# Partnership for the Assessment of Risks from Chemicals

PARC T4.1.2.: Selected biomarkers of exposure for PARC Aligned Studies

PARC Partnership FOR THE Assessment OF Risks FROM Chemicals

Co-funded by the European Union

This partnership has received funding from the European Union's Horizon Europe research and innovation program under Grant Agreement No 101057014.

# Selection of biomarkers of exposure for PARC Aligned Studies

This document presents the final selection of exposure biomarkers to be measured in the PARC Aligned Studies. Further details on the process followed for the selection of the biomarkers, including the criteria applied and the rationale behind the decisions taken, as well as the design of the QA/QC programme can be found here.

Two levels have been finally defined, the **PARC Aligned Studies scope** and the **PARC Discovery scope** as follows:

| **1. PARC Aligned Studies scope**<br><br>*Biomarkers to be measured in the PARC Aligned Studies* | **2. PARC Discovery scope**<br><br>*Optional, interesting exposure biomarkers for new hypothesis generation* |
|---|---|
| Commercial standards must be available | Standards must be available |
| Covered in G-EQUAS or possibility to include it in the programme | Not covered by G-EQUAS |
| *For the minimum compulsory set:*<br>• *Known or expected high detection frequency (>60%) in target population at the LOQ requirement set for the PARC Aligned Study*<br>• *A high percentage (at least 60%) of the surveyed laboratories with experience in a given substance group should be capable of analysing the biomarker*. | |

Remarks for study owners and laboratories are provided at the end of this document, please make sure that you read them. If you have any questions, do not hesitate to contact PARC@isciii.es.

The selected biomarkers are provided in the following table. Those also measured in HMB4EU are highlighted in green.

| Substance group | Biomarker | CAS nr | Age group | | | PARC Aligned Studies scope | | PARC Discovery scope |
|---|---|---|---|---|---|---|---|---|
| | | | C | T | A | Complete set | Minimum compulsory set | |
| Bisphenols in urine | Bisphenol A (BPA) | 80-05-7 | X | X | X | Y | Y | |
| | Bisphenol S (BPS) | 80-09-1 | X | X | X | Y | Y | |
| | Bisphenol F (BPF) | 620-92-8 | X | X | X | Y | Y | |
| | Bisphenol E (BPE) | 2081-08-5 | X | X | X | | | Y |
| | Bisphenol AP (BPAP) | 260550-89-8 | X | X | X | | | Y |
| | Bisphenol AF | 1478-61-1 | X | X | X | | | Y |
| | Bisphenol Z | 843-55-0 | X | X | X | | | Y |
| | Bisphenol B | 77-40-7 | X | X | X | | | Y |
| | Bisphenol P (BPP) | 2167-51-3 | X | X | X | | | Y |
| Phthalates in urine | MBzP | 2528-16-7 | X | X | X | Y | Y | |
| | MiBP | 30833-53-5 | X | X | X | Y | Y | |
| | MnBP | 131-70-4 | X | X | X | Y | Y | |
| | MEHP | 4376-20-9 | X | X | X | Y | Y | |
| | 5OH-MEHP | 40321-99-1 | X | X | X | Y | Y | |
| | 5oxo-MEHP | 40321-98-0 | X | X | X | Y | Y | |
| | 5cx-MEPP | 40809-41-4 | X | X | X | Y | Y | |
| | cx-MiNP | 936022-02-5 | X | X | X | Y | Y | |
| | MEP | 2306-33-4 | X | X | X | Y | Y | |
| | OH-MiNP | 898544-10-0 | X | X | X | Y | | |
| | MnPeP | 24539-56-8 | X | X | X | | | Y |
| | MnOP | 5393-19-1 | X | X | X | | | Y |
| | MCHP | 7517-36-4 | X | X | X | | | Y |
| | OH-MiDP | 1372605-11-2 | X | X | X | | | Y |
| | cx-MiDP | | X | X | X | | | Y |
| DINCH in urine | OH-MINCH | 1637562-52-7 | X | X | X | Y | Y | |
| | cx-MINCH | 1637562-51-6 | X | X | X | Y | Y | |
| | oxo-MINCH | 1588520-62-0 | X | X | X | Y | Y | |

| Substance group | Biomarker | CAS nr | Age group | | | PARC Aligned Studies scope | | PARC Discovery scope |
|---|---|---|---|---|---|---|---|---|
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| other phthalate substitutes in urine | 5-cx-MEPTP | 1684398-42-2 | X | X | X | Y | | |
| | 5OH-MEHTP | 1684398-38-6 | X | X | X | Y | | |
| | Mono-2-ethyl-5-hydroxyhexyl adipate (5OH-MEHA) | 134998-71-3 | X | X | X | | | Y |
| | Mono-5-carboxy-2-ethylpentyl adipate (5cx-MEPA) | | X | X | X | | | Y |
| PFAS in serum | PFNA | 375-95-1 | | X | X | Y | Y | |
| | PFDA | 335-76-2 | | X | X | Y | Y | |
| | PFBS | 375-73-5 | | X | X | Y | Y | |
| | PFHxS | 355-46-4 | | X | X | Y | Y | |
| | PFHpS | 60270-55-5 | | X | X | Y | Y | |
| | PFOA | 335-67-1 | | X | X | Y | Y | |
| | PFOS | 1763-23-1 | | X | X | Y | Y | |
| | 4:2 FTSA | 757124-72-4 | | X | X | | | Y |
| | 6:2 FTSA | 27619-97-2 | | X | X | | | Y |
| | 8:2 FTSA | 39108-34-4 | | X | X | | | Y |
| | 9CL-PF3ONS | 756426-58-1 | | X | X | | | Y |
| | PFHxA | 307-24-4 | | X | X | | | Y |
| | PFHpA | 375-85-9 | | X | X | | | Y |
| | PFDoDA | 307-55-1 | | X | X | | | Y |
| | PFUnDA | 2058-94-8 | | X | X | | | Y |
| | PFPeA | 2706-90-3 | | X | X | | | Y |
| | PFBA | 375-22-4 | | X | X | | | Y |
| | PFNS | 68259-12-1 | | X | X | | | Y |
| | EtFOSAA | 2991-50-6 | | X | X | | | Y |
| | MeFOSAA | 2355-31-9 | | X | X | | | Y |
| | Adona | 919005-14-4 | | X | X | | | Y |
| | GenX | 13252-13-6 | | X | X | | | Y |

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| Pesticides-Neonicotinoids in urine | N-desmethyl acetamiprid | 190604-92-3 | X | | X | Y | Y | |
| | Acetamiprid | 135410-20-7 | X | | X | Y | Y | |
| | Imidacloprid | 138261-41-3 | X | | X | Y | Y | |
| | Imidacloprid olefin | 115086-54-9 | X | | X | | | Y |
| | Imidacloprid 5-hydroxy | | X | | X | | | Y |
| Pesticides-Pyrethroids in urine | cis-DBCA | 59952-39-5 | X | X | X | Y | Y | |
| | cis-DCCA | 55667-40-8 | X | X | X | Y | Y | |
| | trans-DCCA | 55701-03-6 | X | X | X | Y | Y | |
| | 3-PBA | 3739-38-6 | X | X | X | Y | Y | |
| | 4-F-3-PBA | 77279-89-1 | X | X | X | Y | Y | |
| | ClF3CA | 68127-59-3 | X | X | X | Y | | |
| Pesticides-Organophosphates in urine | TCPy | 6515-38-4 | X | X | X | Y | Y | |
| Pesticides-Glyphosate/AMPA in urine | Glyphosate | 1071-83-6 | | | X | Y | Y | |
| | AMPA | 1066-51-9 | | | X | Y | Y | |
| Metals in whole blood | Hg | 7439-97-6 | | | X | Y | Y | |
| | Pb | 7439-92-1 | | | X | Y | Y | |
| | Cd | 7440-43-9 | | | X | Y | Y | |
| | Cr | 7440-47-3 | | | X | Y | | |
| | Mn | 7439-96-5 | | | X | Y | | |
| | Co | 7440-48-4 | | | X | Y | | |
| | Se **(in plasma)** | 7782-49-2 | | | X | Y | | |
| Arsenic species in urine | Total As | 7440-38-2 | | X | | Y | Y | |
| | As (III) | 7440-38-2 | | X | | Y | Y | |
| | As (V) | 17428-41-0 | | X | | Y | Y | |
| | MMA | 124-58-3 | | X | | Y | Y | |
| | DMA | 75-60-5 | | X | | Y | Y | |

| Substance group | Biomarker | CAS nr | Age group | | | PARC Aligned Studies scope | | PARC Discovery scope |
|---|---|---|---|---|---|---|---|---|
| | | | C | T | A | Complete set | Minimum compulsory set | |
| | Arsenobetaine | 64436-13-1 | | X | | Y | Y | |
| Metals in urine | Cd | 7440-43-9 | X | | X | Y | Y | |
| | Hg | 7439-97-6 | X | | X | Y | | |
| | Pb | 7439-92-1 | X | | X | Y | | |
| | Cr | 7440-47-3 | X | | X | Y | | |
| | Ni | 7440-02-0 | X | | X | Y | | |
| | Al | 7429-90-5 | X | | X | Y | | |
| | Li | 7580-67-8 | X | | X | Y | | |
| | Mn | 7439-96-5 | X | | X | Y | | |
| | Co | 7440-48-4 | X | | X | Y | | |
| | Pt | 7440-06-4 | X | | X | Y | | |
| | Cu | 7440-50-8 | X | | X | Y | | |
| | Sr | 7440-24-6 | X | | X | Y | | |
| | Zn | 7440-66-6 | X | | X | Y | | |
| | Mo | 7439-98-7 | X | | X | Y | | |
| | Sn | 7440-31-5 | X | | X | Y | | |
| Mercury in hair | Total Hg | 7439-97-6 | X | | | Y | Y | |
| **Additional parameters** | | | | | | | | |
| Cotinine (urine) | | 486-56-6 | X | X | X | Y | Y | |
| Creatinine (urine) | | 60-27-5 | X | X | X | Y | Y | |
| Specific gravity (urine) | | | X | X | X | Y | Y | |

| | Also measured in HBM4EU Aligned Studies |
|---|---|

# REMARKS

- **Only those laboratories with successful participation in the PARC QA/QC programme can analyse samples in PARC HBM studies.**
- **G-EQUAS is the QA/QC programme for all biomarkers in the PARC Aligned Studies scope except total mercury in hair, which will be organised by ISCIII.**
- **Successful participation in the PARC QA/QC programme implies successful results in 2 rounds.**

## General remarks

**For study owners:**

- All biomarkers in the PARC Aligned Studies should be analysed. Mandatory biomarkers must be analysed to receive PARC funding.
- If you already have a candidate laboratory, align their participation in the QA/QC programme with the time for sampling/analysis of your study.

**For laboratories:**

- Laboratories can join the G-EQUAS program since now (round 73). Please be aware that Neonicotinoids will be only included from round 74 (autumn 2024).
- For total mercury in hair, information on the programme will be provided shortly.
- Participation should be aligned with analyses (analyses estimated between Q4 2024-Q1 2027).
- When registering for the G-EQUAS, please make sure that:
    1. you indicate that you are participating in G-EQUAS motivated by PARC interests (in relation to the PARC QA/QC programme)
    2. you accept that G-EQUAS organisers share your contact data and results to ISCIII as part of the work done under PARC.

These options will be available for round 73 from the week of 26th February 2024. If you have already registered, please inform us and send us your acceptance on the sharing of contact data and results from G-EQUAS by email to PARC@isciii.es.

## Specific remarks on the parameters/biomarkers

**For Creatinine**

The recommendation is to join the G-EQUAS for creatinine. If a laboratory participates in another PT, it will be asked to provide the results for evaluation in PARC.

**For cotinine**

G-EQUAS is also the QA/QC programme for cotinine.

**For specific gravity**

Measurements should not be done using urine strips but using a refractometer.

**For Lipid adjustment**

A common recommendation will be provided by T9.3.

**For PFAS**

For the analysis of PFAS, the use of **serum** as matrix is recommended in the PARC Aligned Studies following the recommendations provided in Annex 2.3 to AD4.1 Best Practice for the Collection, Processing and Storage of Human Samples, Part 3: Collecting Blood Samples (Whole Blood, Plasma, Serum). If using blood sample collection tubes that contain a separating gel (not recommended), it must be ensured that the sampling material is free of contamination (the tubes should be tested). This must be indicated in the sampling procedure documents.

For the quality assurance of PFAS analysis in serum the G-EQUAS PFAS in plasma is valid (as indicated by the G-EQUAS coordinators and the PARC QAWG).

The recommendation is that laboratories report both linear PFAS (as in G-EQUAS) and total PFAS.

**For phthalates and substitutes**

Phthalates: specific attention is asked for OH-MiNP, cx-MiNP, OH-MiDP and cx-MiDP. The parent phthalates of these biomarkers are isomeric mixtures resulting in multiple and/or broad peaks in real samples, and the transition used for quantification affects the analysis result. For this reason, the participants that include these four biomarkers in their scope are asked to use the following transitions for quantification:

OH-MiNP: m/z 307 > 121 cx-MiNP: m/z 321 > 173

OH-MiDP: m/z 321 > 121 cx-MiDP: m/z 335 > 187

DINCH**:** DINCH biomarkers are isomeric mixtures typically resulting in multiple and/or broad peaks in real samples, and the transition used for quantification affects the analysis result. For this reason, the participants are asked to use the following transitions for quantification:

OH-MINCH: m/z 313 > 153

cx-MINCH: m/z 327 > 173

oxo-MINCH: m/z 311 > 153

Also, please ensure the acquisition windows for the above compounds are sufficiently wide (at least from 2 min before until 2 min after the retention time of the analytical standard, depending on your specific LC method even longer) to ensure all peaks belonging to the biomarker are measured.

Regarding the enzyme**:** for deconjugation it is important to use glucuronidase enzymes free of non-specific lipase activity / and no aryl-sulfatase. Suitable is e.g. E. coli B-glucuronidase K12 enzyme. Details see: Koch et al. Journal of Chromatography B, 784 (2003) 169–182. doi: 10.1016/S1570-0232(02)00785-7

## Notes on other additional parameters

In the PARC Workshop on the General Survey held in Paris in May 2023, the inclusion of the analysis of the additional parameters haematocrit and ferritin for the interpretation of the results of certain metals was discussed. These parameters are out of the scope of the current PARC QA/QC programme. We provide here further information:

Analysis of haematocrit (whole blood): this not an obligatory analysis for PARC Aligned Studies due to the logistical challenge of analysing haematocrit on fresh whole blood samples within a 12h time window. However, for those partners that perform the analysis anyway, we will foresee the option to include haematocrit levels in the harmonized dataset for PARC. This will give the opportunity to perform some sensitivity analysis at an EU level.

Analysis of ferritin (serum): its inclusion is still under evaluation.