., et al. "Paving the way for application of next generation risk assessment to safety decision-making for cosmetic ingredients." *Regulatory Toxicology and Pharmacology* 125 (2021): 105026. Paving the way for application of next generation risk assessment to safety decision-making for cosmetic ingredients - ScienceDirect Vision of safety assessment + status + future prospects: - Davis M, Boekelheide K, Boverhof DR, et al. The new revolution in toxicology: the good, the bad, and the ugly. *Ann N Y Acad Sci*. 2013;1278:11-24. doi:10.1111/nyas.12086 The new revolution in toxicology: the good, the bad, and the ugly - PubMed (nih.gov) - Hartung, Thomas. "Evidence-Based Toxicology: the Toolbox of Validation for the 21st Century?." *Alternatives to Animal Experimentation: ALTEX* 27.4 (2010): 253-263. Evidence based-toxicology – the toolbox of validation for the 21st century? | ALTEX - Alternatives to animal experimentation - Adler, Sarah, et al. "Alternative (non-animal) methods for cosmetics testing: current status and future prospects—2010." *Archives of toxicology* 85 (2011): 367-485. Alternative (non-animal) methods for chemicals testing: Current status and future prospects - Record details - Embase	- Basics safety assessment - NAMs and NGRA are ready for regulatory use for safety assurance for chemicals - Explanation about different system used for safety assessment of pharmaceuticals - NGRA for safety assessment of cosmetics - Vision toxicity testing for pharmaceuticals - The concept of evidence-based medicine (EBM) has emerged from clinical medicine, which retrospectively assesses the evidence of adequacy of a given approach. - The selected experts were asked to analyse the status and prospects of alternative methods and to provide a scientifically sound estimate of the time necessary to achieve full replacement of animal testing	- What is safety assessment? - Introducing safety assessment • Timeline • Facts about how many chemicals/medicine are tested • How many animals are used vs in vitro - Risk can be assessment with a low animals as possible: • Introducing new approach methodologies (NAMs) • Introducing next generation risk assessment (NGRA)

		- Leist, Marcel, et al. "Validation and quality control of replacement alternatives–current status and future challenges." *Toxicology Research* 1.1 (2012): 8-22. Validation and quality control of replacement alternatives - Current status and future challenges - Record details - Embase	- We describe here the principles of model development and quality control. We also give an overview on methods that have undergone validation. Strengths and shortcomings of traditional approaches are discussed, and new developments and challenges are outlined.	
		- Pognan, Francois, et al. "The evolving role of investigative toxicology in the pharmaceutical industry." *Nature Reviews Drug Discovery* (2023): 1-19. The evolving role of investigative toxicology in the pharmaceutical industry | Nature Reviews Drug Discovery	- New article (via mail from Merel)	
		Law safety testing pharmaceuticals: - Legal framework | European Medicines Agency (europa.eu) - OLRC Home (house.gov) Title 21, Chapter 9 Guidelines organisations safety testing pharma: - Clinical efficacy and safety guidelines | European Medicines Agency (europa.eu) - New Drug Application (NDA) | FDA Law guidelines animal testing: - Directive 2010/63/EU on the protection of animals used for scientific purposes. EUR-Lex - 02010L0063-20190626 - EN - EUR-Lex (europa.eu) - AND working party 3Rs EMA 3Rs Working Party | European Medicines Agency (europa.eu) - FDA Modernization act 2.0 (very new!)	- EU - US - EMA - FDA - EU - US	
6	Goals			- Goals big project: • Evidence that NGRA represents a better scientific approach to safety assessment than animal studies; • Transdisciplinary knowledge on NGRA, including its technical aspects and consequences for governing safety assessments; • Transdisciplinary knowledge on governing the acceleration of the transition to animal-free safety assessment; • Enhanced theoretical understanding of the concept of transformative governance. • Increased acceptance and implementation of NGRA, replacing animal studies.

				• Experience with governing the acceleration of transitions, which is relevant for animal-free safety assessment and other sustainability transitions
4	Explanation main research question			- How can the transition to animal-free Next Generation Risk Assessment for pharmaceuticals be accelerated?
5	Explanation sub questions			- Is NGRA a good method for safety assessment of pharmaceuticals? - How is safety assessment/NGRA regulated for pharmaceuticals? - Which stakeholders are involved in the acceptance of using NGRA for safety assessment of pharmaceuticals?

Body

1	NGRA as a possible method for safety assessment of pharmaceuticals	- Rowan AN. Ending the use of animals in toxicity testing and risk evaluation. Camb Q Healthc Ethics. 2015 Oct;24(4):448-58. doi: 10.1017/S0963180115000109. PMID: 26364779. Ending the use of animals in toxicity testing and risk evaluation - PubMed (nih.gov)	- This article discusses the use of animals for the safety testing of chemicals, including pharmaceuticals, household products, pesticides, and industrial chemicals	- Is NGRA a good method for safety assessment of pharmaceuticals? • What is NGRA? ○ Exposure-led, hypothesis-driven risk assessment ○ Not relying on animal-data ○ Tiered approach • Higher tiered levels - Organ-on-a-chip - 3D-cell culture - Organoids - Bio-printed systems - Computational models • How is NGRA used? ○ Chemicals/cosmetics/food as examples which can be implemented to pharma ○ Are there examples in pharma?
		- Sewell F, Aggarwal M, Bachler G, et al. The current status of exposure-driven approaches for chemical safety assessment: A cross-sector perspective. *Toxicology*. 2017;389:109-117. doi:10.1016/j.tox.2017.07.018 The current status of exposure-driven approaches for chemical safety assessment: A cross-sector perspective - PubMed (nih.gov) Pharmaceuticals: - Not published data: but a new paper is coming from Pelin Candarlioglu (in Altex) about the ecosystems of regulators and how validation can occur faster for pharmaceuticals.	- Challenges per industry	
		- Turner, Jan, et al. "Incorporating new approach methodologies into regulatory nonclinical pharmaceutical safety assessment." *ALTEX-Alternatives to animal experimentation* (2023). Incorporating new approach methodologies into regulatory nonclinical pharmaceutical safety assessment	ALTEX - Alternatives to animal experimentation	- Very new article! About NAMs for pharmaceuticals. (Paul Carmichael)
		- Ahuja V, Sharma S. Drug safety testing paradigm, current progress and future challenges: an overview. J Appl Toxicol. 2014 Jun;34(6):576-94. doi: 10.1002/jat.2935. Epub 2013 Oct 29. PMID: 24777877. Drug safety testing paradigm, current progress and future challenges: an overview - Ahuja - 2014 - Journal of Applied Toxicology - Wiley Online Library	- Drug safety testing of pharmaceuticals	
		- Ford KA. Refinement, Reduction, and Replacement of Animal Toxicity Tests by Computational Methods. ILAR J. 2016 Dec;57(2):226-233. doi: 10.1093/ilar/ilw031. PMID: 28053075.	- This review serves to introduce the concepts of computational	

		Refinement, Reduction, and Replacement of Animal Toxicity Tests by Computational Methods - PubMed (nih.gov)	toxicology and evaluate their role in the safety assessment of compounds, while also highlighting the application of in silico methods in the support of the goal and vision of the 3Rs (in pharmaceutical companies).	
		- Vichare AS, Kamath SU, Leist M, Hayes AW, Mahadevan B. Application of the 3Rs principles in the development of pharmaceutical generics. Regul Toxicol Pharmacol. 2021 Oct;125:105016. doi: 10.1016/j.yrtph.2021.105016. Epub 2021 Jul 21. PMID: 34302895. Application of the 3Rs principles in the development of pharmaceutical generics - PubMed (nih.gov)	- In this review, we discuss the application of 3Rs in nonclinical testing requirements for differentiated pharmaceutical generics.	
		- Parish ST, Aschner M, Casey W, Corvaro M, Embry MR, Fitzpatrick S, Kidd D, Kleinstreuer NC, Lima BS, Settivari RS, Wolf DC, Yamazaki D, Boobis A. An evaluation framework for new approach methodologies (NAMs) for human health safety assessment. Regul Toxicol Pharmacol. 2020 Apr;112:104592. doi: 10.1016/j.yrtph.2020.104592. Epub 2020 Feb 1. PMID: 32017962. An evaluation framework for new approach methodologies (NAMs) for human health safety assessment - PubMed (nih.gov)	- A framework that presents a consistent set of criteria, universal across initiatives, to evaluate a NAM's fit-for-purpose was developed by a multi-stakeholder group of industry, academic, and regulatory experts.	
		- Chapman KL, Holzgrefe H, Black LE, et al. Pharmaceutical toxicology: designing studies to reduce animal use, while maximizing human translation. *Regul Toxicol Pharmacol*. 2013;66(1):88-103. doi:10.1016/j.yrtph.2013.03.001 Pharmaceutical toxicology: designing studies to reduce animal use, while maximizing human translation - PubMed (nih.gov)	- Designing studies to reduce animal use, while maximizing human translation → Very good points for pharma!	
		Chemicals: - In silico specific: Ram, Rebecca N., Domenico Gadaleta, and Timothy EH Allen. "The role of 'big data'and 'in silico'New Approach Methodologies (NAMs) in ending animal use–a commentary on progress." *Computational Toxicology* 23 (2022): 100232. The role of 'big data' and 'in silico' New Approach Methodologies (NAMs) in ending animal use – A commentary on progress - Record details - Embase	- The current status of in silico methods is discussed, with input from researchers in the field.	
		- Carmichael, Paul L., et al. "Ready for regulatory use: NAMs and NGRA for chemical safety assurance." *ALTEX-Alternatives to animal experimentation* 39.3 (2022): 359-366. Ready for regulatory use: NAMs and NGRA for chemical safety assurance | ALTEX - Alternatives to animal experimentation	- NAMs and NGRA are ready for regulatory use for safety assessment in chemicals	

		- Pham, Ly Ly, et al. "Estimating uncertainty in the context of new approach methodologies for potential use in chemical safety evaluation." *Current Opinion in Toxicology* 15 (2019): 40-47. Estimating uncertainty in the context of new approach methodologies for potential use in chemical safety evaluation - ScienceDirect	- Information about NAMs: understanding the sources of uncertainty of the results when using NAMs, is important for the broad acceptance in chemicals	
		- Middleton, Alistair M., et al. "Are non-animal systemic safety assessments protective? A toolbox and workflow." *Toxicological Sciences* 189.1 (2022): 124-147. Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow | Toxicological Sciences | Oxford Academic (oup.com)	- Examples NAM toolbox for toxicological safety assessment in chemicals	
		- Pallocca G, Moné MJ, Kamp H, Luijten M, Van de Water B, Leist M. Next-generation risk assessment of chemicals - Rolling out a human-centric testing strategy to drive 3R implementation: The RISK-HUNT3R project perspective. ALTEX. 2022;39(3):419–426. doi: 10.14573/altex.2204051. Epub 2022 Apr 7. PMID: 35404467. Next-generation risk assessment of chemicals - Rolling out a human-centric testing strategy to drive 3R implementation: The RISK-HUNT3R project perspective - PubMed (nih.gov)	- The RISK-HUNT3R NGRA strategy will be developed to address the implementation of a comprehensive NAM toolbox into the regulatory framework for chemicals.	
		- Fragki, Styliani, et al. "New approach methodologies: A quantitative in vitro to in vivo extrapolation case study with PFASs." *Food and Chemical Toxicology* 172 (2023): 113559. New approach methodologies: A quantitative in vitro to in vivo extrapolation case study with PFASs - ScienceDirect	- Most of the real drive for NAMs comes from the chemicals and environmental field. (Paul Carmicheal)	
		- Paini, A., Leonard, J. A., Joossens, E., Bessems, J. G. M., Desalegn, A., Dorne, J. L., ... & Tan, Y. M. (2019). Next generation physiologically based kinetic (NG-PBK) models in support of regulatory decision making. *Computational Toxicology*, *9*, 61-72. Next generation physiologically based kinetic (NG-PBK) models in support of regulatory decision making - ScienceDirect	- Extra information PBK models in chemicals (searched for specifically for information of a PBK model)	
		Cosmetics: - Berggren, Elisabet, et al. "Ab initio chemical safety assessment: A workflow based on exposure considerations and non-animal methods." *Computational Toxicology* 4 (2017): 31-44. Ab initio chemical safety assessment: A workflow based on exposure considerations and non-animal methods - ScienceDirect	- Examples NAM toolbox for toxicological safety assessment in cosmetics	
		- Desprez, Bertrand, et al. "A strategy for systemic toxicity assessment based on non-animal approaches: the Cosmetics Europe Long Range Science Strategy programme." *Toxicology in Vitro* 50 (2018): 137-146. A strategy for systemic toxicity assessment based on non-animal approaches: The Cosmetics Europe Long Range Science Strategy programme - ScienceDirect	- This strategy shows safety assessment on cosmetic relevant chemicals, but is applicable to any type of chemical (LRSS).	

		- Projects & Research - LRSS - Integrated research programme for non-animal safety assessment (lrsscosmeticseurope.eu) - https://one.oecd.org/document/env/jm/mono(2017)27/en/pdf - Dent, Matthew, et al. "Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients." *Computational Toxicology* 7 (2018): 20-26. Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients - ScienceDirect - Dent, M. P., et al. "Paving the way for application of next generation risk assessment to safety decision-making for cosmetic ingredients." *Regulatory Toxicology and Pharmacology* 125 (2021): 105026. Paving the way for application of next generation risk assessment to safety decision-making for cosmetic ingredients - ScienceDirect - Rogiers, Vera, et al. "The way forward for assessing the human health safety of cosmetics in the EU-Workshop proceedings." *Toxicology* 436 (2020): 152421. The way forward for assessing the human health safety of cosmetics in the EU - Workshop proceedings - ScienceDirect - Baltazar, Maria T., et al. "A next-generation risk assessment case study for coumarin in cosmetic products." *Toxicological Sciences* 176.1 (2020): 236-252. Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products | Toxicological Sciences | Oxford Academic (oup.com) Higher tier levels: - Wang, Hongbing, et al. "3D cell culture models: Drug pharmacokinetics, safety assessment, and regulatory consideration." *Clinical and translational science* 14.5 (2021): 1659-1680. 3D cell culture models: Drug pharmacokinetics, safety assessment, and regulatory consideration - Wang - 2021 - Clinical and Translational Science - Wiley Online Library - Nitsche, Katharina S., et al. "Implementing organ-on-chip in a next-generation risk assessment of chemicals: a review." *Archives of Toxicology* (2022): 1-31. Implementing organ-on-chip in a next-generation risk assessment of chemicals: a review | SpringerLink	- Basic information LRSS - The workflow of Desprez is as well implemented by the OECD more broad for chemicals - Principles of NGRA of cosmetics - NGRA for safety assessment of cosmetics - NAMs and NGRA for safety assessment of cosmetics. NAMs consist of *in chemico, in silico, in vitro* and *ex vivo* methods. - Example NAMs and NGRA for safety assessment in a specific cosmetic - To improve the nonclinical prediction of drug safety and efficacy, researchers continue to develop novel models to evaluate and promote the use of improved cell- and organ-based assays for more accurate representation of human susceptibility to drug response. - Organ-on-a-chip research for NGRA for chemicals	

		- Hogberg, Helena T., and Lena Smirnova. "The Future of 3D Brain Cultures in Developmental Neurotoxicity Testing." *Frontiers in Toxicology* 4 (2022). The Future of 3D Brain Cultures in Developmental Neurotoxicity Testing - PMC (nih.gov)	- 3D-cell culture for toxicity testing	
		- Kastlmeier, Miriam T., et al. "Lung Organoids for Hazard Assessment of Nanomaterials." *International Journal of Molecular Sciences* 23.24 (2022): 15666. IJMS | Free Full-Text | Lung Organoids for Hazard Assessment of Nanomaterials (mdpi.com)	- Organoid research for hazard assessment	
		- Alépée N, Bahinski A, Daneshian M, De Wever B, Fritsche E, Goldberg A, Hansmann J, Hartung T, Haycock J, Hogberg H, Hoelting L, Kelm JM, Kadereit S, McVey E, Landsiedel R, Leist M, Lübberstedt M, Noor F, Pellevoisin C, Petersohn D, Pfannenbecker U, Reisinger K, Ramirez T, Rothen-Rutishauser B, Schäfer-Korting M, Zeilinger K, Zurich MG. State-of-the-art of 3D cultures (organs-on-a-chip) in safety testing and pathophysiology. ALTEX. 2014;31(4):441-77. doi: 10.14573/altex.1406111. Epub 2014 Jul 14. PMID: 25027500; PMCID: PMC4783151. State-of-the-art of 3D cultures (organs-on-a-chip) in safety testing and pathophysiology - PubMed (nih.gov)	- This review summarizes the state of the art concerning commonalities of the different models	
		- Pridgeon CS, Schlott C, Wong MW, et al. Innovative organotypic in vitro models for safety assessment: aligning with regulatory requirements and understanding models of the heart, skin, and liver as paradigms. *Arch Toxicol*. 2018;92(2):557-569. doi:10.1007/s00204-018-2152-9 Innovative organotypic in vitro models for safety assessment: aligning with regulatory requirements and understanding models of the heart, skin, and liver as paradigms - PubMed (nih.gov)	- This review provides an overview of recent developments in the field of toxicity testing with in vitro models for three major organ types: heart, skin, and liver. This review also examines regulatory aspects of such models in Europe and the UK, and summarizes best practices to facilitate the acceptance and appropriate use of advanced in vitro models.	
		- Schneider, Marlon R., et al. "Applicability of organ-on-chip systems in toxicology and pharmacology." *Critical Reviews in Toxicology* 51.6 (2021): 540-554. Full article: Applicability of organ-on-chip systems in toxicology and pharmacology (tandfonline.com)	- In the present study, we reviewed issues and opportunities related to the application of OoC in the safety and efficacy assessment of chemicals and pharmaceuticals, as well as the steps needed to achieve this goal. (Peter van Meer)	

		- Ewart, Lorna, et al. "Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology." *Communications Medicine* 2.1 (2022): 154. Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology | Communications Medicine (nature.com) Integrated approaches: - Caloni F, De Angelis I, Hartung T. Replacement of animal testing by integrated approaches to testing and assessment (IATA): a call for in vivitrosi. Arch Toxicol. 2022 Jul;96(7):1935-1950. doi: 10.1007/s00204-022-03299-x. Epub 2022 May 3. PMID: 35503372; PMCID: PMC9151502. Replacement of animal testing by integrated approaches to testing and assessment (IATA): a call for in vivitrosi - PubMed (nih.gov) - Maertens A, Golden E, Luechtefeld TH, Hoffmann S, Tsaioun K, Hartung T. Probabilistic risk assessment - the keystone for the future of toxicology. ALTEX. 2022;39(1):3-29. doi: 10.14573/altex.2201081. PMID: 35034131; PMCID: PMC8906258. Probabilistic risk assessment - the keystone for the future of toxicology - PubMed (nih.gov) - De Wever B, Fuchs HW, Gaca M, et al. Implementation challenges for designing integrated in vitro testing strategies (ITS) aiming at reducing and replacing animal experimentation. *Toxicol In Vitro*. 2012;26(3):526-534. doi:10.1016/j.tiv.2012.01.009 Implementation challenges for designing integrated in vitro testing strategies (ITS) aiming at reducing and replacing animal experimentation - PubMed (nih.gov)	- Pharma are investing heavily in the use of MPS (micro-physiological systems) and organ-on-chip – they are currently sitting on a lot of data in that space and there have been a few good papers emerging. (Paul Carmicheal)	
2	Regulation of safety assessment	- Pant AB. The Implementation of the Three Rs in Regulatory Toxicity and Biosafety Assessment: The Indian Perspective. *Altern Lab Anim*. 2020;48(5-6):234-251. doi:10.1177/0261192920986811 The Implementation of the Three Rs in Regulatory Toxicity and Biosafety Assessment: The Indian Perspective - PubMed (nih.gov) EU regulations: - Legal framework | European Medicines Agency (europa.eu) - Clinical efficacy and safety guidelines | European Medicines Agency (europa.eu) - Directive 2010/63/EU on the protection of animals used for scientific purposes. EUR-Lex - 02010L0063-20190626 - EN - EUR-Lex (europa.eu) - European Commission: Animals Used for Scientific Purposes, Replacement, Reduction and Refinement – the "Three Rs Animals used for scientific purposes - Environment - European Commission (europa.eu)	- Indian perspective on regulation drug development - Rules EU safety assessment pharma - Guidelines safety assessment pharma - Rules EU animal testing (Sonja Beken)	- How is safety assessment/NGRA regulated for pharmaceuticals? • How are specific laws/organisations regulating safety assessment in the EU? ○ European Union: wants to phase out animal testing since 2007 + European Parliaments called out the European Commission to create an action plan to phase out animal tests ○ ECHA with REACH: even though this law is there, still a lot of animal experiments are performed? → Revision REACH ○ EMA ○ EPAA ○ ECVAM ○ EU-ToxRisk ○ TPI ○ SCCS ○ EFSA • How are specific laws/organisations

		- COMMISSION STAFF WORKING DOCUMENT Progress report on the assessment and management of combined exposures to multiple chemicals (chemical mixtures) and associated risks Accompanying the document COMMUNICATION FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT, THE COUNCIL, THE EUROPEAN ECONOMIC AND SOCIAL COMMITTEE AND THE COMMITTEE OF THE REGIONS Chemicals Strategy for Sustainability Towards a Toxic-Free Environment - Overton - REPORT FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT AND THE COUNCIL on the development, validation and legal acceptance of methods alternative to animal testing in the field of cosmetics (2018) - Schiffelers MJ, Blaauboer BJ, Bakker WE, et al. Regulatory acceptance and use of 3R models for pharmaceuticals and chemicals: expert opinions on the state of affairs and the way forward. *Regul Toxicol Pharmacol*. 2014;69(1):41-48. doi:10.1016/j.yrtph.2014.02.007 Regulatory acceptance and use of 3R models for pharmaceuticals and chemicals: Expert opinions on the state of affairs and the way forward - ScienceDirect ➔ ECHA with REACH for chemical/cosmetic safety (more about the chemicals that are produced while producing pharmaceuticals) - Fentem, Julia, et al. "Upholding the EU's Commitment to 'Animal Testing as a Last Resort'Under REACH Requires a Paradigm Shift in How We Assess Chemical Safety to Close the Gap Between Regulatory Testing and Modern Safety Science." *Alternatives to Laboratory Animals* 49.4 (2021): 122-132. Upholding the EU's Commitment to 'Animal Testing as a Last Resort' Under REACH Requires a Paradigm Shift in How We Assess Chemical Safety to Close the Gap Between Regulatory Testing and Modern Safety Science - Julia Fentem, Ian Malcomber, Gavin Maxwell, Carl Westmoreland, 2021 (sagepub.com) - Ball, Nicholas, et al. "A framework for chemical safety assessment incorporating new approach methodologies within REACH." *Archives of toxicology* 96.3 (2022): 743-766. A framework for chemical safety assessment incorporating new approach methodologies within REACH | SpringerLink - Knight, Jean, et al. "Continuing animal tests on cosmetic ingredients for REACH in the EU." *Alternatives to Animal Experimentation: ALTEX* 38.4 (2021): 653-668. Continuing Animal Tests on Cosmetic Ingredients for REACH in the EU (uni-konstanz.de)	- Progress report on the assessment and management of combined exposures to multiple chemicals (chemical mixtures) and associated risks - Progress report on the development, validation and legal acceptance of methods alternative to animal testing in the field of cosmetics - Points of view on regulatory acceptance of pharmaceuticals and chemicals in the EU - NGRA is embedded in EU guidance for safety assessment of cosmetics and food, but not for the regulation of chemicals → shift is needed in how we assess chemical safety - Development of a framework with *in silico, in vitro and in vivo* methods to meet the requirements of REACH	regulating safety assessment in the US? ○ FDA ○ EPA ○ ICCVAM ○ NTP • Global ○ OECD ○ ICM

		- Pistollato, Francesca, et al. "Current EU regulatory requirements for the assessment of chemicals and cosmetic products: challenges and opportunities for introducing new approach methodologies." *Archives of toxicology* 95.6 (2021): 1867-1897. Current EU regulatory requirements for the assessment of chemicals and cosmetic products: challenges and opportunities for introducing new approach methodologies | SpringerLink	- In vivo research is often still needed to fulfil REACH, even though there is a ban on in vivo research for testing cosmetics	
		- Wetgeving - ECHA (europa.eu)	- REACH legislations	
		- https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1907	○ Initial text (2006)	
		- Animal testing - REACH - Chemicals - Environment - European Commission (europa.eu)	○ Only *in vitro* is not enough	
		- REACH Revision - cefic.org - Revision - REACH - Chemicals - Environment - European Commission (europa.eu)	○ Revision will take place this year	
		➔ EMA (more focussed on pharma!) - 3R working party 3Rs Working Party | European Medicines Agency (europa.eu)	- 3R working Party (Peter Theunissen + Sonja Beken)	
		- Ethical use of animals in medicine testing | European Medicines Agency (europa.eu)	- Implementation 3Rs In the EU the concept of 3Rs is already introduced in 2016. The FDA introduced a roadmap only in 2018	
		➔ EFSA - Guidance Document on Scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals - Addressing the new challenges for risk assessment. - Outcome of the public consultation on the draft EFSA Guidance Document on Scientific criteria for grouping chemicals into assessment groups for human risk assessment of combined exposure to multiple chemicals'	- Development of a roadmap for risk assessment of food and feed components	
		- Escher, Sylvia E., et al. "Development of a Roadmap for Action on New Approach Methodologies in Risk Assessment." *EFSA Supporting Publications* 19.6 (2022): 7341E. (EFSA NAM roadmap) Development of a Roadmap for Action on New Approach Methodologies in Risk Assessment | EFSA (europa.eu) - de Jong, Esther, et al. "Roadmap for action on Risk Assessment of Combined Exposure to Multiple Chemicals (RACEMiC)." *EFSA Supporting Publications* 19.10 (2022): 7555E. Roadmap for action on Risk Assessment of Combined Exposure to Multiple Chemicals (RACEMiC) | EFSA (europa.eu)	- Development of a roadmap for risk assessment of multiple chemicals in the regulatory domains of pesticides, food contact materials, contaminants, food additives, as well as in the overarching	

			domain of chemicals.	
		EU Initiatives ➔ EPAA (initiative European Commission, includes pharmaceutical companies) European Partnership for Alternative Approaches to Animal Testing (europa.eu)	- Basics about the EPAA	
		- Westmoreland C, Bender HJ, Doe JE, et al. Use of New Approach Methodologies (NAMs) in regulatory decisions for chemical safety: Report from an EPAA Deep Dive Workshop. *Regul Toxicol Pharmacol*. 2022;135:105261. doi:10.1016/j.yrtph.2022.105261 Use of New Approach Methodologies (NAMs) in regulatory decisions for chemical safety: Report from an EPAA Deep Dive Workshop - PubMed (nih.gov)	- Information EPAA on chemical safety assessment	
		➔ EURL ECVAM (initiative European Commission): *The Commission is also active in the field of developing alternative test methods, for example by the current Framework Programme for Research. The Commission operates the European Centre for the Validation of Alternative Methods (ECVAM), which is a global leader in the field* (Animal testing - REACH - Chemicals - Environment - European Commission (europa.eu))		
		- EU Reference Laboratory for alternatives to animal testing (EURL ECVAM) (europa.eu)	- Basics about EURL ECVAM	
		- The EURL ECVAM search guide - Publications Office of the EU (europa.eu)	- Helps with researching for animal alternatives	
		- EURL ECVAM status report on the development, validation and regulatory acceptance of alternative methods and approaches (2018).	- Status rapport 2018	
		- But this one is from 2021: EURL ECVAM Status Report 2021 on Alternative Methods published - EUROoCS	- Status rapport 2021	
		- Validated test methods - health effects (europa.eu) TSAR: https://tsar.jrc.ec.europa.eu/	- The EURL ECVAM has contributed to the validation of several test methods: TSAR + DB-ALM. In TSAR there is a difference between validated and accepted. The acceptance can take a long time, this is not that practical	
		- ESAC - EURL ECVAM Scientific Advisory Committee (europa.eu)	- The EURL EVCAM has a scientific advisory committee	
		➔ EU-ToxRisk (project group, funding from EU) - EU-ToxRisk - About EU-ToxRisk	- Basics about project group	
		- Graepel, Rabea, et al. "Paradigm shift in safety assessment using new approach methods: the EU-ToxRisk strategy." *Current Opinion in Toxicology* 15 (2019): 33-39. Paradigm shift in safety assessment using new approach methods: The EU-ToxRisk strategy - ScienceDirect	- EU-ToxRisk is 38-partner European research project. This review article provides an	

			overview of the project, its aims and approach and the methodologies that are being used.	
		➔ TPI - van Meer, Peter, et al. "Animal free applications in the development of cell-based therapies." *Authorea Preprints* (2020). Animal-free applications in the development of cell-based therapies - Meer - 2021 - British Journal of Clinical Pharmacology - Wiley Online Library - Home | Transitie Proefdiervrije Innovatie - Home | Animal Free Innnovation TPI (animalfreeinnovationtpi.nl) ➔ Vac2Vac - Home | Vac2Vac (europevaccine.wixsite.com)	- Information about the TPI - Basics about the TPI - Basics about vac2vac	
		US regulations: - FDA Information about FDA can also be found in the thesis of Inke Looman - Center for Drug Evaluation and Research | CDER | FDA - New Drug Application (NDA) | FDA Federal Food, Drug and Cosmetic Act of 1938 - Part II: 1938, Food, Drug, Cosmetic Act | FDA - OLRC Home (house.gov) FDA Modernization Act revision in 1997 - James, J. S. (1997). FDA reform signed into law. Food and Drug Administration. *AIDS treatment news*, (284), 6-7. FDA reform signed into law. Food and Drug Administration - PubMed (nih.gov) FDA Modernization Act 2.0 in 2022 - Han, Jason J. "FDA Modernization Act 2.0 allows for alternatives to animal testing." (2023). FDA Modernization Act 2.0 allows for alternatives to animal testing - Han - Artificial Organs - Wiley Online	- Information about CDER which is a part of the FDA and monitors drug safety - Guidelines organisations safety testing pharma: - Background info: The new law brought cosmetics and medical devices under control, and it required that drugs be labelled with adequate directions for safe use. - The whole Act nowadays (title 21) - In 1997 the FFDCA from 1938 was reformed to include a goal of speeding research, innovation and access to care. - 29 December 2022 Biden signed into law the FDA Modernization Act 2.0. It	

		Library - - The FDA Modernization Act 2.0 - What does it mean? - CN Bio (cn-bio.com) - H.R.2565 - 117th Congress (2021-2022): FDA Modernization Act of 2021 | Congress.gov | Library of Congress - S.2952 - 117th Congress (2021-2022): FDA Modernization Act of 2021 | Congress.gov | Library of Congress - S.5002 - 117th Congress (2021-2022): FDA Modernization Act 2.0 | Congress.gov | Library of Congress - FDA workshop: Baran SW, Brown PC, Baudy AR, Fitzpatrick SC, Frantz C, Fullerton A, Gan J, Hardwick RN, Hillgren KM, Kopec AK, Liras JL, Mendrick DL, Nagao R, Proctor WR, Ramsden D, Ribeiro AJS, Stresser D, Sung KE, Sura R, Tetsuka K, Tomlinson L, Van Vleet T, Wagoner MP, Wang Q, Arslan SY, Yoder G, Ekert JE. Perspectives on the evaluation and adoption of complex in vitro models in drug development: Workshop with the FDA and the pharmaceutical industry (IQ MPS Affiliate). ALTEX. 2022;39(2). doi: 10.14573/altex.2112203. Epub 2022 Jan 21. PMID: 35064273. Perspectives on the evaluation and adoption of complex in vitro models in drug development: Workshop with the FDA and the pharmaceutical industry (IQ MPS Affiliate) - PubMed (nih.gov) - Avila, A. M., Bebenek, I., Mendrick, D. L., Peretz, J., Yao, J., & Brown, P. C. (2023). Gaps and challenges in nonclinical assessments of pharmaceuticals: An FDA/CDER perspective on considerations for development of new approach methodologies. *Regulatory Toxicology and Pharmacology*, 105345. Gaps and challenges in nonclinical assessments of pharmaceuticals: An FDA/CDER perspective on considerations for development of new approach methodologies - ScienceDirect - Avila AM, Bebenek I, Bonzo JA, et al. An FDA/CDER perspective on nonclinical testing strategies: Classical toxicology approaches and new approach methodologies (NAMs). *Regul Toxicol Pharmacol*. 2020;114:104662. doi:10.1016/j.yrtph.2020.104662 An FDA/CDER perspective on nonclinical testing strategies: Classical toxicology approaches and new approach methodologies (NAMs) - PubMed (nih.gov) - Schechtman LM. Implementation of the 3Rs (refinement, reduction, and replacement): validation and regulatory acceptance considerations for alternative toxicological test methods. *ILAR J*. 2002;43 Suppl:S85-S94. doi:10.1093/ilar.43.suppl_1.s85	refutes the FFDCA of 1938. This law now allows NAMs. - Background info - Law introduced to the House - Law introduced to the Senate - Law passed the Senate - This article covers the output from a workshop between the Food and Drug Administration (FDA) and Innovation and Quality Microphysiological Systems (IQ MPS) Affiliate. - Newer article than the one underneath this one - View of FDA about NAMs. FDA/CDER also encourages communication with stakeholders regarding NAMs and is committed to exploring the use of NAMs to improve regulatory efficiency and potentially expedite drug development. - In the US the ICCVAM and NICEATM are incorporated in the federal law, this is discussed with the FDA	

		Implementation of the 3Rs (refinement, reduction, and replacement): validation and regulatory acceptance considerations for alternative toxicological test methods - PubMed (nih.gov) - Walker EG, Baker AF, Sauer JM. Promoting Adoption of the 3Rs through Regulatory Qualification. *ILAR J*. 2016;57(2):221-225. doi:10.1093/ilar/ilw032	- Regulatory qualification, a formal process defined at the the U. S. Food and Drug Administration and the European Medicines Agency, hinges on a central concept of stating an appropriate "context of use" for a novel drug development tool (DDT) that precisely defines how that DDT can be used to support decision making in a regulated drug development setting.	
		➔ EPA - The Frank R. Lautenberg Chemical Safety for the 21st Century Act | US EPA (17-02-2023) - Thomas, Russell S., et al. "The next generation blueprint of computational toxicology at the US Environmental Protection Agency." *Toxicological Sciences* 169.2 (2019): 317-332. Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency | Toxicological Sciences | Oxford Academic (oup.com) US initiatives ➔ ICCVAM (which is composed of representatives from 17 U.S. federal regulatory and research agencies) - ICCVAM (Interagency Coordinating Committee on the Validation of Alternative Methods). 2018. A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States. Available: https://ntp.niehs.nih.gov/go/iccvam-rdmp. A Strategic Roadmap for Establishing New Approaches to Evaluate the Safety of Chemicals and Medical Products in the United States (nih.gov)	- The EPA in the US is there to protect the environment and is more focussed on chemicals. The TSCA act helps with this. - NGRA computational toxicology seems promising in US - This strategic roadmap is a resource to guide U.S. federal agencies and stakeholders seeking to adopt new approaches to safety and risk assessment of chemicals and medical products that improve human relevance and replace or reduce the use of animals	

		Global initiatives: ➔ OECD - OECD encourages the development of non-animal test methods for the detection of thyroid disrupters - OECD ➔ ICH - DETECTION OF REPRODUCTIVE AND DEVELOPMENTAL TOXICITY FOR HUMAN PHARMACEUTICALS S5-R3_Step4_Guideline_2020_0218.pdf (ich.org) - https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-q2r2-validation-analytical-procedures-step-2b_en.pdf	- The OECD encourages the development of NAMs - ICH guideline on toxicity for pharmaceuticals (Peter Theunissen) - Validation of analytical procedures (powerpoint dal Negro)	
3	Parties involved in the acceptance of NGRA	Basics about a stakeholder analysis in the thesis of Inke Looman - Abarkan, Fatima Zohra, et al. "Identifying Key Factors for Accelerating the Transition to Animal-Testing-Free Medical Science through Co-Creative, Interdisciplinary Learning between Students and Teachers." *Animals* 12.20 (2022): 2757. Animals | Free Full-Text | Identifying Key Factors for Accelerating the Transition to Animal-Testing-Free Medical Science through Co-Creative, Interdisciplinary Learning between Students and Teachers (mdpi.com) - Schiffelers MJ, Blaauboer BJ, Bakker WE, et al. Regulatory acceptance and use of 3R models for pharmaceuticals and chemicals: expert opinions on the state of affairs and the way forward. *Regul Toxicol Pharmacol*. 2014;69(1):41-48. doi:10.1016/j.yrtph.2014.02.007 Regulatory acceptance and use of 3R models for pharmaceuticals and chemicals: Expert opinions on the state of affairs and the way forward - ScienceDirect - Pant AB. The Implementation of the Three Rs in Regulatory Toxicity and Biosafety Assessment: The Indian Perspective. *Altern Lab Anim*. 2020;48(5-6):234-251. doi:10.1177/0261192920986811 The Implementation of the Three Rs in Regulatory Toxicity and Biosafety Assessment: The Indian Perspective - PubMed (nih.gov) - Mahony, Catherine. "Building confidence in non-animal methods: Practical examples of collaboration between regulators, researchers and industry." *Computational Toxicology* 10 (2019): 78-80. Building confidence in non-animal methods: Practical examples of collaboration between regulators, researchers and industry - Record details - Embase • Chapman KL, Holzgrefe H, Black LE, et al. Pharmaceutical toxicology: designing studies to reduce animal use, while maximizing human	- Focus areas to phase out animal studies in medical science - Focusses on which stakeholder groups are involved in de EU for the regulation of the acceptance of chemicals and pharmaceuticals - Indian perspective on stakeholders - Open discussion with stakeholders is critically important to build confidence in moving away from reliance on animal toxicity data and allow for development and eventual uptake of the approaches (cosmetics). - Chapter 3.1 about stakeholders	- Which stakeholders are involved in the acceptance of using NGRA for safety assessment of pharmaceuticals? • End users/Society • Researchers • Expert on NAMs • Information specialists • Organisations that sell the pharmaceuticals • Regulators • Politicians

		translation. *Regul Toxicol Pharmacol*. 2013;66(1):88-103. doi:10.1016/j.yrtph.2013.03.001 Pharmaceutical toxicology: designing studies to reduce animal use, while maximizing human translation - PubMed (nih.gov)		

Appendix A4: Information obtained from the experts that were contacted by email and the responses received

Dr. Peter J. K. van Meer - Non-clinical assessor pharmacology, toxicology and pharmacokinetics in the Medicine Evaluation Board of the College ter Beoordeling van Geneesmiddelen	- Never heard of NGRA, EMA uses Novel Approach Methodologies (NAM) - Publications about how risk can be assessed. With as a characteristic less animals: • Chien, Hsiao-Tzu, et al. "Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight of evidence approach." *Regulatory Toxicology and Pharmacology* (2022): 105329. Re-evaluating the need for chronic toxicity studies with therapeutic monoclonal antibodies, using a weight of evidence approach - ScienceDirect • Schneider, Marlon R., et al. "Applicability of organ-on-chip systems in toxicology and pharmacology." *Critical Reviews in Toxicology* 51.6 (2021): 540-554. Full article: Applicability of organ-on-chip systems in toxicology and pharmacology (tandfonline.com) • van Meer, Peter, et al. "Animal free applications in the development of cell-based therapies." *Authorea Preprints* (2020). Animal-free applications in the development of cell-based therapies - Meer - 2021 - British Journal of Clinical Pharmacology - Wiley Online Library • Ferreira, Guilherme S., et al. "Levelling the translational gap for animal to human efficacy data." *Animals* 10.7 (2020): 1199. Animals | Free Full-Text | Levelling the Translational Gap for Animal to Human Efficacy Data (mdpi.com) • Prior, Helen, et al. "The use of recovery animals in nonclinical safety assessment studies with monoclonal antibodies: further 3Rs opportunities remain." *Regulatory Toxicology and Pharmacology* (2023): 105339. The use of recovery animals in nonclinical safety assessment studies with monoclonal antibodies: further 3Rs opportunities remain - ScienceDirect	By email: - Emailed: 23-01-2023 - Email back: 23-01-2023
Dr. Peter T. Theunissen - Non-clinical assessor pharmacology, toxicology and pharmacokinetics in the Medicine Evaluation Board of the College ter Beoordeling van Geneesmiddelen	- Background: 3RsWP site: 3Rs Working Party | European Medicines Agency (europa.eu) - Qualification of alternative methods (ICHS5R3 guidance (Annex II): S5-R3_Step4_Guideline_2020_0218.pdf (ich.org)	By email - This colleague was emailed by Peter van Meer: 23-01-2023 - Email back: 23-01-2023
Prof. dr. Paul L. Carmichael - Works in the Safety & Environmental Assurance Centre (SEAC) of Unilever in the UK, developing and implementing of NAMs for assuring human and environmental health	- "Published solid examples from Pharma are as rare as unicorns" - Output of EPAA which includes pharmaceutical companies. "But I have been deeply disappointed by the lack of ambition": European Partnership for Alternative Approaches to Animal Testing (europa.eu) - Pharma are investing heavily in the use of MPS (micro-physiological systems) and organ-on-chip – they are currently sitting on a lot of data in that space and there have been a few good papers emerging: • Ewart, Lorna, et al. "Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology." *Communications Medicine* 2.1 (2022): 154. Performance assessment and economic analysis of a human Liver-Chip for predictive toxicology | Communications Medicine (nature.com) - Most of the real drive comes from the chemicals and environmental field: • Fragki, Styliani, et al. "New approach methodologies: A quantitative *in vitro* to *in vivo* extrapolation case study with PFASs." *Food and Chemical Toxicology* 172 (2023): 113559. New approach methodologies: A quantitative *in vitro* to *in vivo* extrapolation case study with PFASs - ScienceDirect - New article:	By email - Emailed: 23-01-2023 - Email back: 23-01-2023 And 24-01-2023 with a new article

	• Turner, Jan, et al. "Incorporating new approach methodologies into regulatory nonclinical pharmaceutical safety assessment." *ALTEX-Alternatives to animal experimentation* (2023). Incorporating new approach methodologies into regulatory nonclinical pharmaceutical safety assessment | ALTEX - Alternatives to animal experimentation	
Dr. Katya Tsaioun - Director of Evidence-based Toxicology Collaboration at Johns Hopkins Center for Alternatives to Animal Testing, Bloomberg School of Public Health	- Article about a study they published a couple of years ago useful to start framing my thinking about this problem: • Dirven, Hubert, et al. "Performance of preclinical models in predicting drug-induced liver injury in humans: a systematic review." *Scientific reports* 11.1 (2021): 1-19. Performance of preclinical models in predicting drug-induced liver injury in humans: a systematic review | Scientific Reports (nature.com) - Subscribing to newsletter Evidence-Based Toxicology Collaboration (EBTC) network which will put you in touch with thought leadership on evidence-based methods and their application to adoption of NGRA among other things: EBTC Newsletter (ebtox.org) - Mentioned a webinar, not clear when this one is	By email - Emailed: 24-01-2023 - Email back: 24-01-2023
Dr. Sandra Coecke Head ECVAM In-house Validation and Training laboratories & Team Leader & EU-NETVAL Coordinator, European Commission, Joint Research Centre	Information from EPAA seminar: - For legislation on medicinal products for human and veterinary use, still a lot of animals are used - Drivers: • Directive 2010/63/EU of the EP and the council: article 4 and 13 state that the when possible no or as little animals should be used with good animal welfare • Reduce drug attrition through better prediction ○ Kola and Landis 2004 ○ Hornberg et al. 2014 ○ Hay et al. 2014 ○ Harrison 2016 - Mentions the same 3R working party as Peter Theunissen • There is also an innovative task force that focusses on the regulatory acceptance of NAMs	By email - Emailed: 24-01-2023 - Reminder: 30-01-2023 No email back was received. The information here was obtained out of the EPAA workshop 2022.

Appendix A5: The eLearnings that were made and the seminars that were watched for my own understanding

eLearnings:

- SYRCLE • SYRCLE (ekphost.nl)	- Writing a systematic review • Clear explanation about the steps of how to write a preclinical systematic review - Compass: Basics about how to search information, come up with a search, evaluate resources and save and use them • Very basic - Compass +: Systematically searching for literature • Different searching machines and how to build a search was explained • A lot of overlap with SYRCLE	- Done: 23-01-2023
- Compass (+) (theoretical) • Compass+: Systematically searching for literature - Universiteitsbibliotheek Utrecht - Universiteit Utrecht (uu.nl)	- Basic information + training how to use PudMed • Good explanation about Pudmed itself and to practice with building a search	- Done: 25-01-2023
- Training PubMed (practical) • Algemeen - New PubMed - LibGuides at Utrecht University (uu.nl) • Introductie - Training new Pubmed - LibGuides at Utrecht University (uu.nl)		- Done: 27-01-2023
- EU-52 • EU-52: Searching for (existing) non-animal alternatives - Education and Training Platform for	- Made in collaboration with the EU to make the searching to known alternatives easier + make clear what the direction of thinking is • Again overlap with SYRCLE and Compass (+), but there was information specific for NAMs	- Done: 01-02-2013

Laboratory Animal Science (etplas.eu) • Information about 3Rs comes in handy for introduction		

Seminars:

- Animal Welfare intergroup • The Revision of EU Chemicals Legislation as a step towards human-relevant, new approach methods | Intergroup (animalwelfareintergroup.eu)	- Experts on New (non-animal) approach methodologies (NAMs) informed the Intergroup on Animal Welfare that it is high time we moved away from using animals in laboratory testing. - The 2022 Annual Conference of the European Partnership for Alternative Approaches to Animal Testing (EPAA) "Accelerating the Transition to Animal-Free, Sustainable Innovation"	- Watched: 16-01-2023
- EPAA workshop 2022 • The 2022 Annual Conference of the European Partnership for Alternative Approaches to Animal Testing (EPAA) "Accelerating the Transition to Animal-Free, Sustainable Innovation" - Streaming Service of the European Commission (europa.eu)	- Clear information about the steps into writing a systematic review • Almost the same information as in the SYRCLE eLearning	- Watched: 17-01-2023 & 18-01-2023
- Young TPI seminar Systematic Review writing • Webinar - introduction to (systematic) reviews Tickets, Fri, Feb 24, 2023 at 4:00 PM | Eventbrite	- Resembled the SYRCLE eLearning a lot	- Watched: 24-02-2023