International Antimony Association

EHS GROUP CALL

13 December 2018

Agenda

- Tour de table
- Approval of the conclusions of the last call
- Questions on the last update sent on the 27.11
- Update on the EHS strategy
  - In vitro inhalation study
  - Genotoxicity studies
  - PSLT workshop
  - Dashboard to communicate on EHS progress
- Next meetings

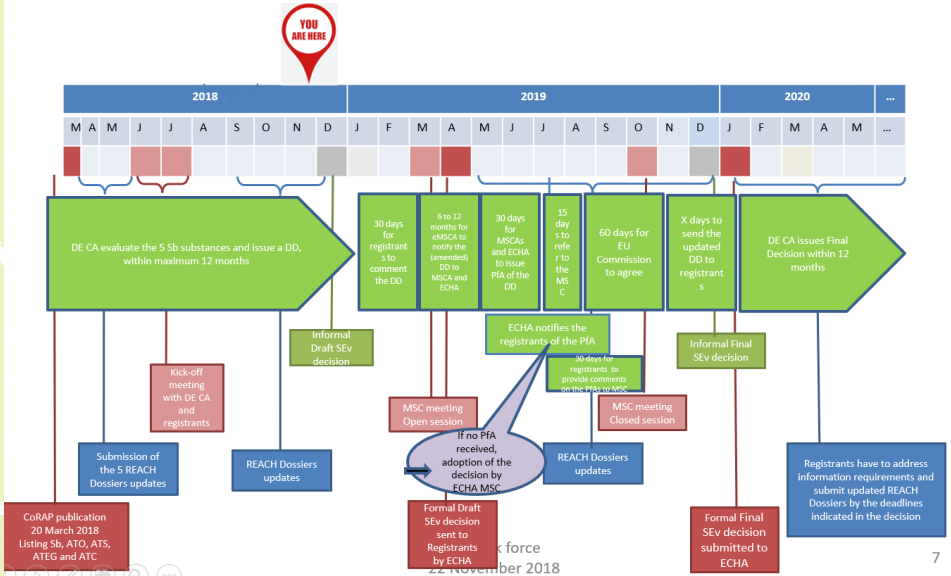
EHS update 27 November 2018

EHS strategy update
Substance Evaluation

Evaluation	

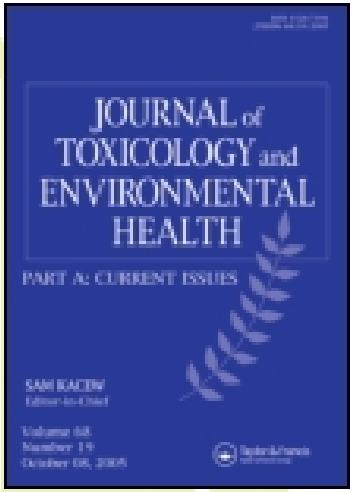
1.1 Substance Evaluation	In the context of the Substance Evaluation, i2a continues to share with BAuA the progress of the research program aiming to tackle the remaining knowledge gaps around the toxicology of Sb substances. An update of the strategy developed under COLLA will be sent to BAuA by end of the year. It will list all the actions i2a has already taken:
	- Set up of the in vivo reproductive toxicity study plan
	- Set up of the tests for the lung toxicity assessment:
	  o Validation of the method for determination of the total antimony dissolved and antimony V and III in simulated physiological media
	  o Bio-elution testing on the 10 antimony substances in simulated gastric fluid and simulated gastric fluid + proteins
	  o In vitro inhalation study on alveolar cells (see in vitro inhalation test section)
	- Set up of genotoxicity testings:
	  o Tox tracker assay on 12 antimony substances
	  o Assay to interpret micronucleus observed in vivo in the NTP study
	- Set up of a workplace monitoring campaign aiming to generate workplace air exposure data.
	- Contacting the Belgian Federal Police to collect exposure data from indoor shooting ranges.
	
	BAuA will be able to comment the strategy but will not issue an official document to agree or disagree on i2a's plan.
	
	A Toxicologists Task Force and a Monitoring Task Force have been set up to enable more in-depth discussions on the reprotoxicity study, in vitro inhalation study and workplace monitoring program among scientific experts of i2a members . Conference calls, webinars or meetings are scheduled every 6/8 weeks on these specific topics. EHS participants are invited to notify i2a their interest to be included in these two TF.
	
	Registrants of the trivalent substances are expecting the formal Draft SEv decision from ECHA in April 2019.
	
	i2a's COLLA/SEv plan is also being used to satisfy i2a's commitment to the Metals and Inorganics Sectorial Approach (MISA). MISA aims to facilitate a more systematic improvement of Registration Dossiers by asking associations to inform ECHA about their internal update plans. By submitting i2a's dossier update work plan through MISA, i2a's planned SEv work becomes visible to the broader ECHA structure and other Member States, thereby decreasing the risk that Sb substances are picked up in parallel REACH or CLP processes.

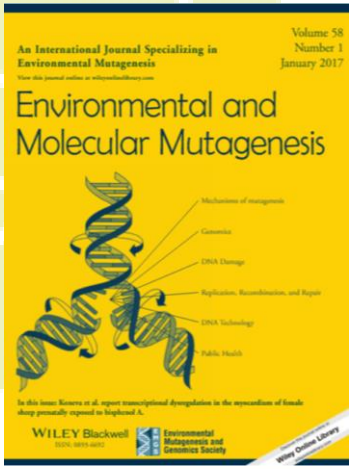
baua: Bundesanstalt für Arbeitsschutz und Arbeitsmedizin



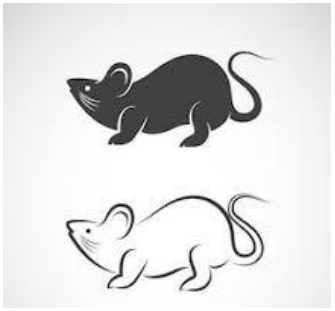
EHS strategy update
Scientific publications

3	**EHS strategy**	
	3.1 Status of scientific publications	- The tox tracker report *"Genotoxic properties assessed by ToxTracker"* is finalized (Annex 1). 12 substances have been now tested (the 10 i2a substances having a Reach dossier and 2 additional substances typically reported in reference literature: antimony triacetate and antimony potassium tartrate). None of the tested antimony compounds showed genotoxic properties in the ToxTracker assay in absence or presence of a metabolizing system. Significant levels of oxidative stress were observed for most of the compounds, but there was no indication that oxidative stress led to indirect genotoxicity. Activation of the unfolded protein response was also observed for most tested compounds. There was no indication for any of the tested antimony compounds that their toxicity was increased due to metabolism by the liver. R. Cortvrindt will now start the drafting of the article analyzing these results through a publication. i2a will need to revert on the identification of the scientist journal to publish. - The second genotoxicity article on "Analysis and synthesis of genotoxicity data on antimony substances" will be drafted by C. Boreiko as main author in 2019. - No new action has been taken on the article on: "The presence of Sb in consumer products". The Sb Day however revealed that information could be collected with Br and PVC producers and users, who have generated leaching data. - The potential article on the reprotoxicity study plan will be placed on the agenda when the planned reprotox study will be finalized (mid-2019).

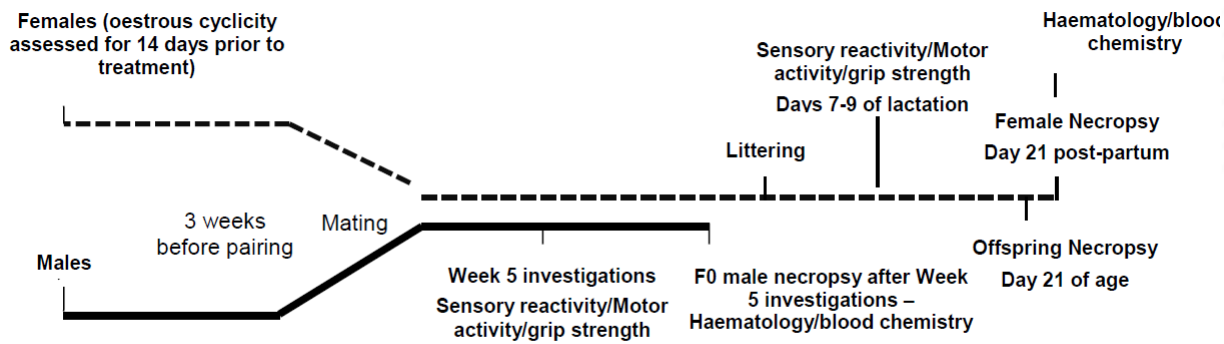




EHS strategy update
Reprotoxicity Study



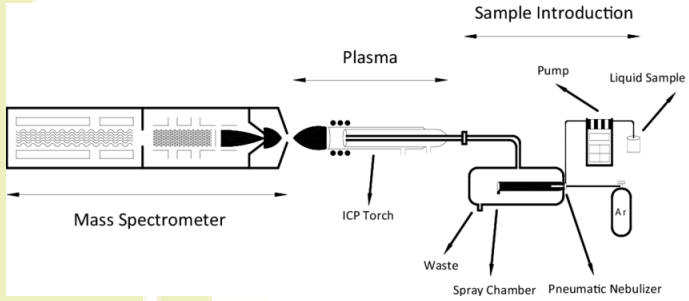
3.3 Reprotoxicity study	As discussed at the last tox TF a Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening (OECD 422) has been designed to generate information concerning the effects of antimony substances orally administrated on male and female rats reproductive performance such as gonadal function, mating behaviour, conception, development of the conceptus and parturition. It has been designed to clarify two questions of specific relevance to Sb substances: the link between the observed foetus toxicity and the maternal toxicity, and the effect of Sb and possible reversibility of the delayed ossification observed in previous studies. This study is coordinated by Lindsay Aveyard, expert in reprotoxicology and will be performed by Envigo. The study will last 28 days + 3 weeks to observe the effects on offspring reared to weaning and selected offspring treated at least from weaning is planned to be; The proposal has been sent for review and comments and is now accepted. The contract with ENVIGO will be signed by the end of November, formalities and laboratory capacity secured in December, and the study will be performed in Q1 2019.



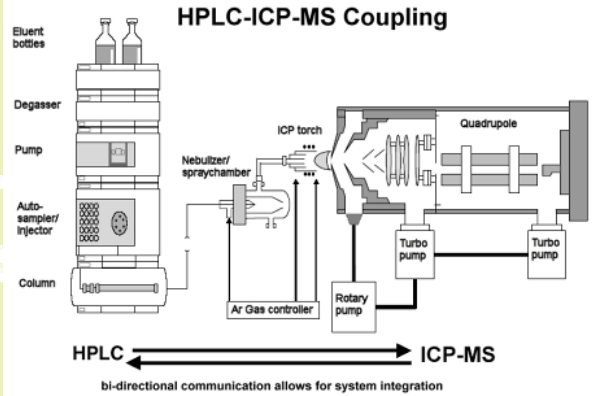
EHS strategy update

3.5 Quantification and speciation method	VITO has finalized the report on the validation of the method for the determination of Sb in several bioelution fluids (Annex 2). The bioelution samples in gastric fluids will be now analyzed through these methods. The Total dissolved antimony will be measured through the ICP-MS method whereas the antimony III and V will be measured through the more complex method, HPLC ICP-MS, able to distinguish both species, antimony III and antimony V. However, it has been shown that the method to identify antimony V in the simulated gastric fluid with proteins was not fully reliable. i2a is investigating what are the implications in the interpretation of the future measurements of this species.

vito



3.4 Bioelution test	ECTX has received from the lead registrants the 10 substances to be tested in the 2 simulated gastric fluids (with and without proteins). The protocol will follow the SOP procedure developed by Eurometaux and ECVAM. The study plan with a timeline and a quote will be addressed to i2a beginning of December and will be shared at the next EHS call. The plan is to collect the bioelution samples 1st half of January and to measure them at Vito site 2nd half of January. These results will allow to inform the choice of the test item for the reproductive toxicity study, and refine the read-across justification for the oral chronic endpoints. The potential need to test on other biofluids (e.g. alveolar, lysosomal or interstitial) is still under assessment. Identification of the relevant proteins for the inhalation route, and the validation of the Sb determinations from these fluids will be needed before these follow-up bioelution tests are launched.



ECTX Ecotoxicology & Biodegradation

EHS strategy update
Workplace air monitoring

3.6 Workplace air exposure monitoring	Following the i2a communication, through the Task Force and the recent antimony days, i2a received a lot of interest in the workplace air exposure monitoring campaign. IOM, selected partner of the program, has presented the different steps of the projects during the antimony days (Annex 3) - Development of the monitoring guidance document for collection of inhalation and respirable samples and supporting contextual information - Collection of respirable and inhalable personal samples and measure particle size distribution (PSD) at producer and DU sites - Development and population of an exposure database - Appropriate agreed statistical analysis of the collected exposure measurement data and reporting At its recent conf call, the Monitoring TF was invited to comment the second draft version of the Monitoring Guidance and companies were invited to come forward and express their interest to participate in the Campaign. First confirmed companies will have the possibility to exchange with IOM to define their specific monitoring strategy and budget requirements, and may also be able to loan the monitoring equipment from IOM, instead of purchasing it. A minimum of two producers and two users of each main use of Sb substances (not only

IOM

EHS strategy update
T25

4.1 AOB	- T25 analysis for ATO has been calculated. The assessment of the applicability of the method is in the hands of Eurometaux and the Cobalt institute. i2a's input and the input of other sectors has been used to obtain recognition that the T25 approach may not be applicable to metals. A dedicated Work Group is being set up by ECHA to further discuss what the best solution would be.

Pharmacol Toxicol. 1997 Jun;80(6):272-9.

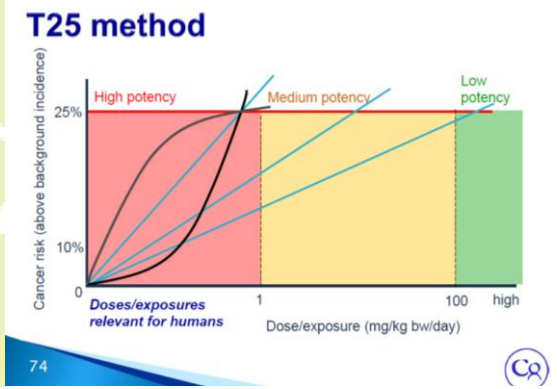
T25: a simplified carcinogenic potency index: description of the system and study of correlations between carcinogenic potency and species/site specificity and mutagenicity.

Dybing E[1], Sanner T, Roelfzema H, Kroese D, Tennant RW.

Author information

Abstract

A simplified carcinogenic potency index, the T25, is proposed as a practical method for the inclusion of potency considerations in carcinogen classification systems. The T25 is the chronic daily dose in mg per kg bodyweight which will give 25% of the animals tumours at a specific tissue site, after correction for spontaneous incidence, within the standard life span of that species. Calculated T25 values of a set of 113 US National Cancer Institute/National Toxicology Program (NC/NTP) carcinogens showed excellent correlation (correlation coefficient 0.96, P < 0.0001) with the carcinogenic potency index TD50 of Peto et al. (1984). The mean of T25 values for 51 transspecies, multiple common site NCI/NTP carcinogens were 10-fold lower than those for 62 NCI/NTP single species, single site carcinogens. For these 113 carcinogens, the mean T25 values were approximately 3-fold lower for agents that were also mutagenic in Salmonella compared to the non-mutagenic agents.



In vitro inhalation study

Summary of the minutes

- Regulatory background:
  - The interpretation of the NTP carcinogenicity study by NTP concluding that ATO is a reasonably anticipated human carcinogen
  - The future new edition of the Report of Carcinogens listing ATO and expected increased regulatory attention in the US (and beyond)
  - The European REACH Substance Evaluation of the Sb 3+ substances possibly leading to a revised CLP classification (and an stricter EU-binding OEL) and questioned read-across

Summary of the minutes

- Current i2a EHS strategy:
  - Validation of the quantification and speciation method for total dissolved Sb and Sb 3+ and 5+.
    - Method identifying Sb 5+ in the simulated gastric fluid with proteins was not fully reliable.
    - Certainly due to the formation of complexes with proteins
    - No alternative method
    - Await full results and decide how to use gastric + proteins fluid after

Summary of the minutes

- Current i2a EHS strategy:

  - Bioelution testings are planned to be launched by first half of January in simulated gastric fluid and simulated gastric fluid with proteins

  - Toxtracker assay on 12 substances :
    - No indication of direct nor indirect genotoxicity
    - Contradictory with in vivo observations
    - Cells used are not cells lungs → only first 'ranking' system, to be followed up with in vitro strategy using lung cells

Summary of the minutes

For discussion

- Lung toxicity design:
  - Preparation phase
    - Grinding of the substances to make them available to the cells
    - Questions to be addressed:
      - Is the substance generally supplied as inhalable powders?
      - Can the substance be produced in inhalable powder form (e.g. upon request from a customer)?
      - May the substance release inhalable dust or become inhalable during use?
      - Additionally to the inhalability of the Sb substance, does the substance remain chemically stable during the preparation for and performance of an in vitro testing?

Summary of the minutes

For discussion

- Lung toxicity design:
  - Preparation phase
    - Agreement to undergo in a fresh characterization of the substances
      - Particle size distribution with laser diffraction
      - Aerosol characterization with APS (aerodynamic particle sizer)
      - BET characterization (determination of the surface area)
  - Preincubation of the test material in the culture medium, followed by a recovery of the supernatant, which would contain the ionic fraction.

Summary of the minutes

For discussion

- Lung toxicity design:
  - First screening on the 10 substances on rat/mouse/human alveolar cells (epithelial and macrophages)
    - Cells exposed to the fresh material (substances particles)
    - Cells exposed to the supernatant recovered from the preincubation (ionic fraction)
    - Allow to determine whether any biological responses were due solely to the ionic fraction.
    - 3 measurements on these media:
      - Cytotoxicity and cell viability (identification of the BenchMark Dose)
      - Oxidative potential, acellular and intracellular
      - Cytokine release

Summary of the minutes

For discussion

- Lung toxicity design:
  - Second screening on selected cells and substances, predicting oxidative stress, detrimental effects of ROS production:
    - intracellular detection and quantification of reduced and oxidized glutathione and malondialdehyde (MDA). These 2 tests are often performed together to allow correlation between them.
    - quantitative gene expression of hemeoxygenase-1 (HO-1)
    - determination of DNA damage – with Comet assay and Cytokinesis Block Micronucleus (CBMN) assay

Summary of the minutes

For discussion

- Lung toxicity design:
  - The in vitro lung deposition model (subsequent phases of the testing strategy) was not specifically discussed as it was agreed to focus on the screening phases at this moment.

Genotoxicity Studies

Genotoxicity assessment – RAAF 2018

- "Sb (III) compounds can produce positive results when tested for genotoxicity in vitro and in vivo. "

  - Clastogenic events (usually the formation of micronuclei) appear to be most commonly observed endpoint.

  - Sb (V) compounds have been tested with less frequency and available data are inconsistent, but it is expected that they would produce no or lower genotoxic effects than Sb (III) compounds

MN Studies

For discussion

- Reliance on existing genotoxicity data may be questionable due to the observation of micronuclei :
  - Metals and metalloids can form cytoplasmic inclusion bodies that can be mistaken for micronuclei if stains used during the assays are not highly specific for DNA
  - Some reports of micronucleus induction by Sb compounds may reflect therefore staining artefacts.
- Need to Verify the possible Micronucleus staining artifacts:
  - Micronucleus tests with staining procedures with high specificity for DNA could provide a rapid means of determining whether staining artifacts have impacted the existing genotoxicity data base.

MN studies

For discussion

- Micronucleus study in CHO-WBL cells by ILS:
  - Treated cells stained with Giemsa stain and microscopically scored for MN by a person.
  - Presumption that antimony III induce MN fairly effectively.
  - The slides will then be destained (the Giemsa removed by solvents) and restained with a stain (acridine orange) that has much higher affinity for, and specific binding to, DNA.
  - Research of DNA in the MN identified by Giemsa staining
    - If no DNA: protein inclusion bodies, mistaken for MN, were induced by Sb. This would explain the artifactual false positive results observed in genotoxicity database (including NTP study one) and would demonstrate **that most of the positive in vitro and vivo genotox studies are not valid.**
    - If DNA is present, MN may not be an artefact and would request investigation.

For discussion

Oncogene Activation Studies

- Another method is under investigation: Next-generation sequencing (NGS) technologies.

- Enable to identify rare DNA sequence changes responsible to spontaneous EGFR oncogene activation, implicated in the development of cancer.

APPLICATIONS OF NEXT-GENERATION SEQUENCING

Enhancing the accuracy of next-generation sequencing for detecting rare and subclonal mutations

Jesse J. Salk[1,2,4], Michael W. Schmitt[1,2,4] and Lawrence A. Loeb[1,3*]*

Abstract | Mutations, the fuel of evolution, are first manifested as rare DNA changes within a population of cells. Although next-generation sequencing (NGS) technologies have revolutionized the study of genomic variation between species and individual organisms, most have limited ability to accurately detect and quantify rare variants among the different genome copies in heterogeneous mixtures of cells or molecules. We describe the technical challenges in characterizing subclonal variants using conventional NGS protocols and the recent development of error correction strategies, both computational and experimental, including consensus sequencing of single DNA molecules. We also highlight major applications for low-frequency mutation detection in science and medicine, describe emerging methodologies and provide our vision for the future of DNA sequencing.

PSLT Workshop

The PSLT discussion

For information

- 23 April 2018 CARACAL subgroup discussion:
  - 'Intrinsic' hazardous property of a substance
  - Translation of TiO2 classification into Annex VI entry
  - How to address hazards of **poorly soluble low toxicity particles (PSLTs**) under Classification, Labelling and Packaging of substances and mixtures (CLP)?
- Approach of Paul Borm & Kevin Driscoll
  - Conduct a survey among experts in academia, industry and regulatory bodies
    - to make an inventory on current consensus in various paradigms in the step-wise process leading to classification of Titanium Dioxide (TiO_2) and perhaps also PSLT as a group.
    - To question on 4 topics : overload, risk/hazard assessment and models, classification and labeling
  - Willingness to set up a workshop with experts in 2019

The PSLT Workshop

For decision

- Goal : How inhalation data is being applied to ensure the best science is used to evaluate, classify as to hazard and assess risk of inhaled particulate materials
  - Is there a technical basis to evaluate PSLTs as a group?, if so, how would they be defined?;
  - Should distinctions be made in classifying materials depending on particle size, for example nano size materials versus larger micron size?
  - How should maximum tolerated doses be determined for inhalation studies with poorly soluble particles and how should responses above those levels be extrapolated?
  - Is the rat lung response to high doses of particles like titanium dioxide and carbon black unique to that species, or is the rat a human relevant, sensitive species";
- i2a sponsoring proposal : 2000 €
- 1-2 April 2019 in Edinburgh (UK)
- Proposal for i2a to participate (Craig Boreiko – Marjorie Huppert)

EHS progress tracking template

Excel tracking sheet with details

For discussion

- Every item/project/action would be described through 3 different parts:
  - Description of the action: regulatory driver, scientific and regulatory aim, potential/selected partner, i2a coordinator
  - Progress tracking: done, left to do, status
  - Expenditure estimation: spent, left to spend , status
  - Tracked through excel
  - Any suggestions for adding useful information missing?

Ex: Excel tracking sheet with details

For discussion

Admin tracking						Progress tracking				Expenditure tracking			
Main regulatory driver	**Work item**	**Scientific aim**	**Regulatory aim**	**External Partner**	**i2a Group/Secretariat**	**Done so far**	**Left to do**	**Status**	**Comment**	**Spent so far**	**Left to spend**	**Status**	**Comment**
EHS Strategy													
REACH Substance Evaluation	Scientific publication	Make publically available to scientific community the right information on antimony hazards and exposure	Strenghten argumentation and reasonning in any regulatory process	Rita Cortvindt Craig Boreiko Dr Rossman L. Aveyard	Tox TF/M. Huppert	Content, poposal agreed	Release of the first drafts. Choice of the scientific journal	Waiting for amended timetable of the authors	Action work in the meantime have delayed the delivery of drafts.	50 000,00 €	50 000,00 €		
REACH Substance Evaluation	Method for quantification and speciation Sb	Compare and rank Sb substances according to bioavailability	Identify relevant test items for reprotox testing	ECTX, VITO	Tox TF/M. Huppert	Identification of the method to measure Sb compounds	Finalize the report	Report available for members	Discussion around the applicability of the method for Sb	None		Not yet signed	
REACH Substance Evaluation	Bioelution gastric	Compare and rank Sb substances according to bioavailability	Identify relevant test items for reprotox testing	ECTX, VITO	Tox TF/M. Huppert	Identification of the substcances Identificaiton of the media Identificaiton of the method to measure Sb compounds	Agree on the protocole and poposal Run de test Analyse the results	Substances to test on site	Test should start in january	None		Not yet signed	
REACH Substance Evaluation	OECD 422 test	Understand internal toxicity and delayed ossification	Postpone and inform/reduce mandatory EOGRTS test	ENVIGO, L. Aveyard	Tox TF/M. Huppert	Design of the study with ENVIGO Agreement on the protocol proposal	Identy substances to test	Study Will start Q1 2019	Waiting for bioelution gastric tests results	None	795 000,00 €	Signed	
REACH Substance Evaluation	In vitro lung test	Compare and rank inhable Sb substances around lung toxicity	Inform read-across/grouping for carcinogenicity classification	IOM	Tox TF/M. Huppert	Agreement on the purpose of the study	Agree on the design of the study	Waiting for proposal		None		Not yet signed	
REACH Substance Evaluation	Workplace exposure data collection	Document latest exposure levels in producing and using sites	1) Demonstrate safe use with hard data; 2) Use in TLV/OEL revisions processes	IOM	Monitoring TF/C. Braibant	Agree on the purpose and contetn of the campaign	Finalize the Monitoring Guidance Organise the Feb WS Organise the sampling	Reception of voluntary participation from members		None	692 000,00 €	Signed	

Progress

For discussion

- Need to track through a timeline
  - 3 colors :
    - Finalized
    - On progress
    - Estimated remaining time
    - Cancelled or Issue
  - How to estimate the time
    - Regulatory deadline
    - Proposal from partner
    - Experience

Date of status : 13 December 2018	2017	2018				2019				2020				2021			
		Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Scientific Publication																	
Method for quantification and speciation Sb																	
Bioelution gastric																	
OECD 422 test																	
In vitro lung test																	
Workplace exposure data collection																	

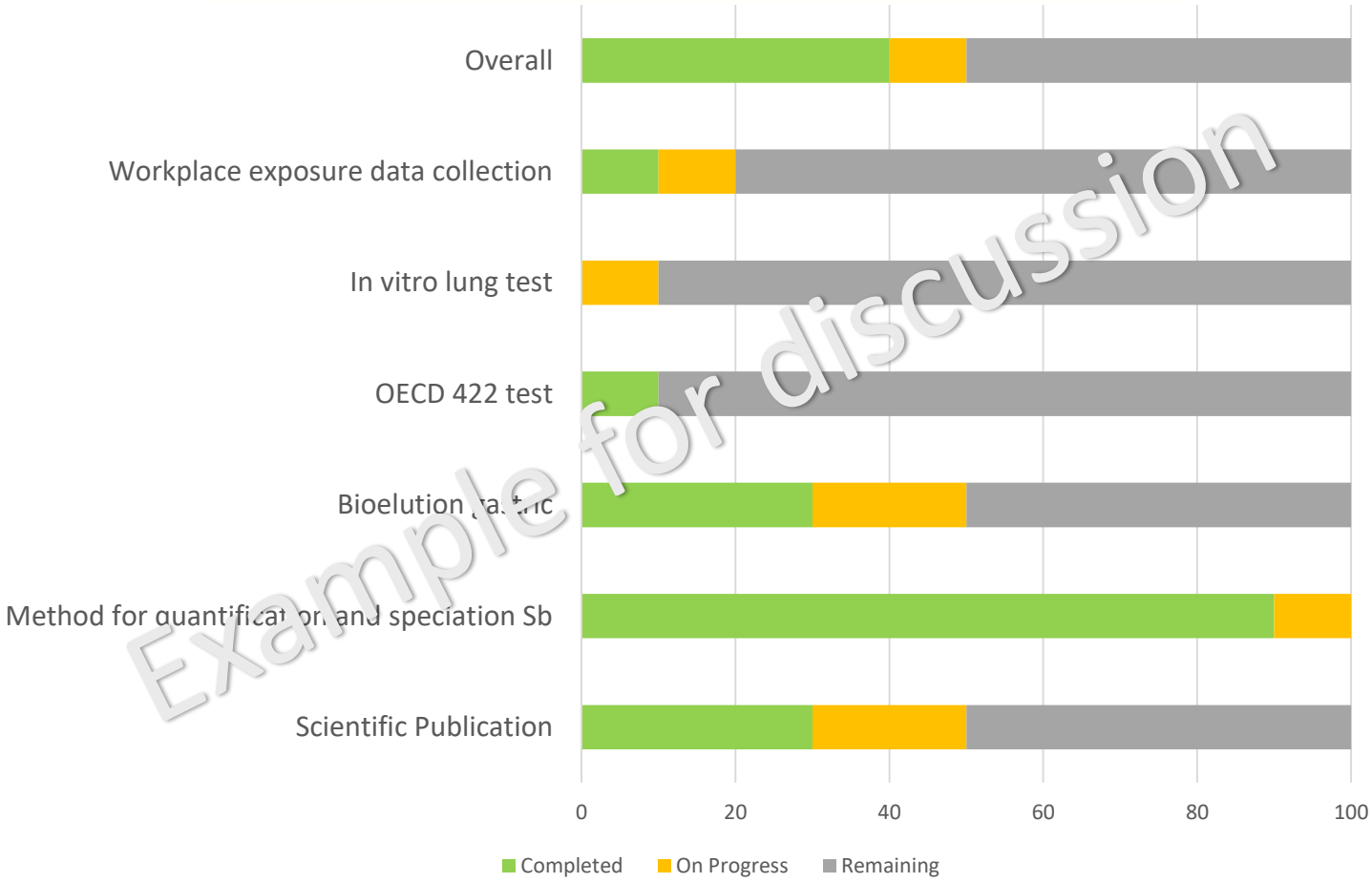
Progress

For discussion

- Need to track by project
  - 3 colors :
    - Completed and on time:
    - On progress but delayed
    - Estimated remaining time
    - Cancelled or Issue
  - How to quantify ? Need to define milestones
    - Identification of the need
    - Identification of the different steps of the project
    - Presentation of the project
    - Communication with members of the strategy
    - Request for external support
    - Agreement of the proposal
    - Presentation of the results
    - Discussion of the results with partners and members
    - Communication of the results
    - Finalization.
  - Suggestions?

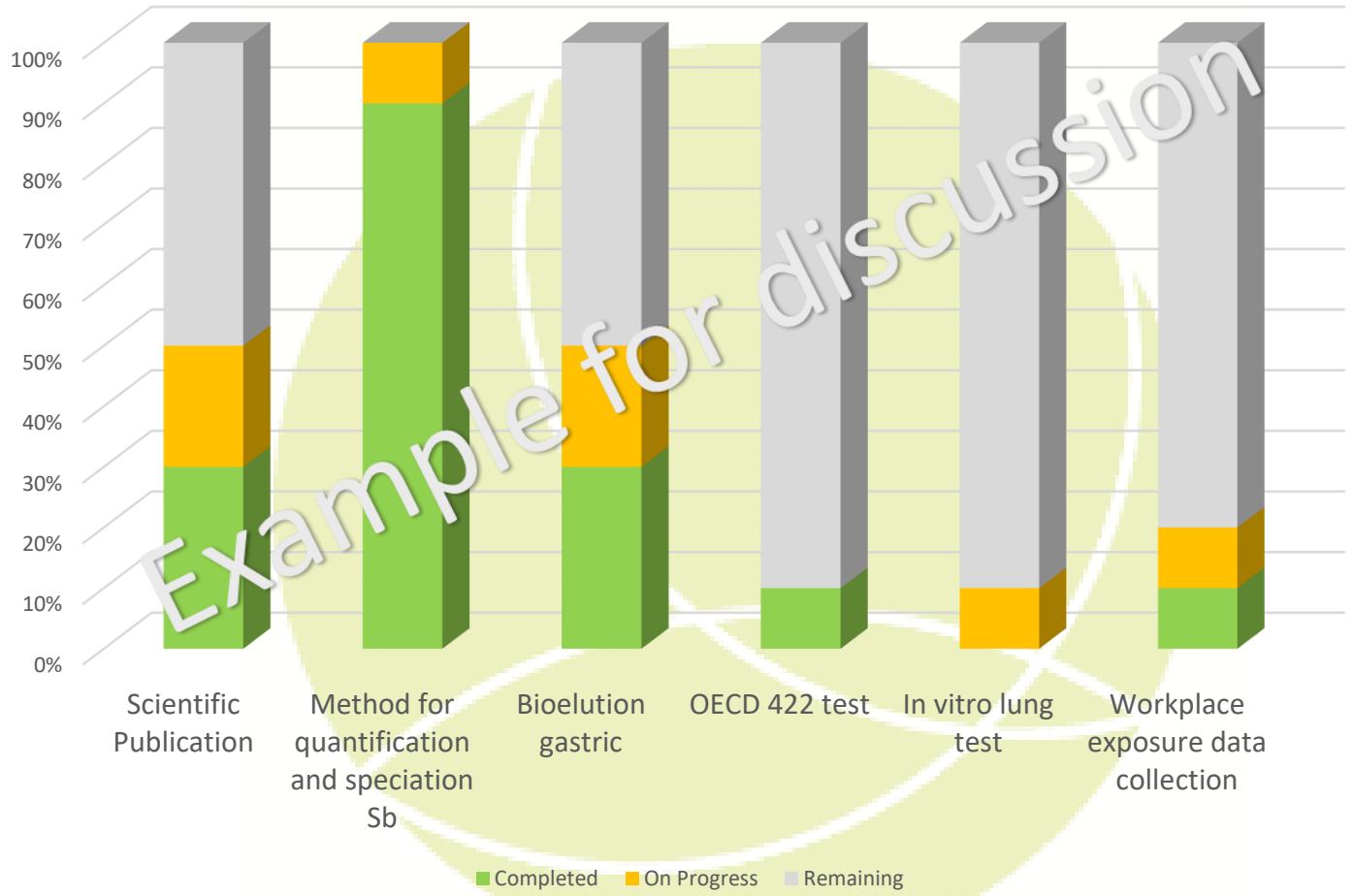
Progress

For discussion



Progress

For discussion



2019 EHS meetings and events

For information

- Next calls – date to be defined :
  - Mid of February
  - Mid of April
  - Mid of June
  - Update end of July
  - Mid of September
  - Mid of October
  - Mid of December

- EHS events:
  - i2a Workplace Exposure Monitoring Workshop: 21 February 2019 in Brussels (Belgium)
  - PSLT Workshop: 1-2 April 2019 in Edinburgh (UK)

Minutes

Minutes

Attendants

- Marie-Laure Ledrich (Traxys), Adam McCarthy (Albemarle), Mark Carpels (Campine), Caroline Braibant (i2a), Marjorie Huppert (i2a), Craig Boreiko (late)
- The participants had no comments on last October call. Comments on the last November update were addressed. The conclusions were approved.
- All actions are in progress and in-time
- The agenda was approved

Comments on the last November EHS update

- Workplace monitoring campaign: 2 samplers will be used
  - Question: why does the guidance recommend 2 samplers, one to capture the inhalable fraction and one for the respirable one.
  - Companies may have different type of samplers. In order to harmonize the data, it is advised to use the samplers referred in the monitoring guidance.
  - The reference to the study comparing samples justifying the choice for two samplers (one for inhalable and for respirable) instead of one (measuring both inhalable and respirable) will be circulated.
- Tox tracker assay
  - Question: how can we explain the effects which happen in in-vivo studies are not happening in in-vitro assays?
  - Question will be raised to the toxtracker experts (Rita Cortvrindt, Giel Hendriks) and to the in vitro inhalation study partner (Matthew Boyles)
  - Similar observations have been made for other metals (Chromium, Cobalt) – not Sb-specific

Minutes

- Lung in vitro inhalation study
  - Design of the study
    - Need to better define how the study will explain the genotoxicity, and the discrepancies of the effects observed between in-vivo and vitro results
    - What can be expected and what cannot be expected from this study?
    - Minutes of the 22 November TF meeting will be sent to the EHS members

- Genotoxicities studies
  - What is the concentration tested in the micronuclei studies? The **limit of solubility** needs to be taken into account.
  - Oncogene activation studies may be part of the genotoxicity assessment. i2a needs to define the strategy with the budget of this assessment of MoA with Craig. Nothing will be launched before discussion and support from the TF

Minutes

- Progress tracking template
  - The participants agree to develop 2 tracking documents, updated monthly
    - An excel sheet with all details of all topics (slide 29)
    - An overview represented by a graph (slide 32)
  - Adding regulatory deadline was suggested
  - Every EHS call will refer to these documents and additional slides will be added to discuss specific topics
- A doodle will be sent to fix the next EHS calls, planned to occur every 2 months


THANK YOU
ALL
AND
MERRY
CHRISTMAS