The use of effect biomarkers in human biomonitoring studies: exposure to hexavalent chromium

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Background

- Hexavalent chromium, Cr(VI) is a human carcinogen (Group 1, IARC), and its exposure has been associated with increased lung cancer risk, especially in exposed workers.

DHHS (NIOSH) Publication No. 2013–128

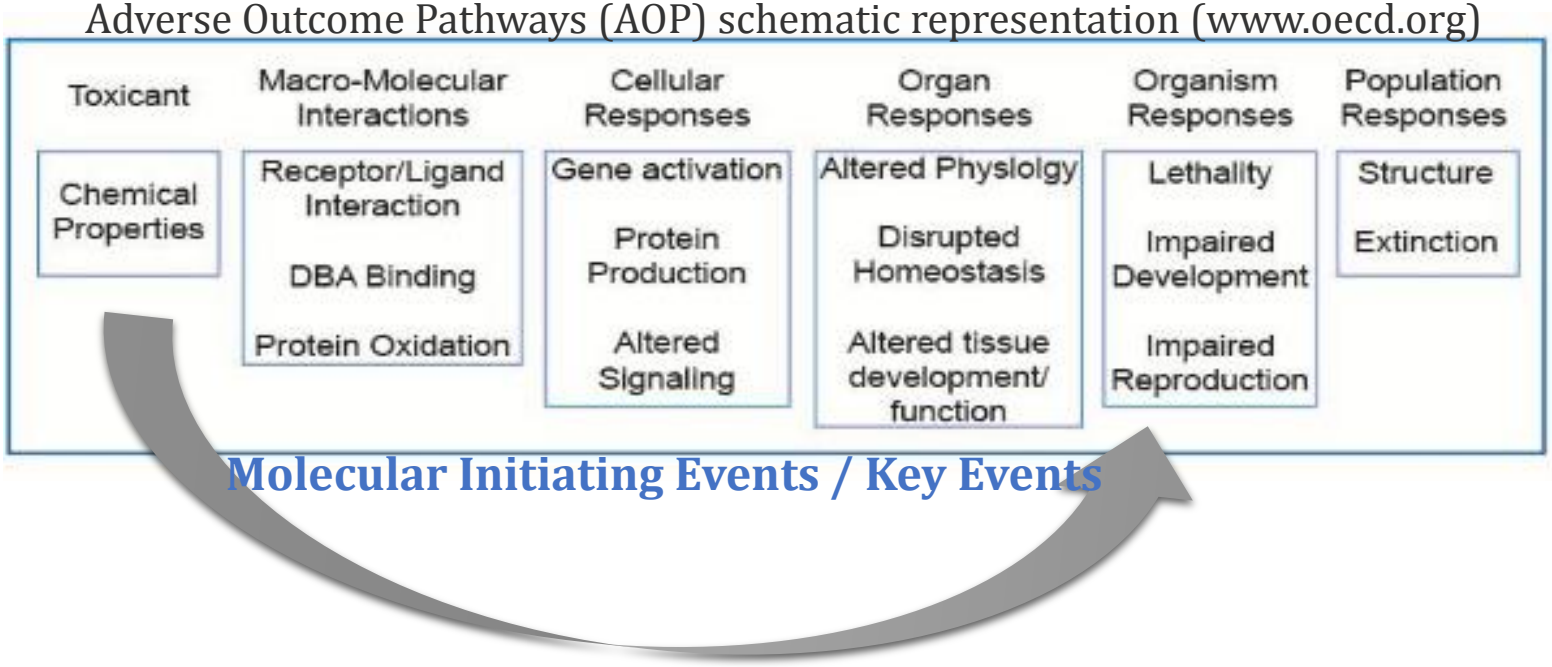
- The general population may be exposed to Cr(VI) through food, drinking water and tobacco smoke, and gastrointestinal cancer may also be developed.

- Under the Human Biomonitoring for Europe Initiative (HBM4EU), Cr(VI) has been considered a priority substance, indicating the need for generating and analyzing data on human exposure and effects, both as single substance and in mixtures.

Background (cont.)

- Although many epidemiological studies have reported data on human exposure to Cr(VI), fewer included effect biomarkers assessment.
- However, these biomarkers are central to identify early biological effects before the onset of any adverse health effect.

Adverse Outcome Pathways (AOP) schematic representation (www.oecd.org)



Objetives

- This work aims at
  - Reviewing effect biomarkers for Cr(VI) analysed in exposed populations, which may be quantitatively linked to adverse health outcomes in humans.
  - Identifying needs for the development of new effect biomarkers and setting up decision criteria associated with the ideal characteristics of biomarkers.

Literature search – criteria and results

Search results	Chromium AND (MeSH Terms OR synonym AND all fields)	Exclusions	Filters
242 references	"environmental monitoring“ “monitoring”	• environmental-only studies • non-human studies • human exposure-only studies	full text 10 years human
196 references	"biomarkers”		
603 references	"toxicity"		

Selection of the 49 references
which contained the most complete information on chromium effect biomarkers

We found 57 effect biomarkers used in Cr(VI) biomonitoring.

Most frequent effect biomarkers – oxidative stress biomarkers

	# of studies in the search	Hazard/ Health Outcome
8-OHdG (oxidized DNA)	11	ROS Mutagenicity/Carcinogenicity
MDA (malondialdehyde)	8	Oxidative stress, Mutagenicity/Carcinogenicity Immunotoxicity
GSH (glutathione)	3	Oxidative stress, Mutagenicity/Carcinogenicity
SOD (superoxide dismutase)	3	Oxidative stress, Mutagenicity/Carcinogenicity Immunotoxicity
LPO (lipid peroxidation)	3	Oxidative stress, Mutagenicity/Carcinogenicity Immunotoxicity

Most frequent effect biomarkers – Genotoxicity

	# of studies in the search	Hazard/ Health Outcome
Comet assay (ss/dsDNA breaks)	10	Genotoxicity
Micronucleus test (chromosome breaks/ loss)	8	Genotoxicity/Carcinogenicity

Candidate novel effect biomarkers –criteria for scoring

- numerical score assigned depending on the characteristics of the specific effect biomarker under study

Has the biomarker been assessed in human matrices?	Non-invasive: Urine: 5 points Saliva: 4 points Placenta: 2 point Invasive: Serum: 3 points Others: 1 point
Is there a plausible mechanism of action (MoA)?	Yes: 2 points No: 0 points
Is an Adverse Outcome Pathway (AOP) reported for this effect biomarker?	Yes: 3 points No: 0 points
Has the biomarker been implemented in epidemiologic studies?	Yes: 5 points No: 0 points
How would you define the feasibility, based on cost, efficacy, specificity, sensitivity and reliability of the biomarker?	Unsure: Indicate your concern(s) and add 0 points Low: 0 points Middle: 2 points High: 5 points

Olea, N., et al. 2018. Criteria for prioritization of biomarkers of effect . Deliverable Report D 14.1 WP 14 Effect Biomarkers. www.hbm4eu.eu.

Novel effect biomarkers –pros & cons

Novel effect biomarker	Brief description of the effect biomarker	Final Score
Gene expression - **DNA repair genes** - **detoxifying genes**	**Strengths**: - Related with the Cr(VI) MoA - low invasiveness: blood samples - low cost, depending of the number of genes studied **Limitations**: - low specificity	17/20
Epigenetics **DNA methylation levels**	**Strengths**: -Related with the Cr(VI) MoA - low invasiveness: blood samples **Limitations**: - low specificity - costs are still high.	12/20

Novel effect biomarkers –pros & cons

Novel effect biomarker	Brief description of the effect biomarker	Final Score
Proteomics	**Strenghts**: -Related with the Cr(VI) MoA (Identification of important deregulated pathways) - low invasiveness: blood samples - more accurate then gene expression - high-throughput data can be obtained **Limitations**: - low specificity - Expensive	12/20
Microarray microRNA expression profile	**Strengths**: - Related with the Cr(VI) MoA - miRNAs can be isolated from many types of biological samples and are stable - miRNA profile indicates affected functions and biological processes and may be related with a health outcome **Limitations**: - expensive - low specificity - interpretation may be demanding	12/20

Conclusions-I

- *Classical effect biomarkers*
  - One common characteristic is the fact that they are **not specific** for chromium exposures.
  - Can be affected by other heavy metals or even by polycyclic aromatic hydrocarbons (Ferguson et al., 2017; Hormozi et al., 2018; Pan et al., 2018)
- *Novel effect biomarkers*
  - Potential to improve specificity, as long as molecular events are identified.
  - Allow high throughput methods, but are more expensive and still lack validation.

Effect Biomarkers - Link to Health Effects

- Adverse health outcomes (AOs) related to Cr(VI): lung cancer, asthma, respiratory irritation, skin irritation, etc.

- Plausible AOPs for the main AOs reported relate to:
  - ✓Cancer- AOP 139, AOP140
  - ✓Respiratory tract sensitization – AOP39
  - ✓Sensitization of the skin- AOP40

Source: HBM4EU WP13 deliverable under preparation

Effect Biomarkers - Link to Health Effects

- **Cancer:** AOP 139 (Alkylation of DNA leading to cancer 1, Under Development)

Events: Molecular Initiating Events (MIE) Key Events (KE) Adverse Outcomes (AO)

	Sequence	Type	Event ID	Title	Short name
Molecular initiating event	1	MIE	97	Alkylation, DNA **Cr(VI)?**	Alkylation, DNA
Key events	2	KE	155	N/A, Inadequate DNA repair	N/A, Inadequate DNA repair
	3	KE	185	Increase, Mutations	Increase, Mutations
Adverse outcome	4	AO	885	Increase, Cancer	Increase, Cancer

Genotoxicity

https://aopwiki.org/wiki/index.php/Aop:139

Effect Biomarkers - Link to Health Effects

❖**The two most commonly referred MoA of Cr(VI) toxicity identified in these abstracts were related to oxidative stress and DNA damage**.

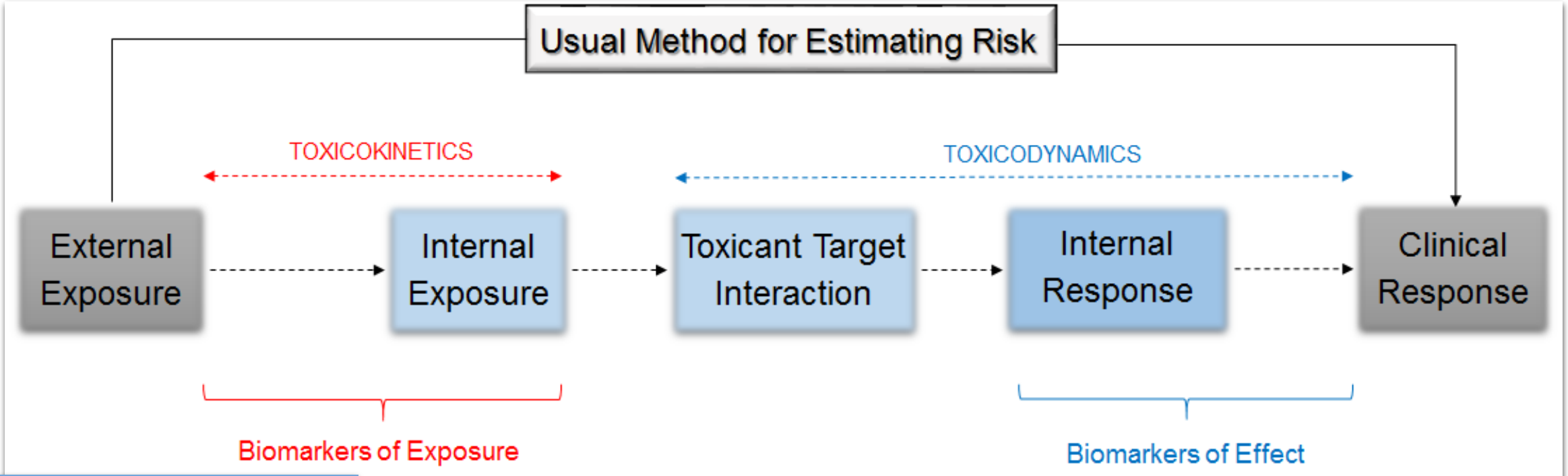
Potential KEs include:

- mitochondrial dysfunction-Xiao et al. 2019
- to chromosomal instability and chromatin organization changes, apoptosis resistance, autophagy and angiogenesis.
- augmentation of ROS and onset of ER stress - Ganapathy et al. 2017.
- chronic wounding of intestinal villi, chronic regenerative crypt cell hyperplasia and increase of crypt enterocytes - Thompson et al. 2013, 2015, 2018
- malignant transformation of several cell types were reported, involving changes in the expression of EGFR, p53, c-Myc, HIF-1alpha, Bcl-2, PDCD4, FBP1, MM1, VEGF, ERK, NF-kB and other proteins involved in signalling pathways related to cell division, proliferation and differentiation. …..

Conclusions - II

- Need to expand knowledge on the AOP framework for lung cancer after Cr(VI) exposure.
- Biomarkers of genotoxicity are relevant effect biomarkers in view of an AO for cancer.
- Similar approach has been performed under HBM4EU for:
  - Bisphenols (coordinated by UGR)
  - Phthalates (coordinated by VITO)
  - PFAS-PFOA (coordinated by AU)
  - Flame Retardants (coordinated by MU and EASP)
  - PAHs (coordinated by NCRWE)
  - Cadmium (coordinated by BfR)

Mustieles et al, 2018. Additional Deliverable AD 14.2. WP14 - Effect Biomarkers . Available at www.hbm4eu.eu



Measurement of
- Cr in red blood cells (more specific for Cr(VI)) or
- Total Cr in plasma or urine or EBC.

Potentially Better Method for Estimating Risk

Measurement in blood cells, urine or EBC
- Reactive oxygen species (ROS)
- Genotoxic and epigenetic biomarkers

can help establishing a relationship between exposure and health outcomes (cancer)

Louro et al. IJHEHealth 222 (2019) 727–737. https://doi.org/10.1016/j.ijheh.2019.05.009

Perspectives – ongoing studies

Identification of mixture health effects- a case study

Occupational exposure to Chromium (VI), nickel and polycyclic aromatic hydrocarbons and lung cancer (INSA, IRAS, UGR, FIOH)

HBM4EU science and policy for a healthy future

HORIZON2020 Programme
Contract No. 733032 HBM4EU

Plan for development of case studies
Deliverable Report
AD 15.1
WP 15 - Mixtures, HBM and human health risk

Need due to:

i) lack of data on PAHs-chromium-nickel mixture effects in humans;
ii) co-exposure that can occur in several occupational settings, such as welding, cars and aircrafts maintenance and much others implicating exposure of a high number of workers;
iii) the published epidemiological data about exposure to each substance and lung cancer does not take into account possible interactive effects;
iv) the existence of suitable biomarkers of exposure and effect regarding these substances

https://www.hbm4eu.eu

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