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Appraisal of strategies

to

monitor (population) folate status

with respect to

the prevention and scientific study

of

congenital anomalies

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Contents

Summary

This study focuses on an appraisal of the quality, relevance and feasibility of the monitoring of the folate status of (pre)pregnant women. The reason for this study is that such monitoring can support health policy as well as scientific investigations concerning folate intake and e.g. birth defects.

The study comprised the construction of a framework for the appraisal, the collection and study of relevant sources of information and finally the appraisal itself of a variety of folate status monitoring scenario's, using the framework and the information collected.

Methods to monitor folate status by assessing intake and by assessing biomarkers were evaluated with respect to the type of folate intake measured, the quantitative estimates used, the subgroups studied, the validity of the methods and the way data are collected. Folate intake is mainly assessed by self-report using self-to-fill-in questionnaires. A sufficiently validated instrument for self-reported intake that might be used for long-term and broadly folate status monitoring does not seem to exist. Commonly used biomarkers are red blood cell folate and plasma folate. These markers can be validly assessed , but their relation with exposures or interventions to be monitored is not clear yet.
A variety of possibly relevant monitoring scenario´s was set up by varying six core characteristics: the estimated factor; the subjects measured;the measurement points/periods; the sampling scheme; the level of analysis; the scope . Each scenario defines a specific way of data collection resulting in a specific dataset. The relevance of the scenario is based on the usefulness of that dataset. The usefullness of the datasets is assessed for different purposes, namely for explanatory scientific research, for the development and evaluation of policy to promote folic acid supplement use and for individual patient care.

Three scenario's are recommended to include in the further analysis. The most relevant one would permanently measure one or more biomarkers of folate status in all pregnancies. It is useful for explanatory case-control studies (and cohort and ecologic studies as well) and also for the development and evaluation of FA-supplementation policy. The second most relevant scenario would also make use of biomarkers but only collected in representative samples of e.g. 1 in 100 pregnancies. The datasets could be used for explanatory ecologic studies of pregnancy outcomes as well as for FA-supplementation policy. And the third most relevant scenario would collect data about folic acid supplement intake in the periconceptional period based on retrospective self-reports.These data can be used for ecologic trend studies and for the purposes of FA-supplementation policy.

The feasibility of the implementation of folate monitoring is studied with respect to the fulfillment of some conditions that would precede actual implementation. These conditions are in the domains: quality of the measurement methods; organisational embedment of data collection; sample and data collection competencies; legal and ethical regulations; cost-benefit expectations. No insurmountable hindrances for the implementation of folate intake monitoring

through self-report methods seem to exist. The same applies to the implementation of a biomarker monitoring, but the compliance with the requirements dictated by legal regulations might cause a considerable hurdle.

It is finally recommended to proceed with two solutions:

A. folate intake monitoring, either through questionnaires or anonymized biomarker measurements, for the purpose of FA-supplementation policy development and evaluation;
B. non-anonymous (regional) census biomarker monitoring for the purpose of explanatory scientific research of birth defects and pregnancy related disorders.

These solutions serve clearly distinct purposes and could both be implemented via separate paths and in different regions. Solution A is furthermore relatively simple and cost-effective if integrated in FA-supplementation policy. Solution B is technically more complex but feasible if integrated in existing procedures of blood sampling in pregnancy. Future work on these solutions should be directed to the further ascertainment of the feasibility and to the exploration of the commitment of decisive stakeholders.

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1 Introduction

The initial reason for this report stems from a generally felt demand for valid and up to date information about the level of implementation of periconceptional folic acid supplementation. The survey based folic acid monitor in The Northern Netherlands is until now the only source in Europe (or even world wide) that provides longitudinal data (1996-2009) on FA-supplementation Zetstra- van der Woude, 2012[1]. However, it is regional (not representative for NL-wide situation), the validity is limited and permanent sources to keep the monitor going are lacking.

An imaginable alternative monitor might be based on biomarkers assessed in blood samples that are already taken at the first prenatal visit for the purpose of prenatal screening on certain infectious diseases and the immune status of the pregnant woman. To further explore this opportunity and prepare a pilot, an initial plan was submitted to be included in the EUROCAT Joint Action 2011-2013 in WP 7- Primary Prevention of congenital anomalies (see Appendix III.1). The plan has thereafter been adapted to the broader scope of "the appraisal of strategies to monitor (population) folate status" (see Appendix III.2). The appraisal comprises data collection using questionnaire surveys and/or biomarkers and the relevance of monitoring scenario's for (explanatory) scientific studies, for policy making and evaluation and for individual care.

The results of the study are presented in this report. The work that had to be done comprised three major domains:

i. the creation of a framework/method for a systematic appraisal;
ii. the collection, study and description of the relevant sources of information and
iii. the appraisal of the scenario's for folate monitoring itself.

This work is in full described in the appendices of this report. The main part of the report gives a general description of the work and its results, referring to the appendices for the details.

2 Methods to assess folate intake and status

2.1 Methods to assess (self reported) *intake* of sources of natural food folates and of folic acid

Several methods of folate intake assessment exist and their use is reported in the literature. With respect to the appraisal of the usefulness of the specific (combinations of elements of) methods, the following core characteristics were described:

- type of folate intake measured;
- the quantitative estimates used;
- subgroups studies;
- validity issues;
- types of data collection.

This list of characteristics is based on the methodological expertise and pre-existing knowledge of the author about methods to assess folate intake. An extensive aforegoing methodological analysis of all relevant characteristics of intake and biomarker monitoring was not done. Chapter 1 in Appendix I lists the detail aspects of the characteristics.

A convenience selection of examples and evaluations of methods to be reported was made, again based on the knowledge of the author about existing and reported assessment methods used, and the exploration of relevant references in these studies. An extensive search and retrieval of all possibly relevant published studies in which assessments were reported was considered not to yield essential new information for the purpose of this appraisal study. The selection of studies is furthermore restricted to studies that were thought to be informative about the assessment method as such. Studies in which these methods were merely applied, and not explicitly evaluated, are therefore not included[a].

Prospective distribution and/or counting of FA-pills is a good method for the assessment of intake. Variants of this method have been or are being applied in intervention studies e.g.[2 3 4 5]. The feasibility for purposes of population monitoring however is at forehand very low and this method is therefore not discussed. Also not included is the (theoretical) possibility of estimating folate intake using data about sales and/or distribution of sources of folic acid (which might e.g. be possible if distribution of FA-supplements would be restricted to pharmacies).

Table I.1 in Appendix I contains an overview of the selected methods and a description of their methodological characteristics. These findings are discussed in the next chapter.

[a] In follow-up work of this report it might be worth to do a search to find all (explanatory scientific) studies that have applied a method of self-reported intake to assess the folate exposure. Such a study might be a source for relevant information about the comparability of estimates between studies and the need for and feasibility of standardization of methods for the sake of the quality of scientific research.

2.2 Methods to assess folate status using biomarkers

Several biomarkers that give an indication of the 'folate status' exist as well as several methods to measure them. Based on the expertise of the author a list of characteristics thought to be relevant to include in the appraisal of the usefulness for folate monitoring, was made. Chapter 3 in Appendix I contains a description of the characteristics.

A convenience search of the literature about biomarkers assessment was done. Special attention was given to methods that were used in/developed for use at larger scale.

Table I.2 in Appendix I contains an overview of the selected biomarkers and their assessment methods and a description of their methodological characteristics. The findings are discussed in the next chapter.

3 Discussion of validity and reliability aspects of methods for measuring folate intake and folate status

3.1 Methods to measure folate intake

Studies on the relationship between both prospectively and retrospectively self-reported intake of FA-supplements on the one hand and the actual intake at the other hand, which would give the best information on validity and reliability, seem to be lacking completely.

The relationship between self-reported intake food folate and/or of supplements and folate status has been documented in four studies [6] [7] [8] [9]. Further insight in the interpretation could be gained by comparing these findings with studies that document the correlation between controlled FA-intake and serum or RBC levels (e.g. [10] [11] [12]).

With respect to the measurement of periconceptional and prenatal intake of folates through self-report it must be concluded that there is not sufficient (hardly any) empirical information that would make it possible to assess the validity and the reliability of the estimate for the actual intake of FA-supplements.

Statements about the validity and reliability can only be based now on the demonstration of some concurrent validity of the instruments and the face-validity of the construction of those instruments. Support for concurrent validity comes from the studies that show a relationship with folate status [6] [7] [8] [9], socioeconomic status[1] and expected Time-To-Pregnancy[13]. Those three characteristics are known to be (folate status) or very likely (SES, TTP) related to folate intake. Because no (informative) reports about the construction process and/or evaluation of the final questionnaires have been found so far, only face validity remains as a source of information (i.e. the impression an expert has about the quality of the questionnaire).

The information mentioned above pertains to self-to-fill-in written questionnaires. Self-report is also measured by verbal methods, e.g. during consultations with pregnant women and documented in the patient file. No evaluation of validity or reliability of these methods have been found and this method therefore is not evaluated separately. There is no reason to assume that these methods would have better reliability or validity than self-to-fill-in questionnaires.[b]

Conclusion: a sufficiently validated instrument for self-reported intake that might be used for long-term and broadly distributed (in several regions/countries in Europe) folate status monitoring does not exist at this time.

[b] Based on personal information of practitioners about existing procedures in The Netherlands.

3.2 Methods to measure biomarkers

Whereas measurement methods for folate intake have a relatively unequivocal reference for their validation, i.e. how well the estimates resemble the intake of supplements and food folate, this is not the case for biomarkers. Biomarkers from the mother's blood could represent (or better: be used in analyses as to represent)

i. levels of recent or of less recent intake of folates and/or could represent
ii. the exposure of the 'mother's own organism' to circulating folate and its relation to e.g. placental functioning and/or
iii. the exposure of the maternal-fetal exchange environment, as an indirect measure of folate levels in the fetal organism.

For making choices with respect to this issue good information about how well specific biomarkers represent one or more of these 'references' would be helpful. However, there is little applicable published evidence on this. The effect of (changes of intake of) folic acid supplements on biomarkers have been studied[c] (showing increases of average values), but good studies the other way round, i.e. predicting past intake from current biomarker levels, do not exist and the available studies cannot explain more than 25% of the variance [9] [6]. The question whether RBC folate or serum folate or a combination of those would be the best predictor cannot be answered. Concerning the 'exposure' of the mother and the fetus to folate, knowledge about the most plausible biological mechanism or pathway related to the outcome of interest might guide the choice for the most appropriate biomarker. But as different outcomes of interests are relevant, both mother-related (i.e. placental functioning that might be related to pre-eclampsia or preterm birth) and fetus-related (neural tube defects and maybe other comparable closure/growth defects as well) there might not be just one most appropriate marker[d].

The reliability and validity of biomarker measurements depend on both the collection and preservation of the samples and on the quality of the biochemical analyses that are done on these samples. As a rule both domains are well documented for measurements that take place either in (relative) routine care or one-off research settings.

An important source of expertise for the further exploration of biomarkers that might be used in monitoring systems is the NHANES- network. NHANES- has issued a Laboratory Manual Procedure for the purpose of standardized and high qualtiy assessment of folate markers nationwide. In 2011 a round table was organized, also based on reviews of historical and actual methodological issues [14] [15] [16] [17].

[c] Any study on modification of effects on biomarkers by MTFHR-polymorphisms?
[d] If a monitor would also have to provide data for studies on other outcomes (e.g. cancer) in the population of interest this might add even more dimensions to take into account.

Conclusion: valid and reliable methods[e] to assess folate biomarkers in (maternal) blood do exist, but their relation with exposures/interventions to be monitored is not (very well) documented yet.

[e] I.e. valid and reliable with respect to the methods to measure the biomarker as such.

4 Relevance of folate intake and folate status monitoring scenario's

4.1 Introduction

In this study the relevance of different scenario's for folate status monitoring is assessed. A scenario describes a specific way of data collection[f] resulting in a specific dataset. The assessment of the relevance is done through a qualitative evaluation of the usefulness of that resulting dataset.

The scenario's do not describe the implementation of the data collection as such yet. For one and the same scenario different appropriate implementations might be possible. In this assessment the feasibility of the implementation of specific scenario's is not taken into account. The feasibility of some general conditions for adequate implementation of the scenario's are discussed separately in Chapter 6.

Appendix II contains the full description of the method that was used for the inclusion and description of each scenario and the results of the qualitative assessment of its relevance.

This Chapter gives a brief overview of the method used.

4.2 Method of assessment of relevance

The relevance of a folate monitoring scenario is assessed with respect to a pre-defined purpose of the use of the datasets that are produced by that scenario. A scenario is in this study defined by six core characteristics, each of which can take on different values. Variation in these values leads to different scenario's with possible different relevance.

Purposes to be evaluated

The relevance is assessed for the main purposes introduced earlier already. The first is that of explanatory scientific research. Within this domain separate assessments are done for case-control designs, cohort designs and ecologic designs. The second purpose is that of policy development and evaluation for which only comparative designs are considered. And the third purpose for assessment concerns the use for individual patient care.

Scenario characteristics

Each scenario is defined by a number of core characteristics which are briefly introduced here.

Estimated factor- Estimated factor pertains to the empirical phenomenon that is to be estimated with the data collected with a specific instrument e.g. the intake of supplements (dosage, frequency, period, substance) or the

[f] Collection concerns: the measurements and the sample

concentration of folates in red blood cells (type of folates, at point in time or average over period). For each specific factor the validity, reliability and (un)biasedness of the data collected with a given instrument and sampling design are strongly related to the usefulness of these data for different types of study.

Subjects measured- The group of subjects for whom data are collected can be different and the following are considered: women 18-40 yrs from general population; all pregnant women; all ('mothers' of) still and live births. These groups are samples from different study populations. The research questions that can be adressed depend on the characteristics of these study populations.

Measurement period(s)/point(s)- Measurements on folate intake or biomarkers can pertain to different periods or moments in the development of the phenomenon under study (maternal folate levels, fetal development, pregnancy stages). Distinction is made in preconception, periconception and 2nd and 3rd trimester. The measurement period/point is not the same as the period/point in time of data collection (e.g. asking after preconception intake at 1st prenatal visit).

Level of analysis *– The level of analysis can vary from matching of all relevant data on individual level to matching on (e.g. country) population level only.*

Sampling scheme- Variations in sampling rates (1:1 vs e.g.1:1000) and sampling periodicity (a month per year vs e.g permanent) have an impact on the relevance and feasibility.

Scope- The scope, geographically and in time/duration, of scenario's can also vary.

Method of assessment

To assess the relevance of different scenario's for folate intake or biomarkers monitoring the core characteristics of each scenario are described and then an overall judgment of the scenario as a whole is made. The overview of the characteristics and the judgments is given in Table II.1 . Table II.2 and Table II.3

The scenario's are at first judged on their potential to provide appropriate datasets for the adequate study of explanatory relations using the different types of designs (case control, cohort, ecologic; see Table II.1). A summary score is given based on the characteristics of the dataset that the scenario can produce. The following rules are applied.

1. For the sake of completeness and for a transparent documentation of the appraisal process each reasonably imaginable scenario that could probably provide appropriate data sets is judged.
2. The judgment is qualitative and leads to one of the following scores: 1. Limited relevance – 2. Moderate or (potentially high) relevance 3. High relevance

In the next step an appraisal was made in the same manner for the use in policy making and evaluation (Table II.2). Finally an appraisal was made for the application in individual care of the data collected (Table II.3).

5 Preliminary selection of the most relevant folate intake and status monitoring scenario's

The selection that is presented hereafter, is made without taking into account feasibility and is restricted to scenario's that are primarily relevant for the domain of reproduction.

1. The most relevant scenario would permanently measure one or more biomarkers of folate status in all pregnancies (see Table II.1 and II.2 the scenario's #a.01.). This scenario would yield valid data about maternal folate status which could be used in explanatory case-control studies (and cohort and ecologic studies as well) and also data that would validly reflect changes in folate and folic acid intake to be used in the development and evaluation of policy to promote periconceptional FA-supplementation.

2. The second most relevant scenario would also make use of biomarkers, which would be collected in representative samples of e.g. 1 in 100 pregnancies (see Table II.1 #c. … and Table II.2 # a.07). This scenario would provide valid data for explanatory ecologic studies of pregnancy outcomes as well as for the afore mentioned purposes of policy development and evaluation.

3. The third most relevant scenario would collect data about folic acid supplement intake in the periconceptional period based on retrospective self-reports (see Table II.1. #c.01 and Table II.2 #a.01). These data can be used for ecologic trend studies and for the purposes of policy development and evaluation. The estimates that are based on self-report have low validity and/or their validity is unkown with respect to the level of actual individual intake, but the data do (very probably) validly reflect average changes over time within (sub)populations[g].

For none of the above selected scenario's it is possible to assess the relevance of it for individual care, because there is not sufficient evidence to assess the advantage and/or to exclude possible harm (see Appendix II Chapter 3) of providing information about folate status in pregnancy.

The evaluation of the relevancy of the biomarker(s) that could be measured in the scenario's 1. and 2. firstly depends on the relation between folate status and outcomes that would be studied using these biomarkers. In general red blood cell (RBC) folate levels reflect the intake back in time longer and over a longer time period than serum folate levels do. Thus, if both measurements would be done on the same blood sample they would not represent the same moment/period and/or duration of exposure of the embryo and the developing fetus to the maternal folate levels. Secondly, this difference is also relevant with respect to the evaluation of the timely (before conception) intake of folic acid supplements.

[g] The validity of self-report instruments is will vary between cultures and countries and unless, it is proven that this variation does not exist, cross sectional ecologic analyses should not be done.

In scenario 3. the data about folic acid intake could be collected through e.g. self-to-fill in questionnaires or structured face-to-face interviews[h]. The application of such instruments for monitoring purpose, would at least require good comparability of the results over time within (sub)populations. Comparability across cultural differences and across populations from different countries would add value, but it has to be demonstrated still that adequate instruments would meet that criterium, can be developed.

The (perceived) relevance of each of these three scenario's also depends on the objectives of its use in relation to the scope of the monitor. If evaluation of national intake patterns is a requirement that should be met, than 1 in 100 biomarkers assessment representatively distributed over the country would do. If however the study into the role of folate status in the prevention of causation of rare birth defects would be a main objective, than the 1 in 1 collection of biomarkers in as many regions in which the birth defect is centrally registered (e.g EUROCAT-regions) would be more apt.

In closing, what ever instrument and/or scope would be preferable in a specific application of a scenario, the three scenario's presented above represent the most relevant solutions and are the ones to be investigated further with respect to feasibility.

[h] Including e.g. standardized questions posed during consultations and registered by the caregiver in the patient file.

6 General conditions for the feasibility of the implementation of the most relevant folate monitoring scenario's

The selected monitoring scenario's can only create the value that is attributed to them, if they can be *adequately* implemented.

If one wants to assess (at forehand) the feasibility of an implementation[i] of a service/facility, a very complete and detailed insight into what this implementation would comprise, is needed. That insight is not available for these scenario's at this stage yet. However, an assessment of the feasibility of the most important general *conditions* that should be met at forehand of the implementation of the scenario, can already be made and is presented hereafter.

6.1 General conditions

6.1.1 The quality of the measurement methods

- It should at forehand be undisputed that measurement methods are available that will guaranteedly produce data with the needed validity, reliability and meaning. These characteristics must thus meet the requirements of its intended use. i.e. the specific purpose for which the monitor is to be used.
- For biomarkers the validity and reliability in general look rather satisfying, but the (biological) meaning of each of the available markers is not clear cut. It should therefore at least be made clear to stakeholders involved, what the markers can be used for (and what not).
- When self-report-estimates (e.g. written questionnaires) of folate intake would be preferred, (new) validation studies would have to be done and yield satisfying results with respect to the validity and reliability.

6.1.2 Organisational embedment

- The implementation of any monitor scenario would comprise several monitor actions (like taking or processing blood samples or personally handing out questionnaires) that are required to be performed at the right time and place *over and over again for a long period*. Implementing new, dedicated facilities that can secure a lasting and adequate performance of these actions, would make a major challenge c.q. threat for the feasibility of monitor scenario's.
- The feasibility would be greater if monitor actions can be integrated into existing services/facilities, preferably as part of routine actions. E.g. if a biomarker can be measured on blood samples that are already collected in prenatal care for other purposes or questions about folate intake that can be integrated in existing routine questionnaires or interviews.

[i] Implementation means: the introduction ánd the maintenance of 'an activity' including all its prerequisites on structure, process and outcome level.

6.1.3 Sample/data collection competencies

- The collection of samples/data that are collected for research purposes require competencies that might not all be standard among the health care workers that are doing this work and/or are not adequately maintained. This might lead to violation of protocols (e.g. how blood samples are temporarily stored, how interviewing is done) and threaten the validity of the methods and/or lead to e.g. incomplete sampling. The organisational embedment must make it possible to have health care workers doing the data collection, that have, or are at least learnable enough to easily acquire, the competencies needed.

6.1.4 Legal and ethical regulations

The collection, storage and use of the monitoring samples and data should meet the existing applicable regulations. The feasibility of the actual implementation of each aspect of that would not do so, is zero.

- The samples and data about folate intake and/or status that are collected in a monitor are primarily meant to be used for scientific research. For the realisation of the full scope of the research possibilities, the following 'sample/data usage' options are required and should be allowed:
    a. long term centralized storage of samples and data with linkage to the individual persons (mother and maybe also the child);
    b. possibility to link data on individual level to other data of the same individual (e.g. prenatal biomarker to an EUROCAT birth defect case);
    c. possibility to collect data that might reveal an individual deviant health status that however cannot be influenced (e.g. a low prenatal folate level, that indicates low protection of the fetus against NTD's in periconception period that cannot be corrected anymore) at the time of data collection[j];
    d. possibility to do as of yet not known measurements on stored blood samples.
- It is imaginable that (some of the) samples/data that are needed for a monitor are collected already for the purpose of (routine) individual care. However, medical research and privacy legislation prohibits the use of such samples/data for the options mentioned above, without the explicit consent of the owner (i.c. the individual of whom e.g. a blood sample is taken).
- Core issues in legislation are whether or not i. the collection concerns body material, ii. stored data are individually traceable or iii. collection and reporting could be seen as a population screening[k].
- If the options mentioned at the first bullet can be met, individual matching of data in case-control studies would be possible, i.e. this condition for the implementation of the scenario with highest relevance for explanatory scientific research would be feasible. On the other end is the scenario of

[j] The collection of such data would be certainly possible in the context of the participation in an approved (by the applicable ethical board) scientific study, but this would probably require an informed consent procedure for each participant. It would also be possible if the screening of biomarkers would meet the rules for a population screening of prenatal FA-status (like lues or HIV-screening).

[k] In the Dutch situation possible applicable laws are 'Wet op het bevolkingsonderzoek (WBO)', 'Wet mensgebonden onderzoek (WMO)' and 'Wet beschermings persoongegevens (WBP)'.

anonymously stored data of self-reported intake, which has high relevance for policy research, but for which none of these options is needed[I].

6.1.5 Cost-benefit expectations

- The implementation of folate monitor scenario's, including the preparatory work and the usage of stored samples/data, is only feasible if 'someone' is willing to pay for i. the initial investments, ii. the costs of the ongoing collection and storage and iii. the usage. The financial means will have to be acquired in competition with other initiatives that address the same resources as folate monitoring initiatives would do.
- Two assets of periconceptional folate monitoring systems might give fund-raising a reasonable chance. The first one is that folate intake monitoring produces evidence that is very helpful to support the plea for investments in FA-supplement intake promotion interventions/campaigns and in the ongoing improvement of such interventions/campaigns. Effective FA-intake promotion campaigns are highly cost-effective with a very competitive price of the QALY's gained , and probably still so if (part of) the monitoring costs are included in the models . The second asset is that especially the collection of periconceptional FA- biomarkers adds importantly to the extension of the use that can be made of the birth defects data that e.g. EUROCAT already collects. And this is true with respect to causal research of the preventive effects and to the safety of FA-intake.

6.2 Preliminary conclusion

The explorations of the feasibility of the conditions for the implementation leads to the preliminary conclusion, that there are no insurmountable hindrances for monitoring that make use of folate intake data using self-report methods. None of the conditions for the implementation of a biomarker monitoring seems to be impossible to realize in principle, but the compliance with legal regulations might be a considerable hurdle.

[I] An important in-between option is that of the use for scientific research of anonymized surplus human body material collected in routine diagnostic procedures, for which (at least in The Netherlands) individual consent is not required (FVMW Code …).

7 Discussion

The information that is brought together in this report covers a broad field and does not straightforward lead to a simple conclusion or recommendation. However, from the appraisal of the methods, the scenario's and the feasibility of some general implementation conditions, it would be best to focus the discussion on two solutions. These are:

A. folate intake monitoring, either through questionnaires or anonimyzed biomarker measurements, for the purpose of FA-supplementation policy development and evaluation;
B. non-anonymous (regional) census biomarker monitoring for the purpose of explanatory scientific research of birth defects and pregnancy related disorders.

Both solutions can produce adequate datasets for its related purposes, although the appraisal made also clear that improvements of methods (like better validated questionnaire for A) or important implementation conditions (like non-anonymity for B) will have to be realized.

Solution A will, if it is based on biomarker assessment, in addition to the main purposes also be informative on undesirable high intake of FA-supplements. The overall relevance of the solution is to serve the achievement of the primary prevention that FA-supplementation can bring us. The primary stakeholders to be involved are health care policy makers, health promotion researchers and health promotion implementation workers.

Solution B will yield high quality data that will help to find valid answers on research questions like the FA-preventable proportion of NTD's and the preventive (or not) effects of FA-supplementation and/or folate status on birth defects of e.g. the heart, lip and palate and urinary tract. From this perspective an extension of the monitoring to other biomakers of interest, like the other B-Vitamins and Vitamin D, might add to the relevance of this solution. For this solution the main stakeholders are birth defect researchers, research institutions and patients/parents organisations that strive towards more prevention of birth defects.

Solution A is not a very complex one, probably a cost-effective one in the short-term if it is indeed integrated in the development and implementation of FA-supplementation policy and is altogether rather feasible.

Solution B, although technically maybe more complex is exactly in that respect rather feasible if it is thought of as an extension to the existing procedures for blood sampling in pregnancy. The direct and potential value for scientific research especially into rare birth defects and pregnancy related disorders will be very large.

The solutions A and B serve clearly distinct purposes and could both be implemented via separate paths and in different regions. For, whereas the

monitoring of folate intake (A) is best done over a number and selection of region's that is informative for national FA-supplements policy, the best regions to implement census biomarker monitoring (B) would be those where excellent data on birth defects are collected (like EUROCAT regions). If good pregnancy outcome data are available on a nation-wide scale, then the implementation on national scale of solution B could serve both purposes at the same time.

In closing, the general recommendation would be to further investigate the practical aspects of the implementation of solutions A and B, which will help to further ascertain the feasibility and at the same time explore the commitment that could and should be gained among respective regional, national or international stakeholders.

Appendix I – Overview and methodological characteristics of methods to measure folate intake or status

1 Characteristics of methods to assess folate intake

The following characteristics were considered relevant to be described (if available in the study reports)

- Type of 'folate intake' assessed
  - food folate intake
  - supplement intake
- Quantitative estimates
  - period/time point measured (interval to assesment, length of reporting period)
  - retrospective/prospective data collection
  - details of reported intake
    - dosages of supplements;
    - frequency of supplement intake;
    - types of supplements (folic acid, folinic acid);
    - size and frequency of food ingredients intake.
- Subgroups studied
  - age;
  - gender;
  - SES.
- Validity issues
  - time window related to conception/pregnancy phases;
  - exposure and reporting context (intervention or intake/education study, public campaign, usual situation);
  - external validity(match between answers and real intake);
  - concurrent/predictive validity(e.g. match between reported intake and folate status)
- Type of data collection
  - telephone interview;
  - face-to-face interview;
  - self-to-fill-in questionnaire;
  - restricted to folate or embedded in broader data collection (folate related only; more topics) ;
  - context (location, encounter type, interviewer type).

2 Overview of methods for measuring folate intake using self reported intake of food folates and of supplements

	Table I.1				
#	**Method**	Source(s)			
	Types of intake	*Quantitative parameters*	*Demographic characteristics*	*Validity issues*	*Method of data collection/ Study population*
a.	**FA-Monitor Northern Netherlands, NL**	Zetstra- van der Woude, 2012 [1]			
	Folic acid supplements	• Period of intake (duration before and during pregnancy). • Dosage • FA or MV-FA	• Age • Gravidity • Parity • SES	• Post-pregnancy reported • Recall from memory • Distinct (SES) and coherent trends over 6 measurement periods indicating concurrent validity	• Data collected at 1st prenatal visit • Self-to-fill-in paper questionnaire • Pregnant women only
b.	**One-off survey ABCD-study Amsterdam, NL**	Sikkens, 2011 [9]			

	Table I.1				
#	**Method**	Source(s)			
	Types of intake	*Quantitative parameters*	*Demographic characteristics*	*Validity issues*	*Method of data collection/ Study population*
	Folic acid supplements	• Period of intake (before pregnancy; as soon as I knew I was pregnant; later in pregnancy)	• Age, parity • Gravidity • SES	• Reported FA-use positively correlated with serum FA (r=0,49)[m] • Possible over reporting of FA-use among specific ethnic groups [n]	• Consent acquired at 1st prenatal visit • Self-to-fill-in mail questionnaire several weeks after 1st prenatal visit • Pregnant women only
					•
c.	**One-off survey West Midlands, UK**	Burton, 2001 [6]			•
	Folic acid supplements	• Period of intake (ever, before pregnancy, now) •		• Reported cumulative FA-use positively correlated with RCF; current use with serum folate. • Broad range of levels within groups of FA-levels reported	• Consent acquired and data collected at antenatal visit (median 13 weeks GA) • Self-to-fill-in questionnaire • Pregnant women only

[m] Implying that 24% of variance in FA-serumlevels can be explained by reported FA-intake.
[n] Based on interpretation that in these groups despite reported high FA-intake more often low levels (<=20nmol/l) were found.

	Table I.1				
#	**Method**	Source(s)			
	Types of intake	*Quantitative parameters*	*Demographic characteristics*	*Validity issues*	*Method of data collection/ Study population*
d.	***Norwegian Mother and Child Cohort Study (MoBa)***	Brantsaeter, 2008 [8]			
	Folic acid supplements by name by Food Frequency Questionnaire (FFQ)	• Period of intake (3-4 months preceding 16-18th week of pregnancy) • Dosage	• Several •	• FFQ correlates 0,26 ($p<0.001$) with serum folate at 17th-18th week • Food Diary (FD) correlates 0,57 • FFQ did not record start of supplement use relative to start of pregnancy • FFQ Q1 vs Q5 with Serum Folate Q1 vs Q5 (MW-U test, $p<0.01$)	• Self-to-fill in mail FFQ sent at 15th week •
e.	**National Danish Birth Cohort**	Mikkelsen, 2006 [7]			
	Folic acid supplements by name by Food Frequency Questionnaire (FFQ)	• Period of intake (4 wks preceding 25th week of pregnancy) • Dosage	• Several	• FFQ compared with RCF and 7days- FD in week *32-38*: $r=0.56$ ($p<0.0001$) for FFQ and FD; $r=0.55$ for FFQ and RCF • FFQ Q1 vs Q5 with RCF Q1 vs Q5 (?-test, $p<0.001$)	• Self-to-fill in mail FFQ sent at *25th* week •

	Table I.1				
#	**Method**	Source(s)			
	Types of intake	*Quantitative parameters*	*Demographic characteristics*	*Validity issues*	*Method of data collection/ Study population*
f.	**One-off surveys miscellaneous**	Examples of one-off surveys among pregnant women or women in prepregnancy period without any specific information on validity (external, trend)			
	Folic acid supplements				
		•			
f.01	***Southampton Women's Survey, UK***	Inskip, 2009 [18]	*	*	

	Folic acid supplements	• FA-supplement intake in past three month's
		  ○ at interview date
		  ○ at 11 weeks gestation
			•	•	• Home interview by research nurses: 100-item food questionnaire
					• Female 20-34 yrs and pregnant within 3 months after the interview

	Table I.1				
#	**Method**	Source(s)			
	Types of intake	*Quantitative parameters*	*Demographic characteristics*	*Validity issues*	*Method of data collection/ Study population*
f.02	***Oral Contraceptive-users survey, NL***	De Jong- van den Berg, [13]	•	•	
	Folic acid supplements	• Current intake and/or intention to	• Expected TTP • Age • Parity • Gravidity	• 'FA-intake/intention to' as reported correlated with expected TTP	• Self-to-fill-in mail questionnair • Mainly non pregnant OC-users: 25-35 years
		p.m.			
f.03	***Deliver Study, NL***	Manniën [19]			
	FA-supplements	• intake periconception • intake prenatal		• no information (yet)	• Self-to-fill in questionnaire • Before 34 wks (20^{th} on average) of gestation • Afters 34 wks gestation
g.	**Dutch National Food Consumption survey**	Van Rossum, 2011 [20]			

	Table I.1				
#	**Method**	Source(s)			
	Types of intake	*Quantitative parameters*	*Demographic characteristics*	*Validity issues*	*Method of data collection/ Study population*
•	• Food folate • FA from fortified food • FA-supplements	• Current intake (➔ habitual intake)	• Pregnancy?, parity? • SES, age	• Estimation of habitual intake	• 2 telephone interviews (independent days, 24 h recall) • general population • female 19-30 yrs:n= 347 • female 31-50 yrs:n= 351
h.	**EUROCAT –NN permanent cases data collection**	EUROCAT-NN, 2012 [21]			
	• FA-supplements	• Period of intake periconception/ prenatal		• No information	• Self-to-fill-in questionnaire after registration of CA at EUROCAT (postnatal up to 1 year)

	Table I.1				
#	**Method**	Source(s)			
	Types of intake	*Quantitative parameters*	*Demographic characteristics*	*Validity issues*	*Method of data collection/ Study population*
i.	**Intake markers as used in explanatory studies on NTD's and other CA's**	e.g. Whitrow, 2009 (Astma) [22] Van Beynum, 2009 (cardiac defects) [23] Milne, 2012 (Brain tumors) [24]	•		
	Folic acid supplements (mostly)			In these type of case-control studies (of which much more exist) folate exposure is estimated in one way or the other. The impression is that these studies do not provide more information about validity than the studies mentioned under a. till e.	

3 Characteristics of measuring folate status using biomarkers

The following characteristics were considered relevant to be described (if available in the study reports).

- Marker of 'folate status' assessed
  - Red Blood Cell (RBC) levels;
  - Serum/plasma levels;
  - Hematocrit Folate (HF).

- Quantitative estimates
  - period/time point measured (length of reporting period).

- Validation
  - intrapersonal variability/reliability;
  - concurrent/predictive (match between folate status and controlled food folate and/or folic acid intake patterns).

- Type of data collection
  - blood material
    - whole blood;
    - blood spot;
    - packaged RBC's.
  - sampling context
    - dedicated procedure;
    - sub sampling (same/other method) embedded in exsisting procedure;
    - sampling from already collected material.

- Subgroups studied
  - age;
  - gender;
  - SES;
  - time window related to conception/pregnancy phases;
  - intervention study groups (exposed to folic acid supplements/ fortified food).

4 Overview of methods for measuring folate status using biomarkers

	Table I.2[o]				
#	**Method**	Source(s)			
	Biomarker	*Quantitative parameters*	*Demographic characteristics/ Outcome*	*Validity issues*	*Method of data collection/ Study population*
a.	**Packed RBC folate assay**	Piyathilake, 2007 [25]			
	RBC-folate	RBC folate in ng/ml	N/A	• Correlates with routine assays using hemolysate from fresh WB (r=0.78)[p]	• Assay of packed RBC's (from centrifuged fresh whole blood samples at 3000 rpm for 10 min's; storage at -80C) • Feasible for epidemiological field study • Used in epidemiological study (MTHFR polymorphisms and folate metabolism)[q]
b.	**Blood spot (dedicated)**	O'Broin, 1999 [26]			•

[o] Abbreviations: WB- whole blood; RBC- red blood cell; DBS- dried blood spot; HF – hemoglobin folate; ICF- ; LC-MS/MS liquid chromatography–tandem mass spectrometry; MA- microbiological assay; CLEIA-competitive protein-binding ChemiLuminescent Enzyme ImmunoAssay; LC-MS/MS liquid chromatography–tandem mass spectrometry; NHANES- National Health and Nutrition Examinations surveys.

[p] Implying that the proportion of explained variance, r^2, is 0,61.

[q] See reference 10 in Piyathilake,2007.

	Table I.2°				
#	**Method**	Source(s)			
	Biomarker	*Quantitative parameters*	*Demographic characteristics/ Outcome*	*Validity issues*	*Method of data collection/ Study population*
	Hemoglobin folate	HF (hemoglobin folate)	N/A	• HF and RBC folate correlate very well (r^2= 0.993)[r]; • r=0.97 for DBS vs WB of normal volunteers	•
c.	**Blood spot (convenience, stored)**	Zimmerman, 2012 [27]		•	•
	HF	HF	N/A	• HF depends on 9 month storage conditions: ranking pertained ➔ useful for (comparative?) epidemiologic studies	• healthy volunteers newborn babies; • as proxy for routinely collected neonatal heel DBS
d.	**Whole blood**	CDC, 2009 [28]		•	•

[r] See reference 10 in O'Broin, 1999.

	Table I.2°				
#	**Method**	Source(s)			
	Biomarker	*Quantitative parameters*	*Demographic characteristics/ Outcome*	*Validity issues*	*Method of data collection/ Study population*
	Serum folate RBC folate		N/A	• MA-used to measure folate (following O'Brion, 1999) • MA- confirmed as preferred method in 2011 [s 14]	• Whole blood samples (0,4 ml serum/ 0,5 WB) • Refrigerated if processed quickly/otherwise frozen samples needed • Intended for pupulation survey
e.	**Whole blood**	Bodnar, 2010 [29]		•	•
	5MeTHF, 5FoTHF, folic acid	5MeTHF:5FoTHF used in analysis)	PTB		• Pregnant < 16 wks (N=313 in analysis) • Nonfasting bloodsamples • LC-MS/MS after Pfeiffer, 2004 [30]
f	**Whole blood**	Yamada, 2012 [31]			•
	Serum folate		PTB, FGR	No information	• CLEIA • First prenatal visit blood sample (N=5075)[t]

[s] This article reports the conlusions of a NHANES roundtable in 2011. (Yetly, 2011)
[t] Population consists of all women attending health facilities (37) for antenatal care in one town (Hokkaido). Recruitment ongoing.

	Table I.2°				
#	**Method**	Source(s)			
	Biomarker	*Quantitative parameters*	*Demographic characteristics/ Outcome*	*Validity issues*	*Method of data collection/ Study population*
g.	**Biomarkers as used in NTD and other CA studies**				
	Serum folate RBC- folate	Daly, 1995 [32] Sikkens, 2011 [9] Magdalijnse [33] Kiefte-de Jong, 2012 [34]		Several one-off studies like these have been conducted or are still running in which folate biomarkers are collected. A first exploration of these studies did not yield more information about other markers/tests and validity than the studies mentioned under a. to f.	
		RCF Serum folate	NTD, Preterm Birth (PTB), Fetal Growht Retardation (FGR),, Astma/Wheezing		

Appendix II – Appraisal of the relevance for science, policy making and individual care of different scenarios for the monitoring of folate intake and/or folate status

1 Introduction

To assess the relevance of different scenario's for folate intake or status monitoring the core characteristics of each are described and then an overall judgment of the scenario as a whole is made. The overview of the characteristics and the judgments are shown in Table II.1 . Table II.2 and Table II.3

In the following Chapter an explanation of the characteristics for the appraisal of the relevance of the scenario's is given.The rules that guide the judgment of the relevance are explained in Chapter 3.

In the discussion of the 'Estimated factor' (2.1 and Table II.1) the concepts validity, reliability and estimator bias will be used as follows. *Validity* is the extent to which the (quantitative) estimate is indeed a measure of the empirical 'factor' (phenomenon/matter) of interest. E.g. in the case of 'fortified food's' whether or not the food product reported by e.g. an interviewee indeed contains folic acid and is not mistakenly assumed to contain it (either by respondent or interviewer) or comparable mismatches in the case of multivitamin use. *Reliability* is the extent to which repeated measurements of the same factor vary, due to noise factors in the (sampling and) measurement procedures[u]. *(Un)biasedness* of the estimator is defined in the statistical meaning, thus whether or not the mean of repeated sample estimates truly reflects the value of the population mean. A biased estimator yields an estimate that is systematically too high or too low. Bias can result from sources like non-validity[v] and non-random faults in the assessment or processing of quantitative measurement data.

[u] Biological variance is considered variance of interest and not as a source of noise.

[v] The appraisal in this paragraph is restricted to the value that folate data from monitoring solutions could have in studies investigating the relation between folate intake/status as a causal determinant of pregnancy outcomes (both mother and child). The value for descriptive studies as such is not treated in this report at all. The value of descriptive time series is appraised in the next paragraph.

2 Scenario characteristics

2.1 Estimated factor

Estimated factor_Validity- It might be relevant in scientific respect to differentate folate intake into the (relative part of) the type(s) of folate that accounts for the intake: natural folates from food; folic acid (PMA) from supplemented foods; folid acid (PMA) from supplements; other synthetic folates (L-5- MTHF salts) both from supplements and possibly food products (future). Both in prospective and retrospective questionnaires (self-to-fill-in written, interview) distinctions for intake from natural food and from supplements can be made validly, because it seems hard to confuse these sources (face validity)[w].
Estimates for the folate intake from unfortified natural food depends on the proper recognition of natural food sources included: this does not seem to be a more than minor threat for the validity (face validity). Estimates for the folic acid intake from fortified food might be more problematic. Some/many (?) of these products are not easy recognizable and might thus not be reported, whereas otherwise products not containing folic acid might be reported to do so.
Empirical studies that have assessed the validity of folate intake assessment by questionnaires in a direct way, i.e. by comparing the estimate on subject level with an 'gold standard'[x], have not been found (yet). In two studies folate intake as reported has been related to folate blood levels (point estimates, not changes over time). Some of the variance of blood folate levels, which can be seen as another indicator of folate intake, can be explained by the reported intake (25% Sikkens, .. Burton).
A prospective distribution of FA-supplements of known strength and rather direct measurement of the intake (based on pill counts) ensures good validity (and probably little bias) and have been (and are) used only in controlled prospective trials and free distribution programmes [MRTC; Czeizel?; Berry/Li; Verona; FzE; NCarolina Morgan[2159]) and not for any monitoring purpose.

Estimated factor_Reliability- The reliability of methods to assess folate-intake cannot be appraised based on empirical data: no studies that reported measurement reliabilities were found. Methods that use prospective or short term (24 hours) retrospective data collection[y] can reasonably assumed to have less variance due to (random) recall-noise than retrospective methods with long recall periods[z]. In the Dutch National Food Consumption Survey [RIVM, …] data are collected twice and

[w] Questionnaires that differentiate between these sources has been used e.g. in [studies]
[x] A feasible proxy of gold standard might be measurement of FA-supplement intake using MEMS boxes (Medication Event Monitoring System) over a sufficiently long period.
[y] Examples: Dutch National Food Consumption Surveys (Van Rossum, 2011), (De Jong van den Berg, 2009)

[z] Dutch folate monitor (Zetstra- van der Wouden, 2012) (1st prenatal visit); ABCD-study Amsterdam en Generation R study Rotterdam (1st prenatal visit); Delivery study Netherlands; EUROCAT-data collection Northern Netherlands

on independent days. The estimator is used as a measure for habitual intake (over a certain period) and this repeated measurement procedure will yield a more reliable estimate for this 'factor' than a single measurement or measurement on dependent (e.g. consecutive) days.

Estimated factor_Unbiasedness[aa]
Several faults in measurement procedures that lead to bias in the estimate are reported or can be imagined. In a questionnaire validation study [Sikkens] the author concludes that quantitative over reporting (social diserable answers specifically related to the context of pregnancy) likely occurs in one ethnic subgroup. Data for this study were from respondents from one town. It is very probable that the presence and effect of this bias vary over many cultural and ethnic differences (in countries and between countries). Another source of bias in the case of supplement measurement is the accurateness and/or completeness of the dosages that are reported. More precise answers will probably be collected if names of brands and products are provided than when open questions are used (it would be worth to compare questionnaires on these aspects). Also the separate answers on frequency and duration of intake might be due to systematic error. More detailed questioning might be expected to yield less biased estimates than broad open questions (which to the impression of DS are most often used now). Regarding measurements of fortified food and natural food folate, the same issues of knowledge of correct dosages or amounts of intake apply as for supplement intake measurement. Another source of bias might a difference between the assumed and really present concentrations of supplemented folic acid (due to –accelerated-decay) and of folates in natural food. The presence and magnitude of both differences probably vary geographically (due to differences in e.g. production methods, regulations and maintenance, storage and distribution etc.)

2.2 Subjects measured

It depends on the explanatory relation that is being investigated, which population of subjects measured is appropriate. 'Subjects measured' refers to the subjects to whom the folate intake/status data are applied as a measure of exposure. In the case of scientific studies and prevention policies of congenital anomalies, these are thus the embryos and foetuses, not their mothers. However, these mothers are the providers of data, unless e.g. umbilical cord blood is used for biomarker assessment, and are therefore in this text figure also mentioned as the subject measured.

If one is interested in the *effect of (changes) in FA-intake levels* ***after*** *the conception* on post-conception outcomes the inclusion of all subjects that succesfully conceived would be appropriate. The completeness of the inclusion of all subjects of such a group depends on several factors. Certain proportions (?) of these subjects wiil:

[aa] Non-validity does not necessarily lead to bias. An estimate with incomplete, but quantitatively balanced, validity will not lead to bias in the estimator.

i. not detect the conception at all, due to not using a pregnancy test (timely enough) and very early miscarriage without recognized or recognizable symptoms;
ii. have an early miscarriage for which they do not seek professional help or that is not registered;
iii. have a termination of pregnancy (for any reason) that is not registered.

This group could be defined as all subjects with a confirmed/confirmable pregnancy. In practice it will be hard to have a good inclusion for such a group if strategies are used that are embedded in routine prenatal and obstretic care as is always the case now.

If the focus of interest is in *effects of (changes) in FA-intake levels* ***before*** *conception* on both conception (yes/no, time till pregnancy (TTP, multiple pregnancies) and post-conception outcomes then the group to be included comprises all subjects that might become pregnant (= all those to whom it might happen, thus conscious and/or in control of it or not). Clearly this group cannot completely be detected in retrospect by prenatal care events, because a (scientifically relevant) proportion will not become pregnant at all (or outside the time window of a monitor implementation). The study of this relation implies the inclusion of subjects in a period before a pregnancy could occur. Like it was done e.g. in the Southampton Women's Survey Study Group with recruitment from general population) (Inskip, 2009) [18] and in a Dutch study among OC-users only (De Jong-Van den Berg, 2009 [13]).

2.3 Measurement period(s)/point(s)

In addition to the distinction between pre- and postconception exposure to folate intake, relevant characteristics of the exposure period are: duration of preconception exposure (relations with outcomes up to periods of one year have been reported (Bukowksi, 2009) [35]); duration of prenatal exposure, often measured per trimester. In many studies the FA-intake was assessed at first prenatal visit or (shortly) after delivery. With respect to validity and reliability repeated measurements during pregnancy are preferable over one time post-delivery measurements.
See also discussion above.

2.4 Sampling scheme

Depending on the questions to be answered a folate intake (or status) monitoring system does not have to collect data from the population of interest permanently and for all subjects, i.e. without interruptions and from each new subject that 'enters' that population. Occurences of (the possibility of a) pregnancy can be considered to be independent (both at a certain point in time and over longer time periods) and therefore a random sample (stratified on any co-variate or not) provides a study group that will allow for conclusions on explanatory (causal) relationships in a case-control design with the same validity that would apply to conclusions based on census population data. Power requirements and feasibility are the factors to be considered in the choice of a sampling scheme.

2.5 Level of analysis

The level of analysis that is possible is relevant for the strength of the relations being studied. It depends on the type of matching of the data from the FA-monitor with the outcome data. The two extremes are matching on the individual level on the one hand and on the level of the whole population (as covered by a FA-monitor) on the other. In between matching on e.g. age, geographic region and dates and/or periods of exposure to FA and occurrence of outcome, might increase the validity and power of ecologic studies.

2.6 Scope

The scope of a monitor solution refers to both the geographic extent and the duration/time span covered. Geographic spread is related to the representativeness of population estimates. The length of the time span that is covered by a monitor determines the opportunities to control for historic trends in effect studies and to study long(er) term effects of folate intake or status.

3 Method for appraisal of relevance

Different FA-monitoring scenario's will yield different types of datasets. The scenario's are judged on their potential to provide appropriate datasets for the adequate study of explanatory relations using the different types of designs. Each scenario is given a summary score. The scores are meant to help and focus further work in this field on those scenario's that can significantly add to our knowledge of the effects of folate intake and status on relevant outcomes (both in child and mother) of reproduction.

The appraisal mentioned above was first done for the usefulness in scientific studies of the data collected. Thereafter an appraisal was made in the same manner for the use in policy making and evaluation. Finally an appraisal was made for the application in individual care of the data collected.

The summary score is based on the judgment of the effect of the characteristics of the scenario on the characteristics of the dataset that can be provided. The following rules are applied.

1. For the sake of completeness and documentation of the appraisal proces each scenario that is imaginable, based on the existing and/or technically feasible 'ingredients' which would be necessary to implement that scenario, ánd that has at first sight potential to serve objectives of either research and/or policy making, is eligible to be treated in Table II.
2. The first judgment is qualitative and concerns the threshold of appropriateness of the datasets. If one or more characteristics of a scenario would make it impossible to provide meaningful datasets, the scenario was considered not eligible and is not mentioned.
3. The remaining scenario's are given one of the following scores: 1. Limited relevance – 2. Moderate or (potentially high) relevance 3. High relevance.

The feasibility of the implementation of a scenario is not taken into account.

3.1 Purposes for the application of data collected in monitoring scenario's to be included in the appraisals

Data collected in monitoring scenario's could be applied for different purposes in each of the three domains of appraisal: scientific research, policy making and individual care. The purposes are listed per domain as well as some characteristics that were included in the reflection on the relevance of the scenario for that specific application.

3.1.1 For scientific research (in general and for each estimate specific).

- for case-control studies;
  - data ready available
    - whether or not data are individual trackable;
  - known validity and reliability;
  - completeness of data sets;
- for cohort studies
- for ecological trend studies
  - census data advantage[bb] (for outcomes required as well; subpopulations)
- for controlled trials (both pre-post and concurrent samples)

[bb] EUROCAT study (Busby, 2005); Dutch trends (De Smit, 2013)

Factors modifying relevance for scientific research
- Concordance/comparibility with estimates used in explanatory studies so far
- Validity of estimates in relation to assocation, causal and explanatory models
- Efficiency of estimates (noise reduction in and natural variance of determinant studied) with respect to power of study numbers included
- Use of same estimates in different regions/countries (pooling of exposure and outcome data)

3.1.2 For policy making

- valid estimates of (not yet) realized NTD-prevention;
- assesment of effects of policy implementations (between regions; pre and post) of folate intake and/or status;
- (early) detection of undesired (scale of) intake of (potentially) unsafe levels of FA-supplements

Factors modifying relevance for policy making
- concurrent collection of data that can guide policy initiatives (ses, knowledge, availabilityof folate rich food/fortified products, FA-promotion efforts in place etc.);
- subpopulations that are included (only pregnant, all women fertile, all adults, all children)

3.1.3 For individual care (folate status only)

- (early) detection of folate defiencies (clinical suboptimal levels)[cc];

Critical factors for relevance
- validity and reliability of marker(s) to be assesed
- what level for which issues is needed in terms of standardization (as a general issue to be appraised for feasibility of implementation)

[cc] (Green, 2011) for validity of assessing folate deficiency;

4 Appraisal of the relevance of folate status monitor scenario's for explanatory scientific studies[dd]

	Table II.1							
#	**Design**	**Estimated factor[ee]**	**Subjects measured[ff]**	**Measurement period(s)/ point(s)[gg]**	**Sampling scheme**	**Level of analysis**	**Scope**	**Relevance**
a.01	Case-control	Folate-intake	All pregnancies	Periconception	Permanent	Individual [hh]	(All) EUROCAT regions[ii]	High
			In this scenario it would be possible to use all (pooled) cases over all periods in all participating EUROCAT registries for case control studies. Relevance increases with number of participating registries.					
a.02			**All (still and) life births**	Periconception	Permanent	Individual	(All) EUROCAT regions	Limited

[dd] The appraisal for the case-control design is in general made with respect to the study of *congenital anomalies and pregnancy outcomes*. The congenital anomalies considered are those that are included in the EUROCAT registers. Most of these anomalies develop in early pregnancy and thus is data about *periconception* exposure to folate essential. If the scope of relevant outcomes would be extended to other health outcomes that might be influenced by exposure throughout the whole pregnancy (like e.g. the immunologic system and cancers) the relevance of scenario's that do collect (valid) data only on 2nd and 3rd trimester and not for periconception exposure, increases.
Furthermore: monitors that provide information on other population groups than mothers-to-be might have value for other outcomes.
[ee] For purposes of conciseness the different estimates of folate intake (mostly self-reported) and for folate status (biomarkers) are in general treated as a group, but where necessary distinctions are made.
[ff] 'Subjects measured' refers to the subjects to whom the folate intake/status data are applied as a measure of exposure. In the case of scientific studies and prevention policies of congenital anomalies, these are thus the embryos and foetuses, not their mothers.
[gg] Period/point concerns the period/point of folate intake measured and nót the period/point of the physical collection of data (e.g. data on periconception intake can be collected at 1st prenatal visit or at end of pregnancy or at 1 year of age etc.)
[hh] For the case control design only scenario's that allow for individual level of analysis are discussed. Case control studies are not possible without matching of data on individual level.
[ii] The scope is defined as the whole population mentioned under 'Subjects measured' in the captive region of a EUROCAT register, thus not only the population of cases of birth defects as registered by EUROCAT.

	Table II.1							
#	**Design**	**Estimated factor[ee]**	**Subjects measured[ff]**	**Measurement period(s)/ point(s)[gg]**	**Sampling scheme**	**Level of analysis**	**Scope**	**Relevance**
			If FA-measurements would be restricted to still and life births (>28 wks) this would mean that no data are available for outcomes that (also) occur before 28 wks of gestation. Neither for cases, nor for controls (data for registered TOP because of NTD would not be available). This drawback is even stronger if data are restricted to life births only.					
a.03			All pregnants	**Periconception and 2nd/3rd trimester**	Permanent	Individual	(All) EUROCAT regions	High
			Outcomes possibly related to 2nd/3rd trimester levels of intake could also be studied					
a.04			All pregnancies	**Post conception and 2nd/3rd trimester**	Permanent	Individual	…	Limited
			For the study of most of the CA's data on exposure in the earliest embryonic stages is crucial. Build up of folate status takes time and even data of immediate postconception intake are not informative on the folate status in the first weeks post conception. However folate intake in 2nd and 3rd trimester is studied in relation to fetal developments in that period of pregnancy (e.g. functioning of immunologic system/astma).					
a.05			All pregnancies	Periconception	**Periodical**	Individual	(All) EUROCAT regions	Limited
			If monitoring would e.g take place one month every year only 1/12 of all subjects would be measured and data on folate intake would only be available for 1/12 of the cases (limiting factor for power) and controls. If however many registers would provide data on outcome, the scope of the monitor could be larger and this could compensate for this loss of numbers.					
a.06			All pregnancies	Periconception	Permanent	Individual	**Outside-EUROCAT regions**	Moderate
			Monitoring outside EUROCAT regions is relevant if other outcome registrations are available (e.g prenatal diagnoses, birth outcomes like birth weight, gestational age and delivery complications are available in obstetric registries). EUROCAT registries are regional with ambition of high quality data on CA, but also limited in numbers of cases.					

	Table II.1							
#	**Design**	**Estimated factor[ee]**	**Subjects measured[ff]**	**Measurement period(s)/ point(s)[gg]**	**Sampling scheme**	**Level of analysis**	**Scope**	**Relevance**
a.07			**Samples (1 in X) of women in general population 18-40**	Periconception	**Periodical**	Individual	(All) EUROCAT regions	Limited (very)
			Periodically repeated measurements in samples of women aged 18-40 would also yield data for women in the preconception period and pregnant women. The coverage however would be very limited (in NL e.g 1 in 20 women aged 20-40 give birth each year; with a 1 in 500 sample only 1/25th of all (future) births would be covered). This loss of power would have to be compensated by a larger scale (see # a.06) of the monitor.					
b.01	Cohort	Folate-intake	All pregnancies	Periconception	Permanent	Individual[jj]	(All) EUROCAT regions	High
			This scenario would yield folate-intake data that would be useful both in prospective and retrospective cohort studies. If (specific) CA's would be the outcome measure large cohorts and/or longer inclusion time frames would be needed.					
b.02			**All (still and) life births**	Periconception	Permanent	Individual	(All) EUROCAT regions	Limited
			Datasets would be non-informative about relations between folate intake and outcomes in spontaneous abortion and pregnancy terminations before 28 weeks. This is a serious bias if cohorts are followed that are formed before conception or directly after confimed pregnancy.					
b.03			All pregnancies	**Periconception and 2nd/3rd**	Permanent	Individual	(All) EUROCAT regions	High
			Relevance comparable as for case-control: see # a.03					
b.04			All pregnancies	**Post conception and 2nd/3rd trimester**	Permanent	Individual	(All) EUROCAT regions	Limited

[jj] For the cohort design only scenario's that allow for individual level of analysis are discussed. Meaningful cohort studies are not possible without matching of data on individual level.

	Table II.1							
#	**Design**	**Estimated factor[ee]**	**Subjects measured[ff]**	**Measurement period(s)/ point(s)[gg]**	**Sampling scheme**	**Level of analysis**	**Scope**	**Relevance**
			See discussion under # a.04					
b.05			All pregnancies	Periconception	**Periodical**	Individual	(All) EUROCAT regions	Limited
			The periodical sampling would cover only part of a cohort for which subjects are included at one time point or in an uninterrupted period of time. The power of the part of the cohort for which FA-data would be available, will be (very) limited.					
b.06			All pregnancies	Periconception	**Permanent/ periodical**	Individual	**Outside-EUROCAT regions**	Moderate
			Considerations as under # a.06					
b.07			**Samples (1 in X) of women in general population 18-40**	Periconception	**Permanent/ periodical**	Individual	(All) EUROCAT regions	Limited (very)
			Data collection would cover only 1: X of subjects in cohorts and the power for CA-studies would thus be very low.					
c.01	Ecologic	Folate-intake	All pregnancies	Periconception	Permanent	Population [kk]	(All) EUROCAT regions	High
			It the data collected can be grouped by time periods that match time periods of outcome registries (e.g. years), time trend series can be studied.					
c.02			**All (still and) life births**	Periconception	Permanent	Population	(All) EUROCAT regions	Limited
			See considerations at # b.02					
c.03			All pregnancies	Periconception	**Periodical**	Population	(All) EUROCAT regions	High

[kk] The individual level is not discussed for ecologic studies, because if that matching would be possibe a (retrospective) cohort design could be used.

	Table II.1							
#	**Design**	**Estimated factor[ee]**	**Subjects measured[ff]**	**Measurement period(s)/ point(s)[gg]**	**Sampling scheme**	**Level of analysis**	**Scope**	**Relevance**
			Periodical monitoring that provides a representative estimate for the population mean over the time period that is analyzed, is approriate for this design.					
c.04		Folate-intake	**Samples (1 in X) of women in general population 18-40**	Periconception	**Permanent/ periodical**	Population	(All) EUROCAT regions	Moderate
			Data from 1 in X samples of the general population could be used if a representative and large enough sample can be selected and a good response is warranted. Collecting data about folate intake with questionnaires in representative population samples is vulnerable for bias in sociodemographic factors. Controlling for this bias in data from the outcome registries is in general not possible.					
c.05		Bio-markers	**Samples (1 in X) of women in general population 18-40**	Periconception	**Permanent/ periodical**	Population	(All) EUROCAT regions	High
			Same considerations as for #c.04 but the response in data collection of bio-markers, e.g. integrated in routine blood sampling, is much less vulnerable to sociodemographic bias.					
c.06		Bio-markers	All pregnancies	Periconception	**Permanent/ periodical**	**Subgroup**	(All) EUROCAT regions	High
			If data on characteristics of subjects from the same (sample of) population are available (e.g. age, parity) ecologic analyses could be done in which controlling for these characteristics would be possible..					
c.07			All pregnancies	**Periconception and 2nd/3rd**	Permanent	Population	(All) EUROCAT regions	High
			See under #a.03					
c.08			All pregnancies	**Post conception and 2nd/3rd trimester**	Permanent	Population	(All) EUROCAT regions	Limited
			See under #a.04					
c.09								

5 Appraisal of the relevance of folate status monitor scenario's for folic acid profylaxis policy making and evaluation

Backgrounds for *policy making*

- valid estimates of (not yet) realized NTD-prevention;
- assesment of effects of policy implementations (between regions; pre and post) of folate intake and/or status;
- (early) detection of undesired (scale of) intake of (potentially) unsafe levels of FA-supplements.

Factors modifying relevance for policy making

- concurrent collection of data that can guide policy initiatives (ses, knowledge, availabilityof folate rich food/fortified products, FA-promotion efforts in place etc.);
- subpopulations that are included (only pregnant, all women of fertile age, all adults, all children)

Design/relation

Relevant evaluations for folic acid profylaxis policy making could be based on several designs and analyses.
Description and/or analysis of trends in folate intake or status are informative about both the (estimable) effect on prevention of FA-dependent outcomes and of (the development) of the level of folate intake.
Comparative designs/analyses like pre-post intervention, with or without pre-post control regions, can be used to assess effects of differences in both long(er) term policies and for one-time evaluations of (implementation of) interventions.

	Table II.2							
#	**Design / relation**	**Estimated factor**	**Subjects measured**	**Measurement period(s)/ point(s)**	**Sampling scheme**	**Level of analysis**	**Scope**	**Relevance**
a.01	Comparative[ll]	Folate-intake	All pregnancies	Periconception	Permanent	Individual	(All) EUROCAT regions	High
			This scenario would allow very rich analyses. It would be possible to make comparisons,using both trends and cross-sectional analyses, in which effects of differences in policies and all demographic characteristics collected, can be studied extensively. Studies both in one region only as well as across e.g all EUROCAT regions, would be both informative.					
a.02			**All (still and) life births**	Periconception	Permanent	Individual	(All) EUROCAT regions	High/ Moderate
			Same considerations as for #a.01 but with less validity, because in this scenario folate intake data would be collected postnatally wich leads to an increases recall bias.					
a.03			**All pregnancies/ All (still and) life births**	**Post conception and 2nd/3rd trimester**	Permanent	Individual	(All) EUROCAT regions	Limited
			Although this scenario would be very informative[mm] about folate intake throughout pregnancy, the relevance for policy making is limited, because the only evidence based indication for FA-suppletion is pre- and early postconception. This variant is therefore not treated in the other scenario's.					
a.04			**All pregnancies/ All (still and) life births**	Periconception	**Periodical**	Individual	(All) EUROCAT regions	High
			Periodical data collection would basically provide sufficient data to conduct all the studies that would be possible in #a.02. For studies into effects of planned interventions, and/or events that might otherwise influence folate intake, too great distances between measurement periods/points might limit the 'power' of such studies.					

[ll] The scenarios #a.01 through #a.11 are primarily appraised for these type of study. The appropriateness for the description of (trends of) prevalences is discussed in summary under #a.12)

[mm] In this scenario it would be rather feasible to collect data on folate intake prospectively (at the consecutive prenatal consultations), which would add to the validity of the data.

	Table II.2							
#	**Design / relation**	**Estimated factor**	**Subjects measured**	**Measurement period(s)/ point(s)**	**Sampling scheme**	**Level of analysis**	**Scope**	**Relevance**
a.05			**All pregnancies/ All (still and) life births**	Periconception	Permanent	**Population**	(All) EUROCAT regions	High
			If only data on folate intake would be collected (thus no other data on any level), the distinction in the level of analysis would be obsolete, because there is only one estimate and that is for the population mean or proportion for folate intake. Studies into effects of interventions and other events are possible and will yield valid results because trends and cross-sectional differences can be estimated on large (census in this scenario) numbers. If non-matchable demographic data would be available for the same samples as for which folate intake data are collected, then studies on ecological level in which demographic characteristics can be controlled for would be possible and changes over time and/or cross-sectional comparisons can then be used to study effects of policy implementations and other events.				
a.06			**All pregnancies/ All (still and) life births**	Periconception	**Periodical**	**Population**	(All) EUROCAT regions	Moderate
			Comparable studies possible as in scenario #a.05, with the possbile limitations of a. too long distances between periods or time-points and b. risk of non-representativeness of demographic data if the sources from which data are collected are not representative samples of the same underlying population.					
a.07			**Samples (1 in X) of all pregnancies/ (still and) life births**[nn]	Periconception	Permanent	Individual	(All) EUROCAT regions	High
			This scenario would allow for the same type of studies as in #a.02 assuming that large enough representative samples are drawn.					

[nn] Feasibility of periodical vs '1 in X'- samples: if data collection in first prenatal consultations then census periodical questionnaire collection might be more practical than random e.g. 1: 10 continuously. If bio-markers are measured in routinely taken blood samples, asking consent for this additional measurement in 1:10 might probably more easily and sufficiently robust be integrated/implemented in the existing procedures.

	Table II.2							
#	**Design / relation**	**Estimated factor**	**Subjects measured**	**Measurement period(s)/ point(s)**	**Sampling scheme**	**Level of analysis**	**Scope**	**Relevance**
a.08			**Samples (1 in X) of all pregnancies/ (still and) life births**	Periconception	**Periodical**	Individual	(All) EUROCAT regions	High
			Same studies possible as with #a.04 as long as sufficiently large samples for point/period estimates can be collected to meet power requirements.					
a.09			**Samples (1 in X) of all pregnancies/ (still and) life births**	Periconception	**Periodical**	**Population**	(All) EUROCAT regions	Moderate/ Limited
			Same studies possible as with #a.05 but with increasing risk of samples that are not representative for and/of from the same underlying populations as compared with census data collection from one and the same population (see comment #a.06).					
a.10			**Samples (1 in X) of women in general population 18-40**	**Periconception / Habitual**	Permanent	Individual	(All) EUROCAT regions	High
			If sufficient representative data for estimates for the subgroup of prepregnant and pregnant women are collected, studies into the effects of interventions and other events on folate intake before and during pregnancy can be done.[oo] Scenario is apt for the assesment of (trends in) folate intake patterns in entire female population 18-40 yrs.					
a.11			**Samples (1 in X) of women in general population 18-40**	**Periconception / Habitual**	**Periodical**	**Population**	(All) EUROCAT regions	Moderate/ Limited
			Same type of limitations as for #a.09 but with even more increased risk because of the smaller sample sizes of (pre)pregnant women. Secenario is apt for the assesment of folate intake patterns in general population(see #a.10)					
a.12	All		**All variants[pp]**	**Periconception / Habitual**	**All variants**	**All variants**	**Outside-EUROCAT regions**	Limited/ Moderate/ High

[oo] With respect to feasibility; would require rather large samples from general population and this on permanent basis ....
[pp] Variants treated in #a.01 to #a.10.

	Table II.2							
#	**Design / relation**	**Estimated factor**	**Subjects measured**	**Measurement period(s)/ point(s)**	**Sampling scheme**	**Level of analysis**	**Scope**	**Relevance**
			The extension of the data collection to more regions will add relevance to all scenario's that are appraised high already. For the extension: i. increases the proportion of the pregnant population(s) for which folate intake can be estimated as a proxy for the proportions of NTD's prevented (for which scenario's #a.01 through #a.10 have a high relevance); ii. makes possible the study of more different and/or differences in interventions and other possibly causal events of folate intake; iii. increases the proportion of the (general) population(s) for which folate intake estimates can be made (#a.10 and #a.11). In the scenarios #a.06 and #a.09 the relevance for one or more types of study is limited by methodological threats and the extension will not increase the relevance for those studies because it is not a remedy against those threats.				

6 Appraisal of relevance of folate monitoring for individual patient care

Introduction

Knowledge about the habitual or incidental folate intake or folate status of individuals might guide the delivery of tailored advice. This advice can be given either in person by a caregiver or through automated messaging (in writing, e-mail, IT-applications). Different possibly relevant applications of this tailored advice are imaginable. These applications are presented in the next paragraph. The appraisal of the relevance is given in Table II.3.
The advice to be given should be directed to actual or future situation of the women that provided the monitor data and of course not to past situations of the subjects measured (embryos and foetuses). When e.g. data about folate intake or status in the periconception period is collected after the delivery, that information should be used to tailor an advice for the current (breast feeding?) or future (remainder of interpregnancy period?) situations of the woman.

Applications of individually tailored advice using data of folate monitoring

I. Folate intake

The advice to be given depends on the current situation of the woman that provided the monitor data. It also depends on what is exactly monitored: habitual intake from natural food and/or intake from fortified food and/or intake from FA-supplements.

1. Women 18-40 of general population
    a. Comparison of habitual intake of food folate equivalents (FFE) with recommended daily average (RDA) and/or tolerable upper level (UL) of folic acid intake could be made and messaged.
    b. If individual information about reproduction characteristics are known (childwish near future, attempting to conceive, pregnant, breast feeding) feed back could be adjusted to this information[qq].
2. Pregnant women

[qq] In the US áll women in fertile age capable of becoming pregnant are recommended to take 400 mcg supplements daily. As far as known no health authority in any EU-country has (yet) issued this advice also.

   a. Two recommendations apply to pregnant women:
      i. sufficient intake needed to meet (increased) metabolic requirements during pregnancy (e.g. in NL from 300 mcg FFE to 400 mcg FFE);
      ii. 400mcg of PMG (folic acid) from 4 weeks before to 8-12 weeks after conception to decrease chance on NTD.
   b. The tailored advice should be adjusted to the phase of the pregnancy.
3. Women in early postpregnancy period
   a. To these women the following recommendations apply:
      i. increased intake needed in case of breast feeding;
      ii. if a next pregnancy within a short interval is probable (e.g. unprotected intercourse), intake of 400mcg is indicated;
      iii. for any pregnancy later in time, intake of 400mcg is indicated;
      iv. ongoing folate supplementation during the complete interpregnancy interval (even in case of contraception in first phase) might be recommendable.[rr]

II. Folate status

The collection of values of biomarkers in the context of the monitoring scenarios treated in Table II.1 and II.2 is probably only feasible in the (early) prenatal period (see also chapter on feasibility). Therefore only the application of a tailored advice based on prenatal folate status is relevant.

1. Pregnant women
   a. Data about folate status can be compared to clinical (pathologic and physiologic) reference values (plasma values: <7 nmol/l - neutrophilic hypersegmentation; < 10 nmol/l - early sign of devlopment of folate deficiency (threshold value for assesment of adequate intake); [LT1827-Gr2008] [LT2334-Gr2003]; to be completed with reference values from other sources; population means by age and sex?)
   b. Folate status could be compared with optimal NTD-protection reference values (RBC folate: >= 906 nmol/l – 80% (? check Daly) protection) with respect to the reminder of the 8-12 wks postconception period and/or with respect to timely use of sufficient folic acid in a possible next pregnancy.

Appraisal of relevance

[rr] (Van Eijsden, 2009) Further study of recovery of folate levels during interpregnancy periodes, might lead to alterations in recommendations of pregnancy related folic acid supplement intake.

	Table II.3			
#	**Application**	**Added value over normal individual care**	**Comments**	**Possible adverse effects**
I	***Monitor: Folate intake***			
1.	Women 18-40 general population			
a.	Habitual intake vs RDA and UL	Pro-active advice on unsufficient or too high intake that might otherwise never be received	This type of monitor would cover only 1: X sample of population. With repeated non-identical samples coverage increases. Participation probably low in groups with highest prevalence of deviations.	None
b.	Reproduction characteristics related intake vs guidelines	Pro-active advice on unsufficient or too high intake in *periconception period* that might otherwise not be received in time.	This will only work if feedback can provided during or shortly after data collection. From first prenatal visit on women could be given the same advice, but actual delivery depends on the level of implementation of food- and supplement advising.	None
2	Pregnant women			

	Table II.3			
#	**Application**	**Added value over normal individual care**	**Comments**	**Possible adverse effects**
a.i	General intake	None.	Prenatal visit to caregiver provides opportunity for tailored advice already	
a.ii	Periconception intake	None.	See 2.a.i	
3.	Women in early postpregnancy period			
	a.i. until a.iv.	None	During postnatal care (home) visits and/or visits to Well Baby Clinics all these advices could be given, but actual delivery depends on the level of implementation of food- and supplement advising.	
II.	***Monitor: Biomarkers***			
1.	Pregnant women			
a.	Comparison with clinical reference values	Detection of (possibly upcoming) folate deficiencies and corrective treatment possible.	This option should be treated as a screening scenario (Applicability of Wilson and Jugner criteria and C-E analysis needed).	None

	Table II.3			
#	**Application**	**Added value over normal individual care**	**Comments**	**Possible adverse effects**
b.	Comparison with optimal NTD-prevention reference values	Detection of unsufficient or ineffective folic acid supplementation.	If data is collected >8 wks of conception the detection of a low protective level cannot lead to a timely correction for this pregnancy. The information is relevant however with respect to possible future pregnancies.	Iatrogenic anxiety in cases of too low protective levels. At least up to any kind of prenatal diagnosis of NTD. [ss]

Feasibility aspects
A prerequisite for the use of these monitoring data is that they are available and identifiable on individual level and that implies, in most if not all instances, an a priori consent of the subjects of the monitoring to use the data collected for this purpose.

Scenario II would in NL fall under the jurisdiction of WBO and it should therefore be not disputed by health authorities that folic acid supplement treatment is an effective treatment of the condition, (upcoming) folate deficiency, diagnosed.

[ss] The impact might however be compared to actual FA-advicing practice in prenatal care. Whereas it is known that any FA-supplementation started 4-8 wks after conception is not (very)effective, many women are confronted with this deficient supplementation upon reading each information leaflet about this as well when questioned during prenatal visits about their FA-intake behavior. In the Hokkaido cohort study (Yamada, 2012) all participants are informated about folate status by their physician three weeks after the blood sample collection. This is of course a research setting in which informed consent is collected, but this type of settings might yield relevance experience on how women deal with the information and what the results of this screening are in terms of therapeutic indications discovered (with respect to monitor II.1.a).

Appendix III- Study plan

1 Initial study plan

The following plan was submitted initially for the WP 7.

"The context.

1. NL has a nationwide implemented programme for prenatal screening on Infectious diseases and Erythrocyte Immunization status. This screening is offered at the first prenatal consultation to all women and after consent the required blood samples are taken. The costs are covered by governmental public health budgets.
2. NL has, as you probably know, a relatively high adequate periconceptional FA-supplement profylaxis prevalence. This is due to the investment in development and implementation of several strategies to promote FA-supplement use. Over the last 10 years a close collaboration regarding FA-matters has been developed between EUROCAT-NN, the department of Pharmacotherapy and Pharmacoepidemiology of the Groningen University (prof.dr. Lolkje de Jong- van den Berg), the department of Community Genetics of Free University Medical Center in Amsterdam (prof.dr. Martina Cornel, former registry leader EUROCAT-NN) and our company (MediClara of which I am the owner and director; by profession I am health care researcher/epidemiologist). The collaboration regards research, development and implementation of strategies and involvement in policy making. It has strongly added to the increase and maintainance of FA-profylaxis in NL. Our group is constantly seeking for innovations in FA-profylaxis strategies.
3. EUROCAT NL has a good track-record in case-control studies into congenital heart defects and FA-exposure.

The rationale

1. If EUROCAT NN (or any other centre as well) would be able to include FA-serum measurements for the assesment of 'supplement consumption' and/or 'profylaxis-status' in its case-control studie, this would add enormous to both validity and power of these studies.
2. If public health bodies would have insight in FA-serum population profiles, this would provide an easy accessable and maintainable monitor of the FA-profylaxis status, to be used for both policy decisions and evaluation of new and existing intervention programmes.
3. If each woman could be informed about her FA-status this might help in professional advice regarding FA-intake in the remainder of that pregnancy as well as pro-active advice regarding FA-intake béfore possible future pregnancies.

Departing from this context and rationale it is our (=the above mentioned collaboration) intention to prepare and execute a demonstration/pilot implementation of the systematic collection and application (for research, FA-policy making and individual care) of FA-serum measurements in the EUROCAT NN- region. In this initiative several regional organisations and players will be involved (labs, caregivers, insurance companies, research groups etc). A pilot

implementation is needed, because several practical, policy, privacy, ethical etc issues have to be settled in such a way that the maximum advantage of the investment in this programme, would it have to become a permanent one, can be realized.

Because permanent FA-serum measurements can add significantly to the efficiency and relevancy of EUROCAT-research into the preventive effect of FA, we think that it is in the interest of EUROCAT (EU-wide) to learn about the feasibility of it. And moreover, if this would appear to be a feasible system for FA-measurements, the system might well be extended to (maybe in some cases only temporarily) broader assesments of nutritional statuses (vitamins, microspores) and even genetic polymorphisms.

Based on the above mentioned relevance for EUROCAT as one of the stakeholders for this pilot, we'd like to explore the possibilities that EUROCAT can offer as a co-investor in this programme."

2 Approved study plan

In the study plan that was contracted, the deliverables were described as follows.

"The main deliverable of this project is a report including

I. *Appraisal of strategies / methods to monitor folate status on a population level with respect to*:

1. the relevance of (permanent) monitoring of the folate status of women shortly before and during their pregnancies for
    a. scientific research;
    b. policy making (evaluation of the promotion of FA intake and monitoring of intake patterns)
    c. individual care and
2. critical factors with respect to the feasibility of the implementation of different methods of (permanent) monitoring for each of these objectives.

II. *Exploration of the feasibility of strategies / methods identified*

The exploration of the feasibility will be focused initially on the Dutch situation and an (pilot) implementation in the EUROCAT region NN.

The report will be based on a review of the applicable scientific and policy literature and consultations with an expert advisory team.

The draft report will be circulated to experts of a selected number (maximum five) of EUROCAT centers for initial comments on essential differences between countries/regions with respect to the conditions and prerequisites for implementation."

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