



National Institute for Public Health
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Ministry of Health, Welfare and Sport

The use of epidemiologic studies for the biomonitoring of harmful substances

RIVM Letter report 2016-0216
B. Tiesjema | J.D. te Biesebeek | M. Mengelers



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Colophon

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Synopsis

The use of epidemiologic studies for the biomonitoring of harmful substances

People are exposed to all kinds of substances, amongst others via food, which can be harmful to their health. To properly estimate the health effects, it is important to determine to what extent these substances are present in the body and to what extent they cause damage. In order to do so it may be helpful to measure concentrations of these substances in body fluids and/or tissues (biomonitoring).

If the population is large enough, these concentrations can then be used to estimate the exposure to these substances. Moreover, biomonitoring, in combination with information collected in the epidemiological studies, may indicate which groups of people may have an increased risk of health problems due to the exposure to a specific substance.

In the Netherlands a large number of epidemiological studies is conducted in the field of public health. The blood and urine collected in some studies may be used for future biomonitoring of harmful substances. The RIVM has examined four studies to determine whether they are suitable for measuring the exposure to harmful substances in blood and / or urine (biomonitoring). This appears to be the case for all four, to a greater or lesser extent. However, the characteristics of the studies differ, so that not only the substance but also the precise questions of the future monitoring study determine which study is most suitable.

For this research it is investigated what requirements a substance must meet in order to be measured via biomonitoring and which biological material is suitable for this. Furthermore, it is described which requirements an epidemiological study should meet in order to qualify for biomonitoring purposes. After that, the most important characteristics of four Dutch epidemiological studies were inventoried. The four studies were chosen because the RIVM has easy access to the data.

Keywords: cohort, biomonitoring, harmful substances, health

Publiekssamenvatting

Het gebruik van epidemiologische studies voor de biomonitoring van schadelijke stoffen

Mensen staan bloot aan allerlei stoffen, ook via voedsel, die schadelijk kunnen zijn voor hun gezondheid. Om de gezondheidseffecten goed in te kunnen schatten, is het belangrijk te bepalen in hoeverre deze stoffen in het lichaam aanwezig zijn en in welke mate ze daar schade veroorzaken. Hierbij kan het helpen om concentraties van deze stoffen in lichaamsvloeistoffen en/of weefsels te meten (biomonitoring). Bij een voldoende grote groep kunnen deze concentraties worden gebruikt om de blootstelling aan deze stoffen te schatten. Bovendien kan biomonitoring, in combinatie met informatie die is verzameld in de epidemiologische studies, mogelijk aangeven welke groepen mensen een verhoogde kans op gezondheidsproblemen kunnen hebben door de blootstelling aan een bepaalde stof.

In Nederland wordt een groot aantal epidemiologische studies uitgevoerd op het gebied van de volksgezondheid. Het bloed en de urine dat bij sommige studies wordt verzameld, kan mogelijk gebruikt worden voor toekomstige biomonitoring van schadelijke stoffen. Het RIVM heeft van vier studies onderzocht of ze geschikt zijn om de blootstelling aan schadelijke stoffen in bloed en/of urine (biomonitoring) te meten. Dat blijkt voor alle vier, in meer of mindere mate, het geval te zijn. Wel verschillen de eigenschappen van de studies waardoor niet alleen de stof maar ook de precieze vraagstelling van de toekomstige monitoringstudie bepaalt welke studie het meest geschikt is.

Voor dit onderzoek is eerst bekeken aan welke eisen een stof moet voldoen om via biomonitoring gemeten te kunnen worden en welk biologisch materiaal hiervoor geschikt is. Voorts is beschreven aan welke eisen een epidemiologische studie zou moeten voldoen om in aanmerking te komen voor biomonitoringsdoeleinden. Daarna zijn belangrijkste kenmerken van vier Nederlandse epidemiologische studies geïnventariseerd. De vier studies zijn gekozen omdat het RIVM eenvoudig toegang heeft tot de gegevens.

Kernwoorden: epidemiologische studie, biomonitoring, schadelijke stoffen, gezondheid

Contents

Summary — 9

1 Introduction — 11

2 Requirements for the biomonitoring of harmful substances — 13

2.1 Typical requirements of biomarkers — 14

2.1.1 Illustrative biomonitoring questions — 14

3 Potential studies to be used for biomonitoring — 19

3.1 Doetinchem Cohort Study — 19

3.2 PIAMA Cohort — 20

3.3 The Netherlands measured (NL de maat genomen) — 20

3.4 Lifelines — 21

3.5 Comparison of epidemiologic studies — 21

4 Comparison of requirements for biomonitoring and properties of epidemiologic studies — 25

5 Conclusions — 27

6 References — 29

Annex I. Overview of specific properties of the epidemiologic studies relevant for the biomonitoring of contaminants — 31

Summary

Biomonitoring studies can provide insight in the body burden of humans to substances. The body burden can then be used to estimate the external exposure or to estimate the risk of specified health effects. If the population is large enough, together with the other information that has been collected in the epidemiologic studies, biomonitoring may also provide insight in subpopulations that have an increased risk in health effects due to a certain substance. In addition, given the long follow-up of some of the cohort studies, analysis of the biological material collected in such studies may provide insight into time trends in the exposure to contaminants in the population.

Within the Netherlands a large number of epidemiologic studies exist in the field of public health. Samples of biomaterials collected and stored in these studies may potentially be used for human biomonitoring studies of contaminants.

For this report, it is first considered which requirements a biomarker has to meet to be suitable for biomonitoring and which biological material is suitable for this purpose. Subsequently, four chemical contaminants have been selected and four typical (illustrative) purposes related to the biomonitoring of these contaminants are given to indicate to which typical requirements an epidemiologic study should comply to be used for a biomonitoring study.

Next, an inventory has been made of the main characteristics of the following four Dutch studies: the Doetinchem Cohort Study, the PIAMA study, 'NL de maat genomen' (measuring the Netherlands) and Lifelines. This includes (amongst others): information on study design, number of participants and life-style characteristics, but also information on sample collection and storage and analysis of (basic) clinical-chemical parameters.

All epidemiologic studies described in this report are, more or less, suitable for biomonitoring studies. However, the characteristics (size, samples stored, background information) differ and this will determine, together with the contaminant of interest *and* the specific research question, which cohort should be selected for future biomonitoring studies. In addition, ongoing cohort studies like the Doetinchem Cohort Study and Lifelines offer the possibility that future sampling and collection of information may be adjusted to specific needs related to (improve) the biomonitoring of contaminants.

1 Introduction

In biomonitoring studies, concentrations of chemical substances (usually exogenous substances) are measured in human body fluids and tissues. These concentrations can be used to study time trends or to estimate the external and internal exposure of humans to these substances. Provided certain conditions are met, the concentration of a substance in a biological fluid can be related to health effects caused by the exposure to these substances. Within the Netherlands a large number of epidemiologic studies exist in the field of public health. In many of these studies blood and/or urine samples have been collected from the participants. In many cases, some, but not all of these samples have been used for analysis of clinical-chemical parameters. When remaining samples are stored, they may potentially be used for other research; for example for biomonitoring studies of the Dutch population. In addition, together with other information that has been collected in the cohort studies, for example on life-style characteristics of the participants, this may provide valuable new insights in which subgroups of a population may have an increased risk in health effects due to a certain substance.

For this report, it is first considered which requirements a harmful substance has to be eligible for biomonitoring and which biological material is suitable for this purpose.

Next, an inventory has been made of the main characteristics of four Dutch epidemiologic studies. Due to time and budget constraints, we have made a (small) selection of studies where RIVM had easy access to the underlying information. This includes (amongst others): information on study design, number of participants and life-style characteristics, but also information on sample collection and storage and analysis of (basic) clinical-chemical parameters.

As finger exercise, four typical (illustrative) purposes related to the biomonitoring of four harmful substances have been used to illustrate to what extent an epidemiologic study may meet requirements related to chemical biomarkers.

The information in this report may be helpful to learn how to select an epidemiologic study for future biomonitoring of harmful substances.

2 Requirements for the biomonitoring of harmful substances

Biomonitoring of harmful substances can be used for many different purposes, such as the determination of exposure, determination of health effect, population screening to identify people at risk for developing a specific disease in an early stage, or trend analysis, for example for assessing the efficiency of preventive measures (1). Depending on the purpose of biomonitoring, many chemical contaminants can serve as a biomarker. It depends on the kinetic characteristics of the substance which biological sample should be used to determine the body burden of the substance. For example, chronic exposure to (methyl)mercury can be measured in hairs whereas chronic exposure to fat soluble POPs (like dioxin) can be measured in breastmilk (2).

We have drawn up some general criteria for the suitability of a biomarker:

1. The biomarker should be chemically stable in a biological fluid like blood or urine;
2. It must be possible to accurately quantify the biomarker in a biological fluid;
3. There should be some kind of correlation between the concentration of the biomarker in the biological fluid and the exposure or effect;
4. The demands of the way of sampling/sample storage should not be too specific/should match the way of sampling/sample storage in the epidemiologic study
5. There should be some understanding of the biokinetics of the biomarker

In order to explore the suitability of an epidemiologic study for biomonitoring we have selected some harmful substances, as an example.

The selection was based on the following approach. Firstly, we selected harmful substances that have a dietary exposure in the Netherlands that exceeds a so-called health based guidance value (HBGV)¹ or that has a small margin of exposure (MoE)². This selection was taken from the RIVM report on the current situation of food safety in the Netherlands (3). Secondly, we have defined some general criteria related to the suitability of a biomarker. Applying these criteria reduced the number of harmful substances.

The following harmful substances were taken from the report on the current position of the Dutch food safety: aflatoxin B1, alternariol, alternariol monomethyl ether, cadmium, lead, acrylamide and carbofuran. The first three substances are mycotoxins, cadmium and lead are heavy metals, acrylamide is a processing contaminant and

¹ A Health Based Guidance Value (HBGV) is a toxicological reference value of the concentration of a substance to which a person can be exposed without an appreciable health risk.

² A Margin of Exposure (MoE) is the ratio between the point of departure (usually a bench mark dose) and the exposure estimate.

carbofuran is a plant protection product (pesticide) not approved anymore within the EU.

In particular criterion 3 of the general criteria narrowed the seven substances down to four: acrylamide, aflatoxin B1, cadmium and lead. The heavy metals cadmium and lead are well-known biomarkers for exposure (4). The protein adducts of aflatoxin B1 and acrylamide are also well-known biomarkers for exposure (5, 6). However, in the process of selecting harmful substances we concluded that each biomarker in combination with a specific research question has its own requirement(s) with respect to the suitability of an epidemiologic study for biomonitoring. Therefore, we choose to describe four typical (illustrative) research questions related to the biomonitoring of the selected substances (see next section).

2.1 Typical requirements of biomarkers

In the following section a typical (illustrative) research question related to the biomonitoring of each substance is given. For each combination of research question and biomarker, some typical requirements are given with respect to the characteristics of the epidemiologic study and the biological samples taken in this study. The requirements for the illustrative biomonitoring questions are summarized in tables 1-3. Due to time and budget constraints we did not investigate detailed requirements like the amount of sample left in storage, the analytical requirements of the chemical method of analysis (detection/quantification limits, temperature of storage, age of sample, etc.).

2.1.1 Illustrative biomonitoring questions

1. A retrospective **trend analysis** to evaluate the measures that have been undertaken in the last years to reduce **acrylamide** levels in food.

There is widespread human exposure to acrylamide. Acrylamide (AA) is formed (amongst others) when certain foods (rich in starch) are prepared at temperatures usually above 120 °C and low moisture. Exposure may also occur through smoking cigarettes. Acrylamide has been classified as a Group 2A carcinogen and therefore, exposure must be as low as possible. In addition, it can cause neurotoxicity. In the last years, several measures have been undertaken to reduce the exposure to acrylamide. Determination of AA and glycidamide (a metabolite of AA) protein adducts, measured in (spot) urine, is now considered the optimal approach for biomarker purposes for a potential health risk perspective, in view of the development of sensitive and specific analytical approaches for these products. In addition, these protein adducts are also useful as a biomarker of exposure, as the data indicate that approximately 50% of administered dose of AA in humans is consistently excreted in urine as AA protein adduct (5, 7, 8). In addition, **urine biomarkers** may be more suitable for determining the AA intake of consumers than the estimation via the AA contents in food and consumption data (9).

Because this research question revolves around a trend analysis,

historical and recent data are required, preferably every two to five years. Effects on all age groups may be investigated for this purpose and a sample size of about hundred samples is thought to be sufficient (although this is not statistically analyzed yet). Because acrylamide exposure is mainly due to food intake, background information on (specific) dietary habits would be very helpful to investigate which measures have been effective.

2. *A pilot study on the **occurrence of aflatoxin B1** (metabolites) in blood/urine.*

Aflatoxin B1 is a potent genotoxic carcinogen, which can cause liver cancer. Only a (theoretical) zero level of exposure will result in no risk. As applies for acrylamide, exposure must therefore be as low as possible. Humans may be exposed to aflatoxin B1 through consumption of contaminated peanuts, nuts, spices and figs (6). A pilot study on the occurrence of aflatoxin B1 in **urine** may provide a first indication of the actual prevalence of aflatoxin B1 exposure in the Dutch population and if further measures are necessary to reduce aflatoxin intake. Aflatoxin B1 forms adducts with albumin, which may be used as biomarker. In addition, the major aflatoxin DNA adduct, aflatoxin N7-guanine (AFB-N7-guanine), in urine may serve as biomarker (10). However, aflatoxin B1 can also be analyzed by LC-MS/MS (11, 12). For this purpose, a sample size of hundred samples is thought to be sufficient (although this is not statistically analyzed yet). **Recent information from all age groups** will be required, to provide an overall picture for the entire population. A **single time point** is sufficient. Background information on age, sex and dietary habits are needed.

3. *A **health impact assessment** of recent and future **cadmium** exposure.*

It is known that cadmium exposure can result in chronic kidney disease. Since current dietary intake assessments indicate that in some populations the intake exceeds the health based guidance value (HBGV), it may be expected that in these individuals adverse kidney effects may occur, depending on the margin of exposure (MoE). Early kidney effects may be found by measuring specific effect biomarkers such as the glomerular filtration rate (**GFR**), **B2-microglobulin, retinal binding protein or microalbumin**. If data on these effect measures are already available, this would be an advantage. To correlate these effects to cadmium body burden, also the **cadmium levels in urine** should be monitored (4). For this purpose, the sample size (number of volunteers) should be much larger than for questions 1 and 2 (exact numbers should be statistically analyzed), since it is not expected that (preclinical) kidney effects due to cadmium exposure will be found in a large percentage of the studied group. For the same reason, only samples from adults are needed. Only **recent and future samples** are needed. It is therefore necessary that the epidemiologic study to be used is still ongoing. Background data on age, sex, urbanization and dietary and smoking habits would be helpful, because this may help to assign kidney effects (due to cadmium) to a specific group of individuals.

4. *Evaluation of the recent **dietary intake assessment of lead**.* People may be exposed to lead via food, but also, to a minor extent, via other routes, such as inhalation of dust. The estimation of lead exposure by the dietary intake assessment probably provides a good estimation of the lead body burden. This can be evaluated by monitoring blood lead levels. For a good comparison of the lead exposure estimated by dietary intake assessment and biomonitoring, samples taken in the same period of the dietary intake assessment are needed, preferably from the same group of participants, however, another representative population could be used as second best. The most recent dietary intake assessment is performed in 2016, therefore, only **recent samples** are needed. For this particular research question, there is no need for historical or future data and samples from about hundreds of individuals is thought to be sufficient (although this is not statistically analyzed yet). For a more detailed evaluation, data on age and sex of the individuals, as well as on dietary habits and some biological factors (such as body weight) are required. The best biomarker for lead exposure is the concentration of **lead** itself in blood. **Whole blood or erythrocytes** should be used.

Table 1. Requirements of the epidemiologic study characteristics needed for the (illustrative) research questions related to the biomonitoring of the selected chemical contaminants (acrylamide, aflatoxin B1, cadmium and lead).

	Acrylamide	Aflatoxin B1	Cadmium	Lead
Historical data	yes	not necessary	no	no
Recent data	yes	yes	yes	yes
Future data	to be determined	no	yes	no
Ages	all	all	adults	adults
Number of participants	hundreds	hundreds	thousands	hundreds
Frequency data collection	every two-five years	once	every two-five years	once

Table 2. Data from the epidemiologic study needed for the (illustrative) research questions related to the biomonitoring of the selected chemical contaminants.

	Acrylamide	Aflatoxin B1	Cadmium	Lead
Background participants	age/sex	age/sex	age/sex/region/urbanization	age/sex
Lifestyle aspects	diet ^a	diet ^a	diet ^a /smoking ^b	diet ^a
Biological aspects	no	no	yes	yes
Biomarkers already measured in blood	no	Albumin	Cystatin C	no
Biomarkers already measured in urine	no	no	GFR	No
Other biomarkers to be measured	AA-Hb adducts	Protein adducts of Aflatoxin B1	β2-microglobulin retinal binding protein microalbumin + cadmium itself	lead itself
Information chronic diseases	no	no	Chronic kidney disease	no

^a consumption data (preferably quantitatively) of food ingredients having a relevant contribution to the daily contaminant exposure

^b data (preferably quantitatively) of the amount of tobacco (number of cigarettes) smoked per day

Table 3. Samples needed for the (illustrative) research questions related to the biomonitoring of the selected chemical contaminants.

	Acrylamide	Aflatoxin B1	Cadmium	Lead
Whole blood				+
Buffycoat				
Erythrocytes				+
EDTA plasma				
NaF plasma				
Citrate plasma				
Serum				
Urine 24h			+	
Spot urine	+	+		
Feces				
Hair				
Breastmilk				
DNA				

3 Potential studies to be used for biomonitoring

In The Netherlands, a number of epidemiologic studies is being, or has been performed. In these studies, a lot of information is gathered on several characteristics of different populations, all with a different purpose. In many cases, also biological samples are collected and stored. Such stored samples might also be useful for our purpose: biomonitoring of harmful substances to study external and internal exposure.

Some of these studies have been carried out by or in collaboration with the RIVM, like the Doetinchem Cohort Study (13, 14), the PIAMA research project (15, 16) and 'the Netherlands measured' (NL de Maat Genomen) (17, 18). Another epidemiologic study, aimed on healthy ageing, is the Lifelines cohort in Groningen (19, 20). Below, these four studies are described. Some examples of other Dutch cohort studies that are not included in this report are the ERGO study (21), the LASA study (22) and the Zutphen Elderly Study (all with elderly) (23), Generation R (with children) (24) and the Amsterdam Growth and Health Survey (a long-term study that started with teenagers) (25). These cohort studies were not included because the involvement of the RIVM and consequently current data access was limited.

3.1 Doetinchem Cohort Study

The purpose of the Doetinchem Cohort Study is to gain insight into trends in lifestyle and health. The main goal is to understand why some people get older without many problems, while others have health problems at an early age. The knowledge obtained from this study, forms the basis for research and advice in the field of public health policy.

The Doetinchem Cohort Study started in 1987 and tracks the health of a group of Dutch adults randomly selected from the Dutch city of Doetinchem. At the start of the study, the participants were between 20-60 years. In the first round, the study provided information of four age groups: 20-29, 30-39, 40-49 and 50-60 year olds.

Every 5 years an investigation of the health and habits of the participants is performed at the local public health service. In the first round, 12,500 people participated (response rate 62%). Data were collected on lifestyle, biological factors, quality of life, physical function, social aspects, chronic diseases and background variables.

Blood and urine samples are obtained from all participants in the Doetinchem Cohort Study during the sequential rounds. The samples are partly used for a number of analyses, such as cholesterol and glucose. Another part is stored for future research. The Doetinchem cohort study is still running. Meanwhile, participants are between 45 and 85 years old and the number of participants has decreased to less than 4500 (13, 14). An application for use and analysis of these samples can be made at the Scientific Advisory Group of the RIVM Centre for Nutrition, Prevention and Health Services (VPZ).

3.2 **PIAMA Cohort**

The Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study was set up with two main objectives: 1) To study the development of asthma and respiratory allergies in the first 8 years of life and monitoring the effect of various risk factors on the development of asthma and respiratory allergies (Natural History Study); 2) To determine whether the use of allergen-proof mattress and pillow covers can prevent the development of asthma and respiratory allergies in young children (intervention study). Only children of allergic mothers participated in the intervention study, because they have the greatest risk to also become allergic themselves. For the Natural History Study, children of both allergic and non-allergic mothers were included. The intervention and the natural history part were integrated into one study: the PIAMA birth cohort study.

The baseline group consisted of 3,963 children born in 1996-1997. Parents of participating children received an annual questionnaire by mail. In addition to the questionnaires for some of the children home visits were made at multiple time points where dust samples were taken from the children's mattresses and floors. Physical examination of some of the children took place at the age of one year, 4 years, 8 years and at the age of 12 years. These medical examinations included blood sampling for allergy testing from both children and parents. Samples of the PIAMA research are stored and can be used for research into asthma and other chronic diseases after request.

After eight years, the study groups of the PIAMA research were joined for a follow-up study at age 11/12, 14-16 and 18 years. This follow-up study consisted of 3 rounds of questionnaires (at 11, 14 and 18 years of age) and 2 rounds of medical examination, including collection of blood samples (at 12 and 16 years of age). Not just asthma and respiratory allergies are monitored, but also other chronic diseases, which increasingly occur in children, such as diabetes (15, 16).

3.3 **The Netherlands measured (NL de maat genomen)**

Commissioned by the ministry of Health, Welfare and Sport, a monitoring study was conducted in 2009-2010 to study the prevalence of (abdominal) overweight/obesity, (parts of) the metabolic syndrome, and undiagnosed diabetes in the general population. The study was called 'Nederland de maat genomen' (The Netherlands measured), abbreviated 'NL de Maat'.

The first phase of the research was conducted among men and women aged 18-70 years from Tilburg, Vlaardingen, Oudewater, Groningen and Haarlemmermeer. Due to a low response in the 18-30 years group, in Phase 2 of the research a more intensive approach was chosen and included only men and women aged 30-70 years living in the cities Amersfoort and Alkmaar. Participants filled out a questionnaire at home, and visited the research centre where height, weight, waist and hip circumference and blood pressure were measured. In addition, blood samples were obtained to determine cholesterol and glucose levels. Residual samples are stored. A total of 250 men and 398 women aged 18-29 years participated, and 1,806 men and 2,059 women aged 30-70 years (17, 18).

3.4 Lifelines

Lifelines is a multidimensional population-based cohort study and biobank. The main goal of Lifelines is to find breakthroughs in the screening, prevention, diagnosis, and treatment of (chronic) diseases as well as in the understanding of the etiology of diseases. It aims to facilitate research in the field of Healthy Ageing, ultimately resulting in an increase in healthy life years. The scientific starting point of Lifelines is that the development of chronic diseases such as asthma, diabetes or kidney disease is a complex interplay of factors.

The cohort study started in 2006. Since 2013, recruitment has stopped, and currently follow up data is being gathered. 167,729 individuals (children and adults) of the northern part of the Netherlands will be followed in a family design over a minimal period of 30 years. Every five years participants are physically examined and biomaterials are collected. In addition, every 1.5 year, participants are invited to complete a questionnaire. Lifelines includes data on environmental exposures, (epi)genetics, psychological and social factors, as well as data on health care use to cover societal impact.

All collected data and samples are available to scientific researchers worldwide. Through submitting a research proposal to the Research Office, scientists can apply for access to Lifelines data or samples. Researchers can request existing data and samples for bioanalysis, or can indicate if they want to perform an add-on study, in which additional data or biomaterial is prospectively collected from a selected group of participants. All proposals are reviewed by Lifelines. Data and samples from the Lifelines databank are only available for research into healthy ageing (19, 20).

3.5 Comparison of epidemiologic studies

The studies differ in size and age groups of the population (see table 4). In the PIAMA cohort, only children (aged 0 at the start of the project and followed up until 18 years) are included, whereas the Doetinchem Cohort Study and NL de Maat only contain data from adults. Lifelines, on the other hand, contains data from all age groups.

Table 4. Overview of characteristics of the described epidemiologic studies (Doetinchem, PIAMA, NL de Maat and Lifelines)

	Doetinchem	PIAMA	NL de Maat	Lifelines
Start date	1987	1996	2009	2006
Still running	Yes	No	No	Yes
Ages	20-60 ^a 45-85 ^b	0 ^a 18 ^c	18-70	all ages
Number of participants	12,500 ^a <4,500 ^b	3,963 ^a 2,984 ^c	4,513	167,729
Frequency data collection^d	Every 5 year: Q, PE, SC	Yearly: Q Every 4 years: PE and SC	Once: Q, PE and SC	Every 1.5 year: Q Every 5 years: PE and SC

^a at baseline

^b at current round

^c at last round

^d Q: questionnaire; PE: physical examination; SC: sample collection

Two of the cohorts (Doetinchem and Lifelines) are still running; data from biological materials are collected every 5 years (see table 4). The fact that these studies are still ongoing means that it is possible to include additional questions or samples to the study, provided that the Scientific Board of the study agrees.

The last data collection in the PIAMA study was in 2014/2015, when the children were 18 years old. It is not yet decided if the study will continue. In NL de Maat, data were collected only once.

In all studies similar information on the background of participants is collected (see table 5), such as age and education level. The same accounts for information on lifestyle factors (such as diet and smoking habits). Nevertheless, there are some differences. For example, in the PIAMA study and Lifelines, information on the physical environment and sleep is included (for detailed information, see Annex I, tables i and ii).

Biological aspects are also measured in all studies. Data on body weight, height and blood pressure are present for all studies. Other biological factors are measured only in some of the studies: In NL de Maat no information is present on cognitive function and lung function and only Lifelines performed an ECG. More details can be found in Annex I, table iii.

Table 5. Overview of available data of the described epidemiologic studies (from no (-) to many (+++) data).

	Doetinchem	PIAMA	NL de Maat	Lifelines
Background participants^a	++	++	++	++
Lifestyle aspects^b	++	+	++	++
Biological aspects^c	+++	++	+	+++
Biomaterials collected^d	+	++	+	+++
Biomarkers measured in blood^e	++	+	+	+++
Biomarkers measured in urine^f	+	-	-	+
Information on chronic diseases^g	+++	+	++	++

^a such as age, education level. See for more details Annex I, table i.

^b such as nutrition, smoking, psychosocial parameters. See for more details Annex I, table ii.

^c such as body weight, blood pressure. See for more details Annex I, table iii.

^d See for more details Table 3.

^e See for more details Annex I, table iv.

^f See for more details Annex I, table v.

^g See for more details Annex I, table vi.

Blood samples are collected in all studies, although the way that plasma is collected is not the same in all studies (in EDTA tubes, in NaF or citrate tubes). It may also differ whether blood samples are collected under fasting or fed conditions, however, this was not further analysed for this report. Also the protocol for urine collection varies: spot (or even more specified early morning spot) urine versus 24h urine. In addition to these common samples (serum, plasma and urine), in the PIAMA study and a recent substudy of Lifelines breastmilk of the mothers of the participating babies was collected and in Lifelines also samples of faeces and hair are stored (see table 6).

Table 6. Overview of biomaterial collection (yes=+ and no=-) of the described epidemiologic studies

	Doetinchem	PIAMA	NL de Maat	Lifelines
Whole blood	-	+	+	+/- ^a
Buffyc Coat	+	+	+	+
Erythrocytes	+	+	+	+
EDTA plasma	+	+	+	+
NaF plasma	+	+	+	+
Citrate plasma	+			+
Serum	+	+	+	+
Urine 24h	-	-	-	+
Spot urine	+	-	-	+ ^b
Feces	-	-	-	+
Hair	-	-	-	+
Breastmilk	-	+	-	-/+ ^c
DNA	+	+	+	+
Cord blood				-/+ ^c

^a Whole blood samples are only collected at the start of Lifelines

^b Early morning sample

^c Breastmilk and cord blood are collected in a recently started subpart of Lifelines: Lifelines NEXT

Some of the biological samples are already used for clinical-chemical analysis necessary for the respective studies (see table 2). Glucose and cholesterol are measured in all studies. Other analyses differ. By far the most analyses have been performed in Lifelines (see also Annex I, tables iv and v). Results of these analyses may also be useful in additional (biomonitoring) studies, depending on the exact research question.

Finally, all studies have gathered, in varying degrees, information on the presence of chronic diseases of the participants (see table 2 and annex I). Information on asthma and diabetes is present in all studies. In addition, the PIAMA study contains information on allergy of the airways. The other studies contain information on the incidence of cardiovascular diseases, migraine and cancer. Moreover, the Doetinchem Cohort Study also contains information on arthrosis, musculoskeletal complaints, incontinence, depression and oral health (see also Annex I, table vi).

4 Comparison of requirements for biomonitoring and properties of epidemiologic studies

Comparing the requirements tables (tables 1-3) to the characteristics of the epidemiologic studies (tables 4-6), it becomes clear that for some features there is an overlap with multiple studies (for example information on sex, age and waist and hip circumference), but for some features there is no information in either of the studies or only in 1 of the studies (for example information on subcutaneous fat tissue or ECG). Whereas in some cases, this may narrow down the studies that may be used for your biomonitoring question, because it is information on a 'hard' requirement (such as the fluid sample needed for analysis), in other cases it may not, because it regards information that is only preferred as an extra (such as information that may give you more information on certain subpopulations, while the principal question is about the whole population) or in the case of information that can be obtained at a later stage (such as information on specific biomarkers). In many cases, the sample needed for analysis of the biomarker in question is the first hard requirement to look at (except in cases where only future data are needed and extra samples may be included in the study). Other hard requirements may be the necessity of multiple time points, recent data, or population sizes. For example, since for all 4 illustrative research questions recent data are needed, the PIAMA study and The Netherlands measured do not meet the requirements. It must be noted that for other biomonitoring purposes, this conclusion might be different.

The specific conclusions for each illustrative biomonitoring question are given below.

1. A retrospective **trend analysis** to evaluate the measures that have been undertaken in the last years to reduce **acrylamide** levels in food.
Acrylamide biomarkers can be measured in spot urine samples, which are collected in the Doetinchem cohort and Lifelines studies. For this question, samples from various time points are required. Both the Doetinchem Cohort study and Lifelines have multiple rounds. The (detailed) differences on the information that has been collected on dietary habits may result in a conclusion which study fits best. In addition, the availability of left over urine samples may help to choose the best suitable study.
2. A pilot study on the **occurrence of aflatoxin B1** (metabolites) in blood/urine.
Aflatoxin B1 can be measured in urine, preferably in an early morning sample, since this is the most concentrated urine. Only in Lifelines, early morning samples are collected. Therefore, Lifelines would be the preferred study for this question. In addition, recent information is required that may come from all age groups. Lifelines also meets these requirements. In addition, since Lifelines has collected data on food and nutrition via an extensive FFQ, this may help to identify specific food items

responsible for aflatoxin B1 exposure, if positive samples are found.

3. **A health impact assessment of recent and future cadmium exposure.**

For cadmium, information on kidney disease would be helpful. This is only available in NL de Maat and Lifelines. However, NL de Maat does not meet the required recent and future data samples but Lifelines does. In addition, 24 h urine samples are required. Such samples are only collected in Lifelines. Therefore, also for this question, Lifelines seems to be the best suitable study.

4. **Evaluation of the recent dietary intake assessment of lead.**

For question 4, whole blood samples or erythrocytes are needed. Erythrocytes are collected in all studies included in this report. However, since recent data are needed, the PIAMA study and NL de Maat drop out. The Doetinchem Cohort study or Lifelines appear to be the most suitable studies. Both studies have (to some extent) collected information on dietary habits. However, it still depends on the exact demands on the blood samples necessary for an adequate analysis of the biomarkers (temperature of storage, age and volume of sample, etc.) if one of those studies can be used.

5 Conclusions

All epidemiologic studies described in this report have stored (remaining) biological samples that may be useful for biomonitoring studies. Obviously, but not to be forgotten, the volume of the samples is limited and may decrease over time, due to additional analyses. In addition, the quality of the samples (in terms of chemical stability) may decrease over time. It should therefore always be checked whether the amount and quality of the available sample is sufficient for the research question.

There are various differences between the epidemiologic studies. Which study will be most suitable will always depend on not only the contaminant in question, but also on the specific research question. An adequate comparison of requirements and epidemiologic studies should therefore be performed for each question on a case by case basis. The information provided in this report may serve as an approach on how to choose the best study. In addition, it is advised to use a multidisciplinary team when investigating which study should be used, including both epidemiologists (knowledge on the epidemiologic studies) and risk assessors (knowledge on toxicology and risk assessment).

Since the RIVM coordinates some of the described studies or participates in them, lines are short and it is probably relatively easy to use remaining samples from these studies for biomonitoring research, as long as the research question is related to healthy ageing and informed consent has been given by the volunteers. An advantage is that it is exactly known which background information is available from the participants, so that this can be taken into account in the interpretation of the biomonitoring data that can be obtained.

Although the selected harmful substances and their illustrative purposes for biomonitoring only serve as an example, some general conclusions can be drawn. Nevertheless, one should keep in mind that the so-called 'hard' or specific requirements (like important information on food consumption, nature of biological sample, etc.) in the end may be decisive. All epidemiologic studies described in this report are, more or less, suitable for biomonitoring studies. However, the characteristics (size, samples stored, background information) differ and this will determine, together with the contaminant of interest *and* the specific research question, which cohort should be selected for future biomonitoring studies. In addition, ongoing cohort studies like the Doetinchem Cohort Study and Lifelines offer the possibility that future sampling and collection of information may be adjusted to specific needs related to (improve) the biomonitoring of harmful substances.

With respect to the research question one should keep in mind that biomonitoring of harmful substances can be used for many different purposes, such as the determination of exposure, determination of health effect, population screening to identify people at risk for developing a specific disease in an early stage, or trend analysis, for example for assessing the efficiency of preventive measures.

If the conclusion for a specific research question is that none of the epidemiologic studies described in this report meet the requirements, it should be noted that there are many other (ongoing) epidemiologic studies in The Netherlands, which might be useful.

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Annex I. Overview of specific properties of the epidemiologic studies relevant for the biomonitoring of contaminants

Table i. Overview of background aspects included in the described studies

	Doetinchem	PIAMA	NL de Maat	Lifelines
Age	20-60	0-18	18-70	0-93
Sex	+	+	+	+
Marital status	+	-	+	+
Education level	+	+	+	+
Employment status	+	+	+	+
Region	-	+	+	+
Urbanization	-	+	+	+
Ethnicity	+	+	+	+
Medical background	+	+	+	+
Religion	-	-	-	-

Table ii. Overview of lifestyle aspects included in the described studies

	Doetinchem	PIAMA	NL de Maat	Lifelines
Food/nutrition	+	+	+ (limited, no FFQ)	+ (FFQ)
Physical activity	+	+	+	+
Physical labor	+ in round 2	-	-	+
Smoking	+	+ (passive)	+	+
Alcohol use	+	+	+	+
Drug use		+	-	-
Medicine use	+	+	-	+
Supplement use	+	+	-	?
Sleep	-	+	-	+
Stress	+	+	+	+
Psychosocial parameters	+	+	+	+
Quality of life	+	+	+	+
Physical environment ^a	-	+	-	+

^a Type of floor covering at home, domestic animals

Table iii. Overview of biological aspects included in the described studies

	Doetinchem	PIAMA	NL de Maat	Lifelines
Body weight	+	+	+	+
Body height	+	+	+	+
Subcutane fat tissue	-	+	-	-
Hip circumference	+	+	+	+
Waist circumference	+	+	+	+
Vascular elasticity	-	+	+	-
Blood pressure	+	+	+	+
Heart frequency	+	+	-	+

Lung function	+	+	-	+
Physical function	+	-	-	
Cognitive function	+	+	-	+
Psychological	+	+	-	+
Hearing	+	+	-	-
Vision	+	+	-	-
Bone health	+	-	-	-
ECG ^a	-	-	-	+
human skin autofluorescence (AGE ^b)	-	-	-	+

^a ECG = electrocardiogram

^b AGE = advanced glycation end-products

Table iv. Overview of biomarkers measured in blood in the described studies

	Doetinchem	PIAMA	NL de Maat	Lifelines
Glucose	+	+	+	+
Hemoglobin	-		-	+
Cholesterol	+	+	+	+
HDL	+	+	+	+
LDL	-	+	-	+
Ureum	-	-	-	+
Ureic acid	+	-	-	+
Hematocrit	-	-	-	+
Monocytes	-	-	-	+
Trombocytes	-	-	-	+
Leukocytes/lymphocytes	+	+	-	+
Erythrocytes	+	+	-	+
Mononuclear cells	-	-	-	+
Electrolytes	-	-	-	+
Hormones	-	-	-	+
Proteins	-	-	-	+
Nutrients	-	+	+	+
HbA1c	+	+	+	+
Triglycerides	+	-	-	+
Lipids	+	+	+	
ALAT	+	-	-	+
ASAT	+	-	-	+
Albumin	+	-	-	+
Creatinine	+	-	+	+
granulocyt	-	+	-	+
Alkaline phosphate	-	-	-	+
TSH	-	-	-	+
Sodium	-	-	-	+
Potassium	-	-	-	+
Calcium	-	-	-	+
Phosphate	-	-	-	+
Free T4	-	-	-	+
Free T3	-	-	-	+
Anti-ds DNA	-	-	-	+
Anti CCP	-	-	-	+

CTD screen	-	-	-	+
Gamma-GT	+	-	-	+
CRP hs CRP	+	-	-	+
CRPH hs CRPH	-	-	-	+
LCRP hs CRP	-	-	-	+
HAL1 Apolipo A1	-	-	-	+
ALPA Apolipo A1	-	-	-	+
HALB Apolipo B100	-	-	-	+
AFPB Apolipo B100	-	-	-	+

Table v. Overview of biomarkers measured in urine in the described studies

	Doetinchem	PIAMA	NL de Maat	Lifelines
Albumin 24-hrs	-	-	-	+
Albumin morning	+	-	-	+
Albumin timed overnight	-	-	-	+
Creatinine 24-hrs	-	-	-	+
Creatinine morning	+	-	-	+
Creatinine timed overnight	-	-	-	+
Leukocytes	+	-	-	-
Nitrite	+	-	-	-

Table vi. Overview of chronic disease prevalence in the described studies

	Doetinchem	PIAMA	NL de Maat	Lifelines
Diabetes	+	+	+	+
Cardiovascular diseases	+	+	+	+
Migrain	+	+	+	+
Cancer	+	-	+	+
Asthma/COPD	+	+	+	+
Allergy of the airways	+	+	-	
arthrosis	+	-	+	
Musculoskeletal complaints	+	-	-	
Incontinence	+	-	-	
Depression	+	+	-	±
Oral health	+	-	-	
Kidney disease	-	-	+	±
Metabolic syndrome			+	±

