

Appendix A1

Recruiting women to the study

The way in which women were recruited to the study was crucial to its success. The following issues were considered in depth:

- when to recruit women
- who would recruit women
- where women would be recruited

Initially, as described in the study protocol, we proposed to recruit women in the antenatal period. Expected advantages of antenatal recruitment were that women would have time to think about the study, and they would be able to contact the study team and ask questions if necessary.

Work to test this approach was initiated. The research midwives compiled and tested draft recruitment forms with small numbers of women, both in the antenatal clinic setting and in parentcraft sessions. The antenatal clinic was used for testing, as there is no selection process; women from all social backgrounds attend. Self-selection occurs in parentcraft classes, and attendance is skewed towards higher social class groups.

In the antenatal clinic setting, not only were the questions in most cases poorly received, but the nature of antenatal recruitment was felt to be invasive, public, and to some degree pressurised and anxiety provoking.

The main problems with the antenatal clinic setting were:

- women had to be approached 'cold' in the antenatal clinic
- approaching a woman made her open to the other people in the clinic and drew what was often felt to be unwanted attention
- there was no privacy, so any questions asked of the woman could be heard by all those in close proximity
- women were reluctant to fill in a questionnaire themselves
- women were reluctant to give full attention, as they needed to be aware when they were called for their appointment
- the research midwives did not know details of the woman's health and pregnancy. In a community clinic, it could be safely assumed the women would be receiving normal antenatal care. However, women attending a hospital clinic might be experiencing problems
- women seemed reluctant to state their feeding intention
- of those who did state breastfeeding, most were very unsure how long they would breastfeed for
- had this form of recruitment continued it might have presented problems with Baby Friendly units. Units with, or attempting to secure, UNICEF Baby Friendly status do not ask women their feeding method, but assume everyone will be breastfeeding
- it was felt that it would have been difficult to recruit to cohort 2 using this antenatal approach, as some women seemed uncertain of their ability to breastfeed, and appeared to see this as another pressure
- the first drafts of the recruitment questionnaire also explained cohort 3, in case women delivered prematurely. This in itself may have provoked anxiety among women at a vulnerable time

Approaching women at parentcraft sessions was a different experience from approaching women in the antenatal clinic:

- the women had the security of being in a group they knew (research midwives never attended at the first session)
- all groups attended were for couples, so women could discuss the study with their partner immediately
- it allowed a valuable opportunity to emphasise the importance of breastfeeding.

In addition to the problems with approaching women in the antenatal clinic, there were other problems with antenatal recruitment. These included:

- difficulties in ascertaining when women recruited antenatally might have delivered their babies
- differences between how the different NHS Trusts collect data and problems with access to data
- issues around data protection. For example, accessing women antenatally would have resulted in SUREmilk staff having to access delivery suite computers on a regular basis to check whether or not SUREmilk women had given birth. This would have allowed them access to details for all women admitted, which contravened good data protection practice. The only alternative was to ask delivery suite staff to fax details of SUREmilk women daily, adding considerably, and unacceptably, to their workload
- probable over-recruitment of women who subsequently chose not to breastfeed

There were a number of advantages to postnatal recruitment:

- target sample are breastfeeding women therefore sample size weighted only for discontinuation rates of breastfeeding
- easy accessibility to participants either on postnatal wards or in homes on routine visits
- community midwives were ideally placed, and were willing, to support postnatal recruitment
- this process was least time consuming for the health care professionals

It was therefore agreed to institute postnatal recruitment for all cohorts and, in addition, to use parentcraft sessions to support recruitment to cohort 2.

Once the rationale for postnatal recruitment was established, the process itself was determined as follows:

Giving information antenatally

One leaflet was designed giving a broad overview of the study, which was to be given to any woman in the antenatal period. Supplies of these leaflets were left at hospital antenatal clinics and antenatal wards, together with posters advertising the study. Community midwives were also given supplies of these leaflets and asked to distribute them to women on their caseloads who might be eligible, at around 32 weeks of pregnancy.

Posters were also displayed in relevant areas (antenatal clinics, parentcraft classes) to promote and advertise the study.

Recruiting women postnatally

For cohort 1: Women would be recruited either at the first postnatal visit in their own homes by the community midwives, or by the research midwives on the postnatal wards (this allowed earlier recruitment and supported shell sample and pump sample collection on day 7).

For cohort 2: Women would be recruited either at the first postnatal visit in their own homes by the community midwives, or by the research midwives on the postnatal wards (this allowed earlier recruitment and supported shell sample and pump sample collection on day 7). In addition, research midwives supported recruitment to cohort 2 by attending Trust parentcraft classes or NCT antenatal sessions.

For cohort 3: Women would be recruited on the neonatal unit, either by neonatal unit staff, or by research midwives.

Recruitment leaflets were designed for each cohort (these are held as 'Study materials' and are available on request).

Appendix A2

Matched group – recruitment and follow up processes and procedures

This small-scale feasibility study complemented the main project. An additional 24 women were recruited solely for this study. Samples of cord blood, meconium (passed within the first 48 hours) and breast milk (collected by breast shell around 7 days post delivery) were requested from participants in this study to allow comparison of levels of residues from each sample type. For rationale, see section 2.2.4, 'Group with matched breast milk, cord blood and meconium samples'.

Information leaflets

The participants' information leaflet was based on those used for cohorts 1, 2 and 3 (held as 'Study materials' available on request). However, particular attention was paid to the following:

- full explanation of taking of the cord blood sample, stressing that this blood sample would be taken from the cord after its separation from the baby
- description of the meconium to allow parents to recognise it

Recruitment and consent forms

The recruitment and consent forms were based on those from cohorts 1, 2, and 3. However, the duplicate copy of the recruitment form was held by the research midwives to complete post-delivery details.

Recruitment of the matched group

All recruitment for this study took place in Leeds, for the following reasons:

- because this was a methodological study, recruiting a representative group of women was less important than collecting complete sets of samples
- this study required a separate ethics application, and only one ethics committee would be involved if only one site was used
- all recruitment and most follow-up was conducted by the research midwives (for rationale, see below) and this reduced the demands on their time
- further, they were on call to collect the samples and to visit the women when they gave birth, which would not have been feasible outside Leeds, where they were based
- delivery suite midwives were involved in collecting cord blood samples and with contacting the research midwives. Using only one centre required that information be given only to midwives in that NHS Trust

The way in which women were to be recruited to this study would be crucial to its success. The following issues were considered in depth:

- timing of recruitment
- where women would be recruited
- by whom women would be recruited
- process of recruitment

It was agreed that the recruitment process would commence in the late antenatal period. The timing of this was complex. Women had to be introduced to the study and given information with enough time to consider participation, while permitting enough time before birth to allow formal recruitment to take place.

The majority of the participants were therefore recruited from antenatal classes, in both units in the city, which they attended late (after 30 weeks) in their pregnancy. This was considered to be the most practical option as:

- regular groups were held in both hospitals
- women attended with their partners, allowing their joint involvement and participation which was considered desirable to secure samples
- the research midwives had access to these antenatal classes
- information could be given to a relatively large group of expectant parents
- the group setting reduced pressure to take part or any anxiety of feeling targeted
- information was given to the whole group, those wishing to take part gave their contact details to the research midwife
- recruitment then took place in the woman's home at a mutually convenient time (usually evening), which allowed time for information giving in a private and comfortable atmosphere

The research midwives recruited this group as:

- the group was small in number. Educating other health care professionals to do this recruitment would have taken more time than actually securing the samples
- close tracking of the women was vital to ensure all samples were obtained. Research midwives had a good working relationship with staff in all areas of the unit and access and information gathering was never a problem
- the women appeared to appreciate the continued personal contact (all women were seen at least twice)

Twenty one home recruitment visits were made by the research midwives. Five women were recruited in hospital, and seven parentcraft presentations were made. Four postnatal visits were also made, to collect samples.

Study approval and procedures

The protocol was submitted to the Local Research Ethics Committee for both Trust hospitals, and the Head of Midwifery was contacted by letter for permission to conduct this study. Approval was received in writing.

As soon as ethics approval was gained, the following procedure was followed:

- a letter was sent to and meetings arranged with senior midwives on delivery suite and postnatal wards
- presentations were given to staff on above ward areas

- antenatal recruitment of women commenced via antenatal classes taking contact details of those interested and giving verbal and written information
- recruitment of women in their homes was conducted, completing recruitment and consent forms
- ‘Alert’ stickers were attached to the front sheet of each woman’s hand held records (available as ‘Study materials’ on request)
- delivery suite staff took cord blood at the birth, and either saved it for the research midwives to collect or sent it to the laboratory via the internal ‘shuttle’ system
- the research midwives were notified of the delivery of each participant, and the ward to which she was transferred
- soiled nappies were saved in a labelled, sealed plastic bag which was then placed in a lidded box on postnatal wards for collection by the research midwives, or if the mother was at home, they were collected from there by the research midwives
- on collection of soiled nappies, the research midwife gave out a shell pack with verbal explanation and written instructions on how to collect the milk sample
- breast milk sample returned to laboratory by first class post as described for cohort 1 and cohort 2

A small group of women (n=5) were recruited on the antenatal wards prior to induction of labour. This was primarily to make up the required numbers, and it also allowed testing of another recruitment route. To ensure that women had chance to consider participation carefully, women were chosen at the beginning of their stay for induction of labour; this can take up to 36 hours for most primiparous women. No woman who was in labour was approached; this was not felt to be appropriate.

Collecting cord blood

Delivery suite staff were asked to secure the sample. To ensure that this process ran smoothly careful planning was needed:

- research midwives informed as many delivery suite staff as possible of the project and the requirements by formal and informal routes
- laminated posters clearly explained the requirements, and all had contact numbers for research midwives (24hour access)
- the women and their partners were asked to remind staff on delivery suite to secure cord blood samples
- ‘Alert’ stickers were clearly and securely fastened to the front of each woman’s notes detailing requirements and giving contact numbers for research midwives
- to minimise the involvement, and room for error, sample bottles were pre-barcoded and placed in the women’s notes with return lab forms
- research midwives were called when blood samples were obtained and the best route for collection was discussed. On weekends and bank holidays, when internal systems did not function, the research midwife collected the sample and kept it in the fridge until the laboratory was open; she then delivered it herself. On week days, the internal shuttle system was used.
- auditing of the mother’s blood group would indicate whether samples were less likely to be obtainable when cord blood was needed for other appropriate testing, such as Kleihauer testing for rhesus negative women, and thalassaemia testing. pH testing may also be conducted, in the case of a difficult birth. The SUREmilk samples were the last ones to be taken, and auditing was intended to indicate whether or not there were circumstances in which collection was not possible
- labelled blood tubes and sample bags were secured in the hand held records to ensure availability at all times and to prevent misplacement

Collecting meconium

In consideration of collecting meconium samples the following factors were noted:

- the recruitment process required a system to alert the research midwives that participants had delivered in order that samples could be collected
- by involving partners at recruitment it was hoped that their involvement in collecting samples would follow
- samples were to be collected as passed in the nappy, to ensure ease of collection for parents and to obtain as much of meconium sample as possible
- nappy liners were to be used to minimise absorption into the nappy itself
- bar-coded bags and nappy liners were prepared and secured into the mother's hand held records to ensure availability and to prevent misplacement
- bags were labelled with date and time of collection to monitor sequence of samples
- samples were placed in labelled receptacle on postnatal ward to prevent misplacement
- regular ongoing presentations were carried out for ward staff, including health care assistants, to ensure their participation in securing samples
- brand of nappies used were noted on recruitment form in case of unexplained findings
- sample from each batch of nappy liners used were saved in case of unexplained findings

Collection of milk

Shell samples were collected as protocol for cohorts 1 and 2.

The results of this study will be published separately.

Appendix A3

Selecting NHS Trusts and maternity units

The study proposal identified West, South and North Yorkshire as the intended regions from which the study sites were to be selected. Using regional maps and a regional Trust index, a total of fifteen units were identified throughout these three regions. Some Trusts had more than one hospital based on different sites. The Trusts fell into three categories; those with more than 3000 births per annum (large); those with 1250-3000 births per annum (medium) and those with fewer than 1250 births per annum (small).

After taking into consideration the research midwives' time and travelling commitments and the recruitment process, it was agreed that it was feasible to target six Trusts.

The decision on which Trusts and maternity units to include in the study was based on the need to achieve a representative sample. Sites needed to represent:

- social, ethnic and demographic variation
- geographical variation
- a mixture of District General Hospitals and Teaching Hospitals
- both urban and rural locations

Also taken into account were:

- the number of deliveries at each unit
- the number of neonatal unit cots
- whether there was an established contact, known to the research team, within the unit

Other factors which had an impact on the final decision were:

- the working patterns of midwives in the units (which impacted on transport of samples)
- the management structure and working of the unit (which had an impact on how possible it was to work directly with the midwives)
- whether or not other ongoing research studies would make this study difficult to conduct

Before the final decision was made on which units to approach, information was collected about each proposed unit. Data were collected from a range of sources, and were not always easy to obtain. Using a network of contacts through the Regional Breastfeeding Coordinators, information was gathered detailing local statistics and facilities, in particular the number of deliveries, number of primiparous deliveries, neonatal units and midwifery staff and useful contacts. Local IT departments were helpful in providing statistics required. Data collected included:

- number of births per annum
- number of primigravid women delivering in the unit
- number of community midwives, and team structure
- number of postnatal beds
- number of neonatal cots
- breastfeeding rates

A list of preferred sites was drawn up in the light of all the above factors. These included: Leeds Teaching Hospitals and Bradford NHS Trusts (large units); Dewsbury and Huddersfield NHS Trusts (medium) and Harrogate and Scarborough NHS Trusts (small). The remaining Trusts were sorted into three categories and served as the back-up list.

Each site was then approached by the research midwives. First contact was through their known contact person, to make informal enquiries about the unit and its potential to be a study site. This contact person was then asked to provide the names of key people and to introduce the research midwives to the maternity unit. Key people included:

- the Head of Midwifery
- team leader for community midwifery
- antenatal clinic sister
- postnatal ward sister
- parentcraft coordinator
- neonatal unit team leader
- consultant obstetricians
- consultant paediatricians

The first formal contact was to the Head of Midwifery. The initial approach was by letter (copies of letters are included in 'Study materials' which are available on request).

The key points of this letter were:

- a brief introduction to the aims of the study
- why the unit had been identified as a site
- what it meant for the unit in terms of staff involvement
- reassurance that the research midwives would lead the project within each unit
- a request to meet and discuss the project further

This approach was well received and each Head of Midwifery approached did request to meet the research midwives for discussion of the project and its implications.

These meetings had common themes:

- concise presentation of what the project was about (using a standard presentation format)
- requested involvement of the unit
- requested involvement of staff

The concerns of the heads of midwifery tended to be:

- staff workloads
- care of the women approached
- possible effects on breastfeeding
- implications for Baby Friendly status

These points were easily dealt with as the research midwives were able to demonstrate that staff involvement would be at a minimum by outlining the following points:

- the number of women we expected each community midwife might recruit was discussed (this was always in single figures)
- all midwives would receive training at their convenience
- all midwives would have an information pack giving concise and precise details on how to recruit
- the length of time for each recruitment was approximately 10 minutes
- pre bar-coded packs saved time
- recruitment was linked to information routinely given regarding breastfeeding and expressing
- women requiring more detailed information should be referred directly to project staff
- community staff would have an in-depth knowledge of the women on their caseload and would approach and recruit only those they felt applicable
- the planned approach to women by the research officers in the postnatal period was only at the permission of the ward staff
- the research officers were practising midwives and appreciated the vulnerability of these women
- the risk to breastfeeding rates was addressed and the project was presented as a device which could be used to encourage breastfeeding, highlighting its importance and the need to protect it
- asking for a day 7 drip breast milk sample was also well received as it was less demanding on the woman and less likely to have an adverse effect on breastfeeding
- Baby Friendly status was not an issue as the information sheets provided were in line with the good practice guidance issued by the BFI, and the aim of the study was to promote and protect breastfeeding. It was also appreciated that the research midwives had a good understanding of BFI

Following initial discussions and negotiations with each maternity unit, five out of the initial six Trusts were willing and able to participate. One NHS Trust declined after the R&D Board was unable to grant approval due to shortage of staff. No replacement was necessary, as one of the large NHS Trusts, which had two separate large units, could generate the numbers needed. One NHS Trust was unable to agree recruitment by their own midwives due to staff shortages, but offered to support recruitment by the research midwives.

Following full agreement by the Trust and staff, and formal permission from R&D and ethics committees, presentations to staff then began.

Presentations to identified key people were at times and locations requested (these are held as 'Study materials' which are available on request, as are copies of the PowerPoint™ presentation). They were made on the wards at frequent intervals as staff tended to rotate, and so became a key part of the visits to the unit. Presentations were well received and seemed to motivate staff to identify eligible women.

One of the first group presentations given by the research midwives was to the Regional Neonatal Nurses. The group were specifically asked about their thoughts on recruitment of women to cohort Group3. No specific concerns were voiced. The views which did emerge, were that the background knowledge we had as midwives obviously meant we had identified the problems in advance and seemed to have dealt with them effectively. It was felt that the right approach should be only to ask for milk which was not needed by the baby, and the best way to do this was using the approach we identified of only collecting milk once the baby was ready to leave the unit. The group also felt that however interested the Neonatal nurses may be in the project, their workload meant that their input would be minimal. Most saw no problems with distributing information leaflets to eligible women. They felt the approach we were intending to use was sensitive and were happy that women should not feel pressurised.

The same approach was then implemented for recruitment on Neonatal Units (NNUs), with the research midwives giving presentations to groups of staff at frequent intervals; this was the only group who requested that the research midwives also present to night staff. This may be due to a higher proportion of permanent night staff. The request was supported.

Appendix A4

Developing and testing written information for women and staff

Four types of written information were developed and tested:

1. antenatal leaflets for all women
2. cohort-specific postnatal recruitment leaflets
3. information for staff
4. posters to support recruitment

All of these 'Study materials' are available as on request.

1. Antenatal information leaflets

The antenatal information leaflet was designed to be accessible to all women. It aimed to give a brief explanation of the study, and to give contact details for any women who wished to have more information. Information used in this leaflet also formed the basis for the postnatal leaflets, the posters and the staff information, so most of the development time was spent on this leaflet.

The preliminary leaflet was based on the experience gained by the research midwives during the pilot of antenatal recruitment. Experience gained by Caroline Harris during her research was also drawn on, as was the experience of a breastfeeding counsellor and researcher who had offered to act as backup to Caroline's study in case of queries from women.

This preliminary leaflet was taken to a local National Childbirth Trust (NCT) group to gain their opinion on the proposed written information. The main themes raised were:

- need for clear messages that a woman's possible inability to express is not related to her ability to breast-feed

- concern that if a woman was left with a breast pump she may be encouraged to express milk and give this to her baby by bottle
- that there should be some mechanism for health professionals to be able to feedback to the research team if any undesirable outcomes were identified
- that it would be appropriate for NCT breastfeeding counsellors in the areas to be included in the pilot to be sent the information leaflets
- that some teachers/counsellors might be happy to give the antenatal information to their groups

The National Network of Breastfeeding Counsellors (NNBC - Yorkshire Region) was also approached to solicit their views on the written information. The response was very similar to that of the NCT, although no one from the NNBC had any concern about women receiving breast pumps. This is likely to be because most units represented were involved in seeking Baby Friendly status, and one of the requirements is that women should receive information on expressing milk.

The style of the information leaflet changed, as several drafts were prepared and edited according to these comments. Further revisions were made in response to input from members of the research team, other researchers working in the same field, and community and acute unit midwives.

The final format of the leaflet was short, succinct paragraphs, with bullet points to reduce the amount of text. A question and answer format was used. The leaflet was presented as a folded sheet rather than A4, as it looked less daunting to the reader.

The word 'contaminant' was never used as this was felt to have the potential to provoke anxiety; as an alternative, the word 'residue' was employed. 'Milk bank' was also avoided, as people then made the assumption that the milk was for consumption. 'Archive' was initially used, but this was found to be a word many mothers were not familiar with. The outcome of this debate was to use the term 'store'.

All information was produced in accordance with Baby Friendly initiative (BFI) guidelines.

2. Postnatal information leaflets

A leaflet was designed for each cohort, giving information about the study, and detailing the requirements for each. These were colour coded for ease of identification. These information leaflets used the same format as the antenatal information leaflet and were in the same layout and design. They were designed to be given at recruitment, as a summary to back up verbal information given by the recruiting midwife, and also as a reference for the woman. Like the antenatal leaflet, the content included a summary of the project. It went on to detail what was asked of the participant and when. Contact details were included should the women need further information or support, and women were encouraged to contact the study team if they had any questions.

These leaflets stressed the fact that many women who successfully breastfeed will not leak milk, or may find it difficult to express milk. This was identified by many commenting on the leaflet as an extremely important message.

All the information leaflets were in accordance with Baby Friendly guidelines, as all of the units involved in the pilot were either actively seeking accreditation or working within the guidelines of

good practice. When shown examples of the finalised information leaflets, no staff had any queries or anxieties about the wording, format or information in general.

3. Staff information

Various types of formats were discussed and costed. The initial plan was to produce a summary flowchart, to be used as a reminder of the project and a guide for recruiting, which would fit into the midwife's own diary. Laminated flash cards were discussed, which highlighted who was eligible and a flow chart of how to recruit. A5 (diary-sized) information booklets were also considered, containing the same information.

The final format was an A4 booklet, which fitted into the folder provided for each community midwife. It gave a brief overview of why the study was being conducted, who we were aiming to recruit and the difference between the cohorts. The guide used bullet points to guide the midwife through the recruitment process, and a summary of the pertinent points of sample collection. This last was requested by at least one midwife at every presentation performed, the usual words being, 'is that written somewhere as an idiot's guide?' A brief overview of what would happen next was also given, allowing the midwife to remind the woman of the follow up by the SUREmilk team.

The A4 leaflet was then given to each community midwife as part of a recruitment pack, which contained all the information sheets and paperwork to recruit a woman.

4. Posters

Two types of poster were developed. A poster introducing the study was provided for each ward area and antenatal clinic for each Trust. Posters were not provided for GP clinics, as the costs precluded this. Posters were also produced for neonatal units and the transitional care ward, in regard to cohort 3. These were located on each breast milk storage fridge and in the areas used to express milk

As before, these were designed to be in line with BFI good guidelines practice. A small pilot was conducted with Leeds midwives to check the language and presentation.

Appendix A5

Reasons given by women for declining participation in SUREmilk

Seventy seven women declined participation when approached by the research midwives. Forty two gave a reason for this; their responses are detailed below. Women who did not give a reason for declining were never pressured to do so. The Bradford Local Ethics Committee specifically requested that women who declined not be asked for a reason.

Reasons given for declining participation included:

Can't decide about participation now (n=20), including:

- wants to think about it, will read leaflet / talk with community midwife (8)
- too tired to discuss it (5)
- too soon to decide (2)
- did not want to discuss, had visitors (2)
- needs husband's permission (2) / wish to discuss with partner first (1)

Might not be carrying on with breastfeeding (n=14), including:

- unsure wants to continue bf / not sure if will succeed with bf / not confident about bf / not settled yet with feeding / feels too early - not sure about feeding / bottle feeding at the moment, will try bf again though / probably won't continue to feed / won't bf for long / would do sample now but not prepared to do one at home (9)
- problems with feeding / finding bf difficult, didn't want more pressure / 'struggling', not sure about taking part (3)
- sore, not interested / too sore and too soon to ask (2)

Not happy about aspects of the study (n=6)

- milk is for babies not for anyone else / rather not - early days and wants all milk for baby / not interested in donating breast milk (3)
- not happy about expressing
- sounded too complicated
- not in mood for research!

Appendix A6

Collecting dietary information to inform SUREmilk analyses

In the collection of dietary information we adhered to the same principles applying to the rest of the study, set out on in the Introduction to Section A. We did not wish to overload participants by placing an excessive burden on them, which might either influence their compliance with other aspects of the study (e.g. milk sample collection), or leave them with adverse views about their participation in the study.

With regard to dietary information we also sought only to collect data which we knew we had the capacity to analyse in terms of exploring their association with contaminants present in breast milk. For exhaustive dietary data collection, information should be collected on both the recency and frequency of dietary intake. Using the former it may be possible to quantify the association between the presence of a dietary ingredient and the presence of a contaminant. Using the latter it may be possible to strengthen this finding, by showing an association between amount of dietary ingredient and amount of contaminant. If, however, the former cannot be demonstrated then there is now value in collecting frequency data. Accordingly, its exclusion might benefit the objectives of the study by not placing an excessive burden of data collection on the mother.

When collecting dietary information, there is also a balance to be reached between the collection of recent, or current, information and historical information. Certain short-lived contaminants (phthalates, phytoestrogens) may be derived almost exclusively from recent diet, while more long-lived, persistent contaminants (OCs/PCBs, dioxins/furans) may be more likely to appear as a result of their release from subcutaneous fat stores at energetically demanding times, such as during lactation. Their appearance, in turn, reflects historical exposure, with diet during adolescence and early adulthood being a potential contributor. Trying to collect accurate, relevant data on diet at this time, which will inevitably be retrospective, is prone to being flawed.

In certain cases, however, the presence of key contaminants (e.g. *p,p'*-DDE & *p,p'*-TDE versus *o,p'*-DDT & *p,p'*-DDT) may permit historical exposure to be distinguished from more recent exposure, given that the parent form may be relatively quickly metabolised, whilst its metabolite might be more resistant, and hence more persistent in the body and/or environment.

A third issue concerns whether the aim is to characterise an individual's normal or typical diet, as compared to their precise intake in an immediately preceding period. Given that pregnancy and lactation are notorious times when a woman may change her diet (for either physiological or intellectual reasons), seeking to characterise her typical diet should relate to the period before she knew she was pregnant (or before conception). Depending upon when dietary data are being gathered this might be nine months to one year previous, and the longer the intervening period the greater the likelihood of recall error. In contrast, if a contaminant of breast milk is likely to reflect recent consumption an accurate rendering of intake over the past week may be more important.

Finally, more qualitative evaluations of a person's dietary lifestyle may be of equal importance for devising dietary intervention programmes. A woman's self-evaluation of her dietary objectives (meat-free/vegetarian, low fat, high fibre) may provide an accurate portrayal of her dietary targets. Giving her altered dietary targets may be part of an intervention, in which case, the accuracy with which she complies with her dietary objectives will also become important.

Accordingly, certain strategic decisions were made concerning the timing of, and amount of, information concerning dietary intake.

Points of dietary data collection:

1. **Recruitment:** The only time that complete data collection could be guaranteed for each participant was at the point of recruitment when women were co-opted into the study. At this point, the woman's responses were recorded on the form by the person recruiting that mother (study investigator or community midwife); these data proved 100% complete. Accordingly, at this point we simply sought to determine whether there were key dietary exclusions (meat, fish, dairy products) or dietary regimens (vegan, vegetarian) to which the participant ascribed. Collection of data at this point allowed us to evaluate whether we were under- or over-recruiting women of a particular type, although this information was never used in a restrictive manner. As these data are available for all participants, whether or not they supplied a milk sample, they allow a comparison of certain dietary characteristics between all women recruited into cohorts 1 and 2.
2. **Dietary data linked to breast milk sample collection:** The focal point of dietary data collection was linked to the collection and provision of breast milk samples. We chose to tie the collection of dietary data to the point of sample collection so that, at each and every point of collection, we enclosed a dietary recency survey.

This 'recency survey' took the form of a dietary questionnaire, in which women were asked about their dietary intake in the 7 days prior to giving each sample. The six-sided questionnaire entitled "Your recent diet" was given or sent to the women with each shell/pump pack, with a request that she complete it at the time she gave each of her samples. Women were asked to tick if they had eaten a food from the given list in the last seven days. The comprehensive food list was developed from the UK Women's Cohort Study (UKWCS) (Calvert et al, 1997) in consultation with Dr Janet Cade, Nuffield Institute, University of Leeds. This list was modified in the light of piloting. There were also free entry text boxes for listing several possible recent changes of diet. These were: food avoided in breastfeeding / food only eaten during breastfeeding / food increased in breastfeeding / other not listed foods. Women were also asked how long it had taken to complete the survey.

The dietary questionnaire comprised 25 broad descriptive categories:

- bread & savoury biscuits
- breakfast cereals
- potatoes, rice & pasta
- dairy & non-dairy products
- butter, spreads & oils
- spreads
- sauces & soups
- grains
- nuts & seeds
- pulses
- eggs, egg dishes
- vegetable dishes
- meat
- other meats
- fish
- vegetables
- fruit
- seasonal fruit
- dried fruit
- sweet snack
- savoury snack
- beverages
- alcoholic beverages
- biscuits, sweets & puddings
- other foods

Under these headings were 245 specified food items, with a further 20 options for including 'other' items not specified, permitting the participant to specify items in an open-ended manner.

The food recency survey was first piloted with ten pregnant women as part of a full diet survey. Women commented that the full diet survey was too complex as it asked about recent and past diet, plus frequency. This took women an average of 22 minutes to complete, with one woman completing the survey incorrectly, detailing only past diet. The recent diet survey took 10 women an average of 9 minutes to complete, so this was adopted. It was felt to be important not to overburden women with paperwork and that responses be as accurate as possible, especially as women were already asked to do a number of other tasks for this study. The survey was also piloted with six members of the project team. Additional foods identified by women and the project team were added into the lists, as was an 'Other' food products box in each food group (e.g. 'Other breads, please name') in response to comments. Alcohol was included as part of beverages in this survey, having been removed from the lifestyle questionnaire to shorten the latter. The final version of this then became the Dietary Questionnaire (DQ), (see Study materials file).

3. Lifestyle Questionnaire: The Lifestyle Questionnaire was sent to all consented participants, irrespective of whether or not they had provided a breast milk sample when (or soon after) their baby was 16 weeks old. The timing of this questionnaire meant that a lower than expected response rate was possible, meaning that certain information might not be secured from the whole sample (c.f. information collected at recruitment).

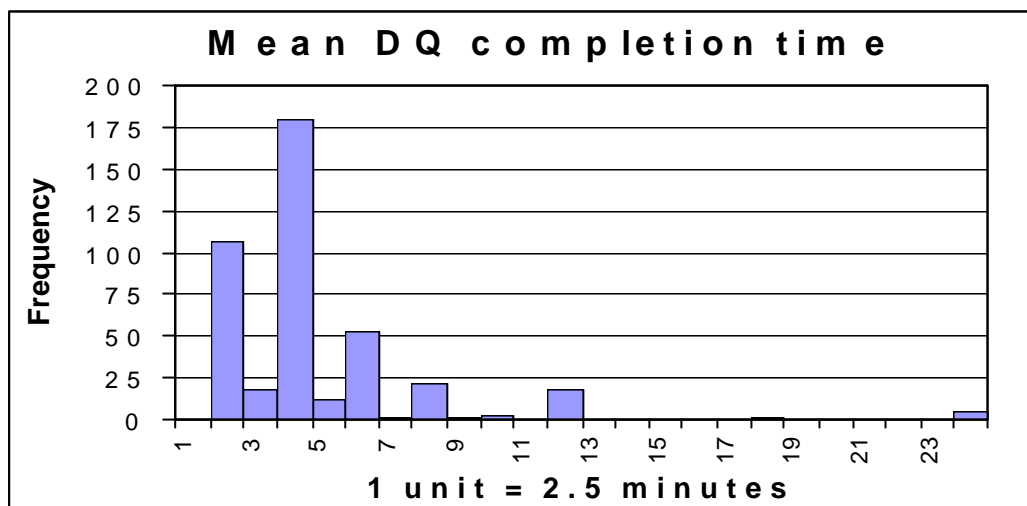
The dietary questions included in this questionnaire were general ones relating to the woman's dietary lifestyle. They were concerned with such issues as: were any vegetables consumed home grown, what type of milk consumed (cow's, goats, whole, semi- or skimmed, etc), what does she do with visible fat on meat, does she eat out much or eat takeaway foods? These questions, therefore, tapped qualitative aspects of a woman's eating habits. The questionnaire also sought to establish whether the woman had changed her diet recently, and if so, for what reasons.

The Lifestyle Questionnaire was completed by 228 of the 379 women recruited into cohorts 1 and 2, 60.2% of the total. Accordingly, data on lifestyle were secured from a marginally smaller subset of the study population than from whom samples were secured (62.0%) suggesting that retrieving data at 16 weeks after birth by a separate process from sample collection is only slightly less effective.

Dietary questionnaire completion times during main study:

Figure A6.1 shows the distribution of the lengths of time taken by participants to complete the dietary questionnaire (DQ) during the study, for both cohorts (1 & 2) and across both sampling methods.

Figure A6.1: Participants dietary questionnaire completion times



There were few differences both between the two cohorts, and as a function of whether the dietary questionnaire (DQ) followed a shell sample or a pump sample. The DQ accompanying a shell sample was invariably completed prior to one accompanying a pump sample, so a small reduction in completion time is likely to have resulted from having undertaken the task once previously. These data are shown in Table A6.1 below.

Only 4 questionnaires were completed in over 30 minutes (3 taking 60 min, 1 taking 45 min); otherwise the majority took 5-15 minutes to complete. The median completion time was 10 minutes under all circumstances.

Table A6.1: Time taken to complete dietary questionnaires

All DQs	Cohort 1		Cohort 2		Overall		
DQ accompanying	a shell sample	a pump sample	a shell sample	all pump samples	all shell samples	all pump samples	All samples
Mean (min)	11.88	10.68	13.18	10.85	12.06	10.78	11.48
n =	181	75	37	110	218	185	403
Median (min)	10	10	10	10	10	10	10

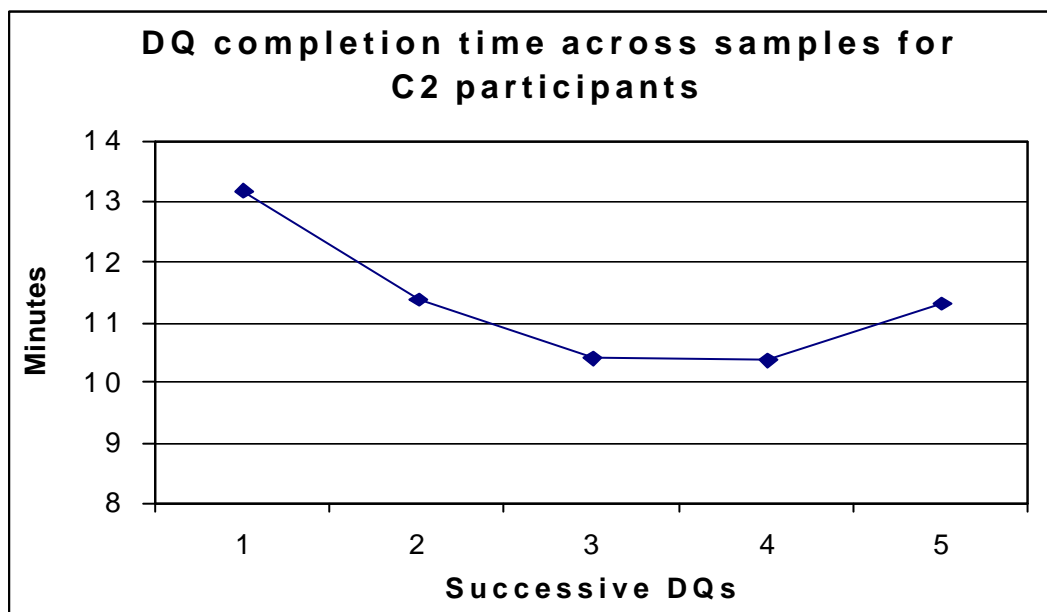
Many more shell samples were received than pump samples, affecting the number of dietary questionnaires received. Accordingly, in Table A6.2, only data from participants who completed a questionnaire after both a shell and a pump sample are included, as this gives the best indication of changes in performance time. The mean completion times are 13.1 and 10.9 minutes, indicating a reduction of difference of just over 2 minutes following prior experience of the dietary questionnaire.

Table A6.2: Time taken to complete dietary questionnaire – both shell and pump samples obtained

Matched DQs	Cohort 1		Cohort 2		Overall		
	shell sample	pump sample	Shell sample	1 st pump samples	shell samples	1 st pump samples	paired samples
Mean (min)	12.81	10.57	13.60	11.66	13.06	10.92	11.99
n =	72	72	34	34	106	106	212
Median (min)	10	10	10	10	10	10	10

Finally, the following figure shows the change in dietary questionnaire completion time by participants in cohort 2 across the repeat pump samples they collected.

Figure A6.2: Change in cohort 2 dietary questionnaire completion time



Dietary data are likely to have variable potential to explain variation in contaminant levels in breast milk; for certain analytes this potential is likely to be quite high (phyto-estrogens, phthalates), with up to 90% of the variance potentially being explained, for others analytes it may be much lower.

Appendix A7

Lifestyle survey

The purpose of the lifestyle survey was to collect background information about the women in the study. It was a four-sided self-completion survey sent to women at least 16 weeks after their baby's birth. Previous addresses were collected - residence in rural areas may have led to potential exposure to some pesticides. Occupational histories were also thought to be important to assess potential industrial exposure to contaminants. Both residence and occupation were collected in the form of census-style questions (based on the UK 2001 Census) for five years prior to the survey rather than full histories as the latter would make the questionnaire too lengthy. A study which collected similar historical data on residence and occupation (the UK Childhood Cancer Study, G Law, pers. comm.) found that children had an average of two changes of address in the first five years of life; some have up to 14 moves in the first 10-15 years of life. The 1991 census found that an average of 6.7% of people move each year (1991 census).

To measure mobility and population mixing, a question about the numbers of times women had moved house was asked. Additional questions were asked about country of birth, and where the respondent spent most of their childhood. Place of study was asked of participants who were still in education. Questions of dietary lifestyle were developed by the SUREmilk team in consultation with Sarah-Jayne Rowles at the Foods Standards Agency, Caroline Harris at MAFF and Shaun White and colleagues at CSL. Questions about source, storage, processing and cooking of food and changes in diet were asked, as were questions on smoking habits.

Of the questions for possible inclusion in the lifestyle survey, it was felt that current occupation (also used for socio-economic classification), religion (as a proxy for dietary exclusion), and the exclusion of meat and other foods, would be the most important variables for analysis and so these were asked on the recruitment form along with current address.

Women were asked how long it took to fill in the lifestyle survey. When originally piloted this took women on average 40 minutes to complete. Only 2 of the 4 women who agreed to complete the draft survey filled in the time it took, and several women refused to complete the survey as it appeared to them too lengthy. The survey was also piloted with six members of the project team. Women commented that the A5 format with 9 point text was too small so this was changed to an A4 format with 11 point text.

As a result of piloting, the questionnaire was reduced in length. Questions about alcohol consumption were removed and alcohol included as part of beverages in the Food Recency Survey. Free text responses for questions about anthropometric measures such as body size and shape were replaced with tick boxes about eating plans and changes in diet. This both reduced the time taken to complete the survey and reduced data entry time. Residence and occupation at one year prior to the survey questions were removed, as responses in the pilots were similar to the 5-years ago question. Socio-economic indicators of car ownership and age at which women left higher education were removed, as an indicator could be derived from current occupation from the recruitment form. Questions of own and family breastfeeding history were removed as women did not always know the information and it involved lengthy and complex questioning. The final version of the lifestyle survey, comprised 36 questions, of which 17 addressed issues of dietary lifestyle, and a further four were on smoking (a copy, held in 'Study materials', is available on request).

Appendix A8

Ethical issues surrounding contaminant levels in women's milk

The ethical issues associated with a study of this nature are enormous, complex and of a far-reaching nature.

The recruitment of women, collection of milk samples, of lifestyle data by questionnaire, and assays of the milk for potential contaminants raise little more than standard concerns to be protected against in a routine manner. The point of merger, however, between the personal details held on the database with data on contaminant levels in milk represents an ethical minefield. There are several interested parties to be considered, seemingly with competing concerns; for instance, one ethics committee raised the concern of litigation in NHS Trusts where women were recruited, should their milk be found to contain contaminants.

Conflicting priorities exist in this situation, which include the researchers' obligations to the woman donating the samples, the woman's obligations to her child, the researchers' obligation to that child, and the individual woman's right to information about herself, that might affect either her or her child's future health. These conflicting priorities are most easily seen in the situation where a milk sample is found to have high levels of a contaminant which might reasonably be expected to cause harm to the infant. The problem, however, is that there are still a number of unanswered questions about whether or not such levels will cause harm.

Questions that might be asked by the research team when finding a raised level of contaminant X in a milk sample would include:

- What do we know about the potential harmful effects of X?
- Do we know what levels of X may be capable of provoking harm?
- Do we know how this infant/child might be affected?
- Are there existing data from past studies?
- Do we have details of what may be regarded as 'safe' levels?
- What are the TDIs for contaminant X?
- Where do these data come from?

The swiftest and simplest way out of this dilemma is that insisted upon by the MREC, which was that at the point at which contaminant data are to be entered onto the database, all personal details by which a donor could be identified are removed from the database. This protection may not in itself be enough to prevent concern, however, as the feedback of general results about the cohort to individual contributors may still provoke alarm, concern, and misplaced fears and assumptions about the identity of key donors.

There are some key questions which need to be asked whenever raised levels are found in samples. These could include:

1. Is there anything we could do now to improve the future health of this child, knowing that it has been exposed to these levels of substance X? (Yes / No)
2. Might the child present with neuro-developmental problems at a later stage? If so, and the issue was raised that the problems might be associated with past exposure to toxic agents, the mother might recall her involvement in the project. If further information were sought from us, and we revealed that we had previously had access to these results but elected not to

disclose them, then might we not be held responsible in some way for withholding such information? The MREC policy decision was intended to protect against such events.

3. Irrespective of the current issues, the results may have an implication for the feeding of future children (as ours is a study of primiparous women). But then, we are not able to extrapolate from the present results to multiparous women.

These ethical issues will be a key concern for the national archive. If neuro-developmental follow-up is planned, as originally outlined, it will be essential to be able to link levels in the milk to the identity of the mother and the child. Protocols will need to be developed to protect all parties in this situation. The experience of the National Blood Authority, who have extensive experience of similar ethical dilemmas, may be helpful in this regard.

Appendix A9

Presentations and training for staff in the participating NHS Trusts

The research midwives devised a plan for visiting the five NHS Trusts in a staged and rolling process, to give formal training sessions on recruitment to as many community midwives as possible, and formal information-only sessions to relevant hospital-based Trust staff. However, as ethics approval for each Trust and even for each maternity unit within a single Trust was gained on a staggered basis, it was more expedient to conduct the sessions according to the order in which ethics approval was gained.

It also transpired that one-to-one sessions at midwives' request were necessary, and opportune informal sessions to take account of staff rotation on the ward areas were carried out. Due to staffing systems in the smaller units, it was easier to access the majority of staff with fewer visits than in the more fragmented systems of the larger units, where staff turnover was rapid. The research midwives also met the requests of the Trust midwives to provide opportune information sessions for students working in the units.

Due to several previous formal and informal visits to all units, meeting with contacts, midwives, Heads of Midwifery, clinical managers and ward managers, the research midwives had ascertained the working set up of each Trust and/or maternity unit and gathered information on staff names, contact numbers, base locations and had secured team meetings invitations.

In the smaller units the community midwives all met at the hospital base at regular intervals, and it was possible to give a formal presentation and give out packs to the majority of staff in the course of these meetings. In addition, however, it was necessary to visit each of the rural outlying community units attached to one of the small Trusts and it was difficult to have access to many of the staff who rotated within these. Staff in the medium Trust were divided into five teams with different bases throughout the area. Ground work with the clinical manager and ward staff was needed before access to these teams was granted. The two large units within the large Trust proved the most costly in terms of time resources. Here, midwives were divided into 12 teams, each with different bases. Due to workload and pressures, meetings were less frequent and more sparsely attended. The research midwives tried to target sessions at all the teams and also at larger PCG/T meetings. In the Trust where exclusive research midwife recruitment took place, valuable visits were made to provide information sessions for the clinical manager and the ward staff, including the background to the study and the recruitment process. This resulted in ease of access to the wards, participants and necessary information from the notes.

Content of the presentations

Draft presentation: Initial drafts of the presentation used words such as ‘environmental contaminants’, talked about possible risks and vulnerable groups. The research midwives then decided that as the written information to women was to be carefully worded to prevent undue anxiety, and was not to include these terms, then neither should they be used when talking to midwives. It would of course be impossible to exclude them from discussion, but they were not to be used on the written information provided. It was hoped that this would reduce the use of these terms by the midwives themselves when they were recruiting women.

The initial draft presentation was quite lengthy, and included information about ‘hot foods’ – a list of food representing potential sources of contamination. The draft presentation also included information about feed-related factors, and quite detailed information about the laboratory side of the project.

The research midwives modified the initial draft to make it accessible to the majority of midwives, based on the following principles:

- midwives may have little time available for recruiting
- messages have to be succinct
- important to focus on what the midwife will be asked to do and why
- important to describe any effect it may have on the women cared for by the midwife
- it is important to describe any effect it may have on the midwife’s workload

This draft was then tested with local midwives to gauge:

- the level of interest
- the level of motivation
- the types of questions asked
- their understanding of the principles of the study

This information was used to modify the draft presentation to its final state, which was succinct, used clear audio-visual aids, and left a sizeable amount of time for questions.

Summary of final presentation

- Who was doing the study and who was funding it - this was very important and secured the initial interest
- What is the study about, and why is it being done
- Emphasis that the study should be used to promote breastfeeding as it highlighted the importance and possibility of protecting breast milk
- The study was a pilot study and therefore all information was extremely useful. So if the midwives found problems with recruitment or returning samples, then this was as valuable as the easy return of a sample. In effect, whatever the midwives did it was going to provide us with useful information. This was an important message to give the midwives, as it seemed to reduce anxiety about their involvement and performance
- The pilot was about testing the information, gaining information on how many mothers would be able to produce a sample, the average sample size, the method of getting the sample back to the laboratory, and about how much the study impacted on the midwives time
- Why three cohorts were being used, and how many samples would be required.
- Use of diet and lifestyle questionnaires, and why this was relevant
- The information to be given to the women and when we would be contacting them
- What exactly we were asking the midwives to do

The level of commitment for each midwife varied depending on their location of work. The community midwives were asked to give the most commitment to the project. They were initially defensive of their time, however, on listening to exactly what we were asking them to commit to, the vast majority saw no problems. The main problem, which was raised by some community midwives, was the fact there was a low breastfeeding rate in their area and they were unsure whether they would have enough women in their caseload who met the criteria. However, once it was explained that if each midwife recruited two women, then the unit's commitments would be fulfilled, again most felt that this was quite feasible. The midwives who were working on the postnatal wards had very little input, and were only required to give women who met the criteria an information leaflet on the study and alert us to women who could possibly be recruited. This was a similar commitment to staff on the neonatal unit, who were asked to support recruitment to cohort 3.

The research midwives also presented information about the study to health visitors whenever circumstances permitted. Although it was recognised that they would have no input into the study, they would have women participating in the study in their caseloads. It was felt that they should have some background information on the study and know who to contact should they have any queries.

Appendix A10

Maintaining contact with NHS Trusts and staff

Head of Midwifery

Contact was maintained with the Head of Midwifery at each unit by letters (held as 'Study materials' available on request). One was sent before the unit was finally selected, one before the meeting to finalise recruitment, one before Christmas to thank her and her staff, and one at the end of the study to inform her that the study had gone well and to thank her and her staff again. Positive feedback was included about the work of the unit in supporting the study, and information was given about how the study was progressing in general. We also requested permission to write or contact the community staff to update them in a similar manner. This was always granted.

Community staff

The community staff were visited whenever possible by a research midwife, and when not feasible (due to their individual team circumstances) a letter with the above information was sent. It was seen as important to keep in contact with staff, but to aim for a balance of being seen as supportive, while not being seen as exerting pressure.

Postnatal ward staff

Contact with the postnatal ward at each maternity unit was maintained by regular visits by the research midwives. The working relationship was extremely easy to establish and to maintain and the staff, although not required to recruit directly, maintained a keen interest in the project. The ease of establishing this relationship was helped greatly when the research midwives were introduced to staff by the infant feeding coordinator.

Ongoing presentations

Time was taken to present the project at regular intervals; this was necessary due to staff rotation and to answer any queries staff had as necessary.

Liaison with staff about women's concerns about infant feeding

When, during an approach, women asked questions about infant feeding (as opposed to about the study), the research midwives answered the queries, but also notified the woman's named midwife about the conversation. This seemed to be acceptable, and ensured that everyone was fully informed of information exchanges and that there was no conflict of advice.

Personal thank you cards

All staff who recruited women to the study were sent an individual thank you card from the SUREmilk project (this was sent on recruitment, and was not subject to the arrival of a milk sample).

Ending recruitment

At the end of the recruitment period for cohorts 1 and 2 each community team was visited, thanked personally, updated on the study, surplus equipment was collected and a token gift of chocolates was left for each team.

Queries from community midwives to the SUREmilk office

Community midwives were encouraged to contact the SUREmilk office if they had any queries; there were always dealt with as quickly as possible. Around ten of these calls were taken by the SUREmilk office. Queries included requests for more packs, and queries about return of the shell sample from the hospital. These were always simple to address.

Appendix A11

Choice of milk collection equipment

Breast milk pumps

When the project was conceived, it was presumed that the collection of breast milk samples would best be facilitated by use of simple, inexpensive hand-operated breast pumps. An evaluation of alternative types of hand breast pump was carried out, based largely on our prior experience of hand pumps in clinical practice, and on economic considerations. Competitive quotes were secured from the two foremost suppliers of hand-operated breast pumps in the UK.

The key considerations were: that they should be simple and reliable to use in practice, have relatively few component parts (both for ease of assembly following cleaning and sterilisation, and to minimise the number of materials from which they were constructed), and that the unit price of pumps should be relatively low. In the absence of evidence to inform us about women's own preferences, our view was that the design which most suited all these needs was the two-handed, piston style hand pump. This is constructed from two polycarbonate cylinders, one of which moves up and down inside the other, sealed at the base by a single, unidirectional rubber seal so that suction is generated by a 'bicycle pump' style action. The level of pressure generated is related to the internal volume of the pump cylinder, which is substantial (100ml). Maximum pressure is generated by 2-3 swift strokes, after which the pressure is held at the nipple surface, to withdraw breast milk. After a short while, the suction pressure tails off and can then be re-applied. This style of pump can be easily cleaned and maintained as it has few moving parts, no complicated seals, and is composed of just two materials, the sourcing of which was determined beforehand.

After initiation of the project, following discussion with the research midwives, one further quality was given consideration. The two-handed breast pump was a relatively old style and design, and women might be more familiar with, and so more ready to accept, newer designs available in stores. These are commonly of a one-handed style of usage, being designed to emulate an electric breast pump (originally designed to emulate a baby's sucking), so are meant to be pumped repeatedly at an approximate cycle rate of one per second. They are perceived as being easier to use, although prolonged use can be tiring. However, they are also constructed from more component parts, can be more difficult to assemble, and are more prone to failure with prolonged use (weeks to months). This latter criterion was not regarded as significant in the context of this study, as the pump would be used once, and once only, by participants to collect a single study sample.

Only one manufacturer still marketed the two-handed pump, which also had the lowest unit price. The one-handed pump produced by the same manufacturer was the next most competitively priced. The decision was made, therefore, to randomise women in the study to use of either the one-handed or the two-handed pump, and to evaluate the outcome in terms of the number of volume of samples returned, and women's views of ease of use of the pumps (see section below on pre-testing pumps).

Expressed breast milk versus drip breast milk

It had also been anticipated, at the outset, that we should collect two samples from each woman in cohort 1, a fixed one at one week post-partum, and a later sample, collected at one point randomly chosen from five times between one week and four months. This would have necessitated purchasing two pumps for each woman, so increasing the project costs substantially. There were also some concerns about women's ability to express milk by pump at such an early stage in their lactation, and we were concerned not to undermine women's views of their ability to produce milk for their baby.

An alternative approach was therefore proposed, to use plastic breast shells to collect drip breast milk at one week post-partum. Women are not universally successful at producing drip breast milk, but neither could we rely on all women being capable of expressing breast milk by hand pump. It is acknowledged that drip breast milk is low in fat, because it is predominantly foremilk, although in the first 7-14 days the fat content is atypically high and was regarded as likely to be high enough to ensure sufficient breast milk fat for assaying fat-soluble contaminants.

We therefore used breast shells to collect a fixed sample of drip breast milk at one week post-partum. These milk samples can be compared, both in terms of their composition and the reliability at securing a sample, with expressed breast milk samples collected at one week. This has the potential to inform a National Archive about the relative merits of alternative collection techniques.

Pre-testing of milk collecting equipment

Prior to initiation of the project we had formulated a protocol (Protocol B in the original application) for evaluating whether the milk collection equipment might itself be a source of contamination. Phthalates were the only contaminant considered for evaluation, so accordingly both pump types and the breast shells were submitted to the protocol to evaluate whether phthalates entered breast milk after prolonged contact with the pump and its component parts.

The results of these analyses are summarised in Table 6.2. The outcome was that the original choice of hand-operated pump (two-handed) had to be rejected for use in this study, and was replaced exclusively by the one-handed pump design. This issue introduced some delays to the

study while replacement stocks of the one-handed pumps were supplied by the manufacturer. The manufacturer of the two-handed pump later identified the seal as the probable source of contamination and the material from which they were constructed was changed immediately (to that used in the other pump design)¹.

Breast shells were found not to introduce contamination with the result that we elected to compare milk collection by the two methods, which were both used with each participant and not randomised between women. We have proposed a follow-up survey to elicit the views of SUREmilk participants regarding the ease of use of both the pump and the shells.

Appendix A12

Assembly of shell and pump packs for distribution to community midwives and to participants

Packs for study participants included all information and equipment that might be needed:

- to remind the mother about what she was being asked to do
- to provide all necessary equipment and instructions about how to use it
- to enable her to return the sample appropriately
- to enable her to contact the SUREmilk team in case of difficulty

Shell packs

Shell packs were assembled in the SUREmilk office, distributed by the research midwives, and given to women at recruitment. Shell packs contained:

- one breast shell and a 60ml sample bottle, both cleaned², wrapped in foil and sealed in a bag
- instructions on use of the breast shell from the manufacturer and from SUREmilk
- recent diet questionnaire labelled with barcode and date sticker
- Freepost envelope for return of diet questionnaire
- plastic bag labelled with barcode and 'midwife to complete' stickers, for return of sample bottle
- breast shell sample bag form labelled with barcode and a spare barcode stapled on, for labelling sample bottle
- four barcode stickers, for the recruitment and consent forms

These were packed into an A4 envelope. Four spare barcodes were stuck inside each envelope in case they should be needed by the research midwives or recruiting midwives.

Pump packs

Pump packs were assembled in the SUREmilk office and posted to women in cohorts 1 and 2 who said they were still willing to provide a sample³. Pump packs contained:

- one hand breast pump and a 60ml sample bottle, both cleaned¹, wrapped in foil and sealed in a bag
- letter to the woman with list of pack contents
- checklist of how to collect and return the sample

¹ We understand that manufacture of the two-handed breast pump had since been discontinued for commercial reasons.

² Please see Appendix A19 for details of the cleaning procedures for equipment

³ See Appendix A13 for details on the process of contacting women prior to sending pump packs.

- instructions on use of the breast pump from the manufacturer and from SUREmilk
- recent diet questionnaire labelled with barcode and date sticker
- Freepost envelope for return of diet questionnaire
- plastic bag labelled with barcode, containing absorbent material to wrap round sample bottle for return by post
- further plastic bag, labelled with barcode and 'This Bag Contains Human Breast milk' sticker, to double-bag the sample bottle as required by the postal service
- padded A5 envelope, pre-addressed and stamped for return of double-bagged sample
- pump sample bag form labelled with barcode and a spare barcode stapled on, for labelling sample bottle

These were packed into a padded A3 envelope. Four spare barcodes were stuck inside each envelope, as for shell packs.

Information pack for community midwives

Community midwives involved in recruiting each had easy access to shell packs, which were delivered to each community midwife base prior to recruitment starting, by the research midwives. Information packs were given to each community midwife to support their recruitment process. These included:

- staff information leaflet x 1
- ethnic origin form x 1 (code list for midwife's reference)
- participants' information leaflet 'Planning to breastfeed?' x 10
- participants' information leaflet for cohort 1 x 5
- participants' information leaflet for cohort 2 x 5
- recruitment form for cohorts 1 and 2 x 5
- consent form for cohorts 1 and 2 x 5
- Freepost envelopes for return of recruitment and consent forms to the SUREmilk office

All of these, except for the envelopes, are held as 'Study materials' which are available on request..

Appendix A13

Procedure for contacting women prior to sending pump packs

The SUREmilk database was used to provide a list each day of participants in cohort 1 and cohort 2 whose pump sample was due to be collected in 14 days or less. These women were telephoned to check that they were still able and willing to receive a pump pack and provide a sample.

Women in cohort 2 had taken on a larger commitment by agreeing to provide a total of five breast milk samples in a twelve week period. All planned contacts with these women, after recruitment, were by the research midwives.

Cohort 1 was a larger group. Each woman had agreed to provide a shell sample and one pump sample at 2, 4, 8 or 12 weeks after the birth. A SUREmilk office-based research associate/midwife contacted women in cohort 1 and co-ordinated both the assembly and posting out of all pump packs.

In line with local and national breastfeeding statistics, it was anticipated that up to half of the women might stop breastfeeding before they had intended and so, perhaps, before they were asked

for their pump sample. The first weeks with a new baby can take parents and families through a range of intense and tiring experiences, and not all mothers or all babies are fit and well at this time. The research midwives/associate therefore devised a check list to semi-structure the telephone conversation:

- the caller identified herself and asked if it was a convenient time to call
- if not, she offered to call later
- she asked how the mother and baby were before asking if the mother would still like a pump pack sent to collect the next sample for the study

Difficulties with phoning and how we dealt with them

Problems encountered in making telephone contact with women, and procedures for dealing with those, are described here:

- no phone number / wrong phone number (often a work number): try directory enquiries. If number not obtained, pack sent anyway, on the basis of the original consent. This was also the procedure when the call was not accepted because we had 'withheld our number' (calls from our office went through the University system and were interpreted by some phone companies in this way)
- no answer: try again (sometimes up to 6 times)
- answering machine: between a third and a half of the contacts with cohort 1 involved leaving messages on answering machines. The first message left said who had phoned from the SUREmilk study and for whom, that it was about collecting the second sample, and that the same caller would ring back. The second message repeated that we would like to send the equipment for collecting the second sample, and if this was no longer required, please ring (giving the office number clearly twice) and tell the team not to send it.
- another person answered the phone, and the mother was not available (this happened more with mobiles): these calls were the most difficult to manage. Rather than leave the answering machine message and be asked to explain it by someone other than the mother, these were treated as 'no answer' calls and the number was called again later.

Many calls were uncomplicated: 'Yes, we're well, send the pump'. When a pump was not required this was usually because the mother was no longer breastfeeding. Some mothers elaborated on this. A small number of mothers and some babies had been readmitted to hospital. Some women told us of family events such as bereavements and moves. The full range of answers was not recorded.

Appendix A14

Selection of appropriate containers for sample transit and storage

Colleagues at the Central Science Laboratories recommended Nalgene™ containers for transporting and storage of milk to minimize container contamination of milk through leaching. Two suppliers were identified of small Nalgene™ bottles and vials: Fischer and Merck.

For storage, 5ml and 18ml vials were available from both companies. Milk would be aliquoted into 5ml portions and 10% space was required for expansion of frozen milk, so 18ml bottles were chosen for milk storage. This had the added advantage that by using larger containers for freezer storage, less fat would cling to the container, and they were easier to wash thoroughly in solvent (12ml vials have become available from Merck and Fischer since the study was completed, but these are thought to be unsuitable due to their size for the reasons above).

60ml wide-necked bottles were identified as suitable for milk transit. These were unavailable from Fischer so Merck was chosen to supply all the Nalgene™ products required; this had the added benefit that a larger discount could be negotiated with Merck than Fischer for supply of equipment as part of wider contract with the University of Leeds.

Cost from Merck at 06/08/02

Bottle wide mouth LDPE with PP cap 60ml, Nalgene™

Nom. cap (ml)	ID neck (mm)	D×O.ht (mm)	Std pk qty	N. Ref.	Cat. No.	Std pack
60	20	38×86	12	2103-0002	215/0397/02	£7.08

Low density polyethylene vials and friction fit snap lids.

Nom. cap. (ml)	Dia.×ht (mm)	N. Ref.	Cat. No.	Pack
18	27.1×52.6	6250-0018	275/0440/05	£5.88

12ml vials are also available from Merck at a cost of £4.17 per 12

[Cost from Fischer per 12: 12ml £4.39; 18ml £6.04; 75ml £12.58]

Appendix A15

Posting breast milk samples to the laboratory

As participating women were asked to send samples back to us by post, it was essential to ensure that such sample transport was safe enough to avoid breakages and leakage. It was important both to maximise the number of usable samples returned to the laboratory, and also to avoid any possible hazards to staff handling the mail. To minimise problems for the participating women, we also wished to use packaging which could be posted in a post box, and did not require a visit to a post office.

Following discussion with the Post Office⁴, a simple and safe system was devised. All material needed was included in the packs sent to women when requesting their sample, with full written information given about what to do (details available as ‘Study materials’ on request). Women were asked to put the milk from the pump or shell into the 60ml bottle provided. They were then asked to wrap it in an absorbent material, Sorbent™; enough was provided to absorb easily the contents of a full 60ml bottle. A barcode sticker was applied to the collection bottle and the mother was asked to place it in a plastic bag, labelled with the word ‘Biohazard’, which also had a barcode sticker applied to it. The bag was then placed in a padded envelope, labelled as a ‘biological sample’, pre-addressed and stamped; the envelope and contents could be posted in a normal post box, thus avoiding a trip to the Post Office.

A tiny proportion of the collection tubes leaked. None leaked enough to compromise our ability to obtain enough breast milk for sampling, and none broke. A few (fewer than five) samples were returned in a different container. Some of these leaked.

Since the study ended, a new product has been developed by the Post Office which may simplify this process for the national archive. This new product might have some problems in use (ability to accommodate collection tubes, cost), so the best means of transporting these samples for the archive will need to be explored further.

⁴ Representatives of ‘Royal Mail’ and ‘Consignia’

Appendix A16

Selection of method for identification of participants

Bar-coding participants

It was viewed as being central to the recruitment of women into the study that each participant should be allocated a unique identification code, which would be applied to all materials either sent to, or received from, that woman. Such a scheme could then be extended to all milk samples donated by participants.

It is commonplace within hospitals for all pathological specimens to be bar-coded by the individual department processing those specimens. The fact that several departments might be using their own bar-code scheme does not present a problem, so long as results are filed in the patient's notes, which are uniquely identifiable either by their NHS number or their hospital number. Alternatively, within the parent department, a master sheet can be held which carries a copy of each of the bar-codes relating to an individual's specimens. We proposed adopting such a scheme for all breast milk and other biological specimens provided by participants in the study, deciding, in addition, to extend this scheme upwards to the bar-coding of individual participants.

This has several advantages, the principal ones being: (i) reliability and accuracy in identifying participants and in allocating data correctly to that person; (ii) scope for ensuring anonymity and confidentiality in data held on individual participants. Any paper-based or electronic system that is reliant on data entry by hand is prone to errors when transcribing information; this can be ruled out with bar-code reading, unless the numerical code, associated with each bar-code, is entered by hand should the scanner malfunction. We used a ten-digit code, the first two digits referring to this particular study's name (SM), leaving eight digits to accommodate up to 99 million participants, this scheme is expandable therefore.

A standardised protocol was followed to ensure maximum benefits from such a system, which was as follows: each participant, once she consented and had been recruited into the study was assigned a unique bar-coded identity (bar-coded labels were applied to her paper-work by the recruiting midwife). All materials given to, or subsequently sent to, that woman (breast shells, pump, collecting bottle and information questionnaires) have the same unique bar-code attached to them by adhesive label, so that all returned materials could be accurately assigned to their donor.

Bar-coding study materials

Bar-coding of all sample collection materials and data collection sheets (including questionnaires) was a natural choice to ensure reliability and accuracy for cross-referencing samples and information received to individual participants.

Each participant had previously been allocated a unique identification code at recruitment. Multiple adhesive labels (24 per A4 sheet) were generated using software from a template. Labels were applied to the contents of a shell pack (sample collection tube, plastic sample containment bag, sample record form, dietary questionnaire) and spare labels stuck to the inside of the package. Once a woman was recruited, spare labels were removed and applied to her consent form and recruitment form. These forms were returned to the SUREmilk office using an enclosed freepost envelope. The pack, with its contents for collecting a shell sample and the seven-day dietary questionnaire, were passed to the mother for her to collect and return her shell sample to the Dept of Chemical Pathology, Leeds, using a stamped, self-addressed envelope (SAE) provided. The dietary questionnaire was to be completed and returned in a separate SAE to the

SUREmilk office. From that point all materials were uniquely identified as belonging to that participant.

When a collecting bottle (60ml capacity) containing the participant's milk sample was received by Chemical Pathology, that milk was aliquoted in smaller amounts (5-10ml) into storage vials (18 ml capacity). Each aliquot was given a new sequential bar-code. Copies of these were placed on the sample record sheet for that participant. This sample record sheet was then returned to the SUREmilk office so that details of her milk sample donation could be entered on the SUREmilk database.

Should it be the case, at a future stage, that an independent processing laboratory (e.g. CSL) uses their own bar-coding system to identify samples and their associated assay results, then copies of their bar-codes will need to be placed on a master sheet for that participant so that all information and results relating to that woman can be unambiguously cross-referenced.

Appendix 17

Selection of software for database design

A flexible and secure database management system (DBMS) was required. It needed the facility for secure and flexible data storage, database maintenance, access to records, and to provide options for upgrade to large data storage capacity for potential use with a national archive. After researching several database software options, Microsoft SQL Server 7.0 (which required a minimum of Windows NT) with a Visual basic front end, was chosen as the database management system for the SUREmilk project. An overview of the salient reasons for choosing this database software is given below.

Security and access: The security architecture was integrated with Windows NT and provided optimal flexibility. Database permissions could be assigned directly to Windows NT users. Connections could be made to SQL Server through Proxy Server, providing secured access to data. Unauthorised users were prevented from connecting to private networks. This kept sensitive data secure by controlling all the permissions and accesses. Access could be blocked to restricted sites by ranges of IP addresses, domains, or individual users to ensure that Internet permissions were used appropriately if the system were to be expanded to a Web-based interface for the national archive.

Capacity: The system has considerable capacity, including utilities for Very Large Database (VLDB) support which include:

- improved online backup (less online contention with the existing server workload)
- improved data placement to reduce storage requirements
- improved bulk data load performance
- back-up and restore operations run much faster, have less performance effect on server operations, and have new features.

Windows 95/98 Support: The program, based on SQL Server 7.0 for Microsoft Windows® 95/98 and Windows NT Workstation, is a fully-featured relational database management system (RDBMS) targeted for workstation and mobile applications. It has a common source code for all platforms, from Windows 95/98 to clustered systems. The visual basic front end allowed easier

data entry and more flexible programming. Routine queries were more easily designed. The Visual Basic front end was used for milk pack management and regular status reports.

Databases built in SQL Server allow both Windows-based queries in Access, and/or command-based queries in SQL Server. It has been developed as a complete study management system, allowing researchers and/or midwives to determine the point each participant has reached with her sample collection, and issue prompts when interrogated.

Most analysis of the recruitment and milk pack management data and attachment of background data was carried out in an Access 2000 front end to the database. The use of Access for custom analysis allowed SUREmilk staff who were non-experts in SQL to access and query the database. Further modifications can be anticipated, such as allowing individual participants to access their personal details on a web-site via the internet; this may permit some self-prompting of sample collection.

A printout of the screens that have been set-up on the database are included among the 'Study materials' which are available on request.

Appendix A18

Accessing postnatal wards and approaching women for recruitment – problems and procedures

A total of 195 visits was made to the participating units by the research midwives for the purpose of recruitment (Table 2.4).

Travel and parking time for visits

The research midwives measured miles travelled and also time spent travelling to visits excluding those at Trusts E and F. As visit times for staff sessions were dependent on convenient times for the staff, travel during peak hours often increased travelling time. Routes were planned in advance to determine most economical travel wherever possible. Average mileage per visit was:

- Trust A 140 miles for the main unit, 237 miles if all units included
- Trust B 42 miles
- Trust C 43 miles
- Trust D 34 miles
- Trusts E and F were very close to the SUREmilk office

Parking was difficult at two of the trusts, resulting in waiting times of up to fifty minutes per visit.

Accessing postnatal wards

All ward areas were locked; waiting time varied from 2 minutes to 10 minutes depending on how busy the unit was. If the ward was busy then staff were not always immediately available to direct research midwives to eligible women. Most units were happy for the research midwives to access mothers' notes, but if the ward was busy the notes trolley was generally in use. Research midwives took a back seat in these situations, reassuring staff that they would be happy to wait. This helped foster a good working relationship with the units.

Afternoon and evening were generally found to be inconvenient times to visit the ward to recruit. Lunchtime and teatime were generally avoided by research midwives as the women were eating and it was consider inappropriate to approach women at this time. From lunchtime until 8.30 pm

most units had open visiting, and women were reluctant to discuss the project if they had visitors. After 8.30 pm was considered inappropriate to approach women, as many were settling themselves and their baby for sleep. Mornings were therefore found to be the most successful time to approach and secure women's interest in the project. Although the wards were generally busy, the women seemed to accept that many members of staff would approach them at this time.

The research midwives always gave precedence to midwives, medical staff, dieticians and physiotherapists, all of whom regularly visited the wards during the morning. This often meant the research midwife waited to approach the women (time waiting ranged from 5 minutes to 45 minutes). Again this was felt to be appropriate to maintain a good working relationship with the staff on the unit.

Time spent at each visit

This was determined largely by the nature of the visit but also the work pressures within the units at the time of visit. As described above, the research midwives were mindful not to encroach on ward procedures such as ward rounds when they were taking place, when ward handovers were in progress, when staff were providing clinical care for women and their babies and other such work commitments. They were also careful to respect the time commitment of the participants, so time was also spent waiting for women to be free from personal hygiene procedures and tending to their baby. Visits to a unit could also result in several visits to a particular ward during the visit time in order to accommodate either the staff or women's commitments. The visit time varied between approximately 1-5 hours. No unit was significantly better or worse than any other in this regard; waiting times were dependent on individual circumstances.

Procedure for recruitment visits

1. Access ward and ask for permission from staff
2. Identify eligible women with help of ward staff
3. Access woman's notes
4. Approach eligible woman, confirm correct person
5. Introduce with name and title of research midwife
6. Ask permission to talk about research
7. In No, accept, thank for time, and move on
8. If Yes, tell women:
 - about study itself - introduce it, explain that it is government-funded, and based at Mother and Infant Research Unit, University of Leeds
 - about background to the issue, along the lines of the wording in the leaflets, regarding residues in the atmosphere, food, and water
 - that residues do get into breast milk, and that small studies suggest that this is within safe limits
 - that the government sees breast milk as such an important food that they want to establish a system to monitor breast milk, and therefore to be better able to protect it
 - that the role of this study is to look at how best to do this, in terms of how to collect samples of breast milk, how much milk to expect from each woman, and how to get the sample back to the laboratory
 - that the study wished to recruit first-time mothers who were breastfeeding their baby
 - exactly what would be required of them, if they remained interested.

Mothers' concerns at recruitment

- Many mothers, when first approached, expressed anxiety about being able to produce a drip breast milk sample. This in many cases led to a discussion on the physiology of

lactation, and reiteration of the purpose of the study (ie information-gathering). The research midwives felt this was vital, so that the study was not seen as anxiety-provoking

- The discussions around physiology of lactation could sometimes lead to further discussions on the management of breastfeeding. These discussions were always continued, in an attempt to reassure mothers of their ability to breastfeed. Following any discussion the research midwife always asked permission of the mother to report the discussion to the midwife responsible for her care. Again this increased the research midwives' time on the unit
- The unit midwives always appeared happy with the reported discussions regarding breastfeeding issues

Appendix A19

Preparation of pumps and shells for use by mother

Pumps and shells were separated into their individual component parts and immersed for two hours in a wash solution (5% Decon™ detergent solution, made up with distilled water) in a 10L Nalgene™ bucket. Aluminium foil was placed over the surface of the Decon™, and weighted down, to ensure complete immersion of the components.

After the two-hour soak the wash solution was drained off and the parts transferred to a bucket of clean distilled water for rinsing. Following the rinse components were placed in 5% nitric acid solution (again made up with distilled water), carefully submerged, and left for two hours. The nitric acid was then drained off and the parts rinsed twice with distilled water before being placed on aluminium foil covered trays in a drying cupboard.

Three buckets, partially filled with fresh hexane, were assembled in a fume cupboard and the dry component parts were rinsed sequentially in each bucket of hexane. After the third rinse components were placed on foil-lined trays for final drying. When all the hexane had evaporated the pumps and shells were reassembled and wrapped in aluminium foil, before packaging in sterile autoclave bags and sealed ready for assembly into packs and their later despatch to study participants.

Appendix 20

Anonymisation procedure

The key concern is that no-one should be identifiable from the data set. Two options were considered:

1. Postal address anonymisation

Using part of the postcode for anonymisation was possible. Postcodes are developed in hierarchy from postal areas e.g. **LS** for Leeds, to postal districts e.g. **LS2**, to postal sectors e.g. **LS2 9**, and then to individual postcodes e.g. **LS2 9JT**. In 1991 97% of postcodes in the UK had fewer than 50 delivery points, although approximately 84 thousand out of 24 million postcodes had only one delivery point (many of these are commercial however rather than residential), e.g. **LS2 9JT** for central administration at the University of Leeds. The average number of residential addresses in a *wholly* residential postcode in 1991 was 12 (Raper et al, 1991).

There were 8,820 postcode sectors in 1991 (e.g. LS2 9), these were formed by grouping postcodes to give areas with more than 50 delivery points (Raper et al, 1991). There were 2,679 postcode districts (e.g. LS2) formed from these with upwards of around 160 delivery points. Postcode districts may be suitable in many circumstances for anonymisation purposes, but in districts covered by the study, such as LS2, there were a large number of commercial addresses and fewer residential ones, so it was considered that this might pose a risk of disclosure. This risk could be increased if the study was conducted nationally including possible areas such as business districts in London.

2. Electoral area anonymisation

Electoral wards may be used to anonymise the data as their distribution is based on the geography of electoral areas rather than on postal ones. Electoral wards are designed to avoid having small numbers of voters, and hence adults, living in them. Though they may be smaller than postal districts they have less potential for variation in their numbers of residents, especially at the lower end (Dale and Marsh 1993). The numbers of adults aged 15-59 ranged from 9,200–20,500 in Leeds wards and 500-5,400 in wards in Harrogate in 1998. The ward containing LS2 9JT had 16,300 adult residents aged 16-59 in 1998. (ONS, 2002)

References

- ONS. 2002. Oxford University population estimates for wards in England, mid 1998. <http://www.neighbourhood.statistics.gov.uk/tables/eng/TableViewer/Wdsview/disviewwp.asp?ReportId=183&area=load> (Accessed 9th September 2002)
- Raper J, Rhind D, Shepherd J. 1991. Postcodes: The new geography. (Essex: Longman)
- Dale A, Marsh C. 1993. The 1991 Census User's Handbook. (London: HMSO)

Appendix A21

Overview of technical aspects of database

Data collection

The mother will be recruited by a midwife (details not given here). The midwife will complete a 'Recruitment form' in the mother's presence, and ask her to complete and sign a 'Consent form'.

Recruitment form

1. Recruitment form completed in presence of ALL mothers approached by midwife. The form, at this point, does not contain an ID.
2. If the mother declines to participate, her post-code is recorded as a minimum, in order to permit comparison of the characteristics of recruits and non-participants.
3. If the mother agrees to participate and signs the Consent form, a study ID is then allocated and affixed to the two forms. These are produced in no-carbon-required (NCR) so that a duplicate copy can be placed in the mother's notes (hand-held record).
4. The top copy of the forms are returned to the SUREmilk co-ordinating centre so that her details and questionnaire data can be placed on the SUREmilk database, and a record made that of that ID having been allocated.
5. This dramatically reduces printing costs; there will be more forms printed than women recruited.
6. Paper record is archived.

Milk sample

1. Notification of new recruit made through receipt of the 'Recruitment form' and 'Consent form'.
2. Pump, sample collection tubes, 'sample bag forms' and dietary questionnaire sent to mother. Sample tubes, bags and forms are all identified by the bar code which represents the mother's study ID (along with other information such as the mother's name if required). All the bar codes are the same so that the mother does not need to match up the bags with the forms.
3. Mother expresses milk, places it in collection tube, stores in fridge and completes form.
4. Milk and form is transported to Chemical Pathology (system to be determined).
5. Sample is aliquoted and stored in freezer. Each local laboratory will use their local system for storage. Each laboratory must attach a bar code to the 'sample bag form' which can link to the aliquots. This links the milk sample stored in a freezer to the mother (via the sample bag form bar codes). It is important that each aliquot is labelled uniquely and linked to the sample bag form (using duplicate stickers attached to form).
6. At this point there are two options. Either the laboratory is:
 - a. one of the 'core' laboratories (say, up to 10) who have the database management system designed to allow direct input of the data in the 'sample bag form' into the data repository. The bar code of the form and the bar codes of the aliquots are recorded onto the database; or:
 - b. not one of the core laboratories. Therefore the request is to store the milk, allowing their own sample management system to store the frozen aliquots. Then the form with the bar codes of the aliquots is sent to the SUREmilk co-ordinating centre for entry onto the database. The details of the laboratory are also required on the form.
7. The milk samples are then:
 - a. analysed in the local laboratory (e.g. for fat content) where the results are input directly to the laboratory database (core laboratories) or sent to the SUREmilk co-ordinating centre (non-core), and
 - b. sent to the Central Science Laboratories at York for analysis. Results are sent to the SUREmilk co-ordinating centre electronically. The best format will be simple ASCII text file, in a pre-determined order. This will allow an operator to upload this data onto the database with an automated system (as part of the management shell).

Specifications for the units involved

The units involved in the study and the specifications are as follows:-

Data Repository at the SUREmilk co-ordinating centre

Actions and events

The actions the Data Repository are responsible for are:-

1. Input 'recruitment form', assign a study ID and attach the printed bar code.
2. Store the paper.
3. Milk sample collection packs sent directly to the mother (bar coded).
4. Sample bag forms to be input where necessary (with local laboratory bar code system).
5. The notification of receipt of the milk samples will be recorded.
 - a. late receipt may be chased.
 - b. progress of sample collection may be monitored.
6. Management of the project can be conducted from the data on the server.
7. Database set up will allow data analysis.

Technical specification

SQL server version 7 installed onto a NT server PC at 22 Hyde Terrace. It will be networked to allow remote access to the data for input, analysis etc. The security will be set in order to control access. This will be in the form of password accounts which are given permission to access each part.

The repository will require regular backups. The best way for this to be done is:

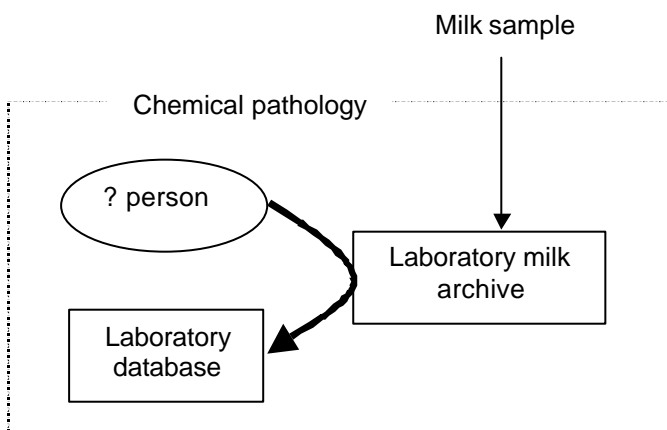
1. Write SQL server to a spare hard disk. SQL server does not have to be stopped as it takes a snap-shot of the data.
2. Tape-backup to record the data from the copy on the other disk.

Other PC's used for inputting data will require a 'client' to allow access to the SQL server. This will probably take the form of an Access front-end, using linked tables.

Another possibility is to allow the SQL server to be physically situated within Healthcare Studies (Baines Wing). They would provide hardware support, but not SQL server support.

Chemical Pathology

Actions and events



1. The milk sample will arrive at the laboratory.
2. Aliquots will be created from the sample bag. The locally used bar coding system (or indeed simple numbering system) will be used for labelling the aliquots. These bar codes/numbers will be recorded onto the 'sample bag form'.
3. Receipt of the sample will be recorded on the laboratory database to allow retrieval.
4. The 'sample bag form' will be returned to the SUREmilk co-ordinating centre with the bar codes of the aliquots attached, but not their locations. It is up to the local laboratories to deal with storage issues.
5. The laboratory may also be asked to notify on the quality of the sample relatively soon after receipt.
 - a. adequate volume of milk
 - b. transported correctly
 - c. aliquoted and stored successfully
6. The samples will be measured for certain qualities.
 - a. local measurements may be made and the SUREmilk co-ordinating centre informed (either manually or using the direct update system)

- b. samples will be sent to CSL for measurements. These will be notified from SUREmilk co-ordinating centre.

Technical Specification

Laboratories will fall into two distinct groups

Core laboratories

'Core' laboratories co-operating with the study. These will be provided with a simple database management system which links directly with the data repository stored by the SUREmilk co-ordinating centre. These will require a specification yet to be determined. But it is likely the following will be required

1. Pentium PC at least 350MHz, 64Mb RAM, running Windows 98/NT4/2000.
2. Linked to the Internet.

Installation of a Virtual Private Network client allows access through the local network. The laboratory technician will open the database management system supplied from the SUREmilk co-ordinating centre, a front-end for logging the arrival of samples. This front-end will have direct access to the Data Repository over some open (e.g. Internet) or closed (e.g. NHSnet) network. There are options for the form and development of this front-end.

1. Access database. Quick to build, moderate speed (probably adequate for this application). Drawback is that the front-end should be a complete 'bundle' with no access to the underlying objects, which is not entirely the case with an Access database.
2. Access Application. As Access except allows a Visual Basic Executable to be created allowing distribution without fear of tampering. Drawbacks are that we have not used this approach and the Developers Kit is expensive.
3. Delphi application allows an executable to be created. It can perform all tasks we may wish to do and is quick. However, there will be a longer development time. There is considerable expertise within this unit on this package.

The data to be recorded on the arrival of the milk sample is included in the 'sample bag form'. The technician will attach locally used bar codes to the aliquots (for tracing within the local freezer management systems) and duplicates to the 'sample bag form'. After completion of the inputting the form will be sent to the SUREmilk co-ordinating centre for archiving.

Non-core laboratories

Smaller/non-'core' laboratories will need to be sent instructions on how to add to the 'distributed archive'. These will perform the same duties as the core laboratories except the form will be sent directly to the SUREmilk co-ordinating centre with no data input locally (except for recording the location of the samples within the local storage system). They will not need a PC for the use by the SUREmilk study. However, it is likely they will need a database management system for their local storage of samples.

Local situation

The laboratory PC at chemical pathology (LGI) is on the University network and is an NT machine connected to the Novell servers. AutoFile™ is a sample location management software.

External laboratory services

The milk samples will be sent to CSL in York for further processing and measurements. The notification to the local storage laboratories will be made directly from the SUREmilk co-ordinating centre. The request can be sent as a letter (or any other method felt appropriate) with

the appropriate Aliquot ID's from our database. These ID's could be sent as printed barcodes also.

Information transfers

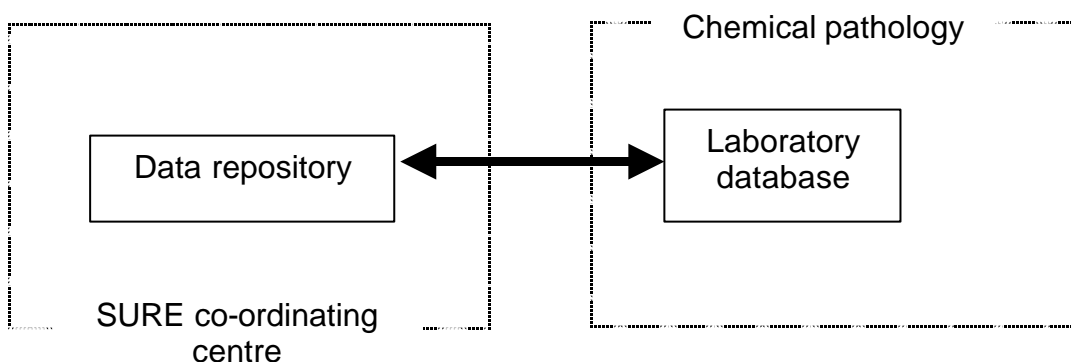
This is the most complicated section of the project. The electronic transfer of data is always preferable to repeated data input. This is due to speed, time and transcription errors.

Laboratory to Data Repository

An electronic link is only proposed for the core laboratories. Data transfer from the non-core laboratories should be by paper and by post.

Local situation

The laboratory PC is on the same network as the SUREmilk co-ordinating centre. This allows a direct access from the Laboratory to the information stored on the main data repository.



Data input on the PC in the laboratory would automatically be stored at SUREmilk co-ordinating centre. The data transfer and viewing in the opposite direction will be restricted to those analysing the laboratory data. We will discuss the options, but the simplest solution will be to provide an Access database with Attached Tables to the relevant information. This is a well-trodden path for this unit.

Nationally

We must look into the issues of confidentiality and data protection. We may collaborate with Mike Thornton, Healthcare Studies, to look at the pitfalls of electronic data transfer from laboratory to SUREmilk co-ordinating centre.

CSL to SUREmilk co-ordinating centre

Data can be returned either using an XL spreadsheet or text. It is planned to use a text file and build into the management shell an ability to upload results automatically onto the system. The data may be obtained either on diskette or via electronic transfer (e.g. Email, FTP etc.).

Table specification

Recruit = 'recruitment form' for the mother

Table.Mother

Column Name	Datatype	Source	Comment
StudyID	int ?		tba
Demographic			
FamilyName	varchar[50]	Recruit	
GivenNames	varchar[100]	Recruit	
Address1	varchar[50]	Recruit	
Address2	varchar[50]	Recruit	
Address3	varchar[50]	Recruit	
Address4	varchar[50]	Recruit	
AddressPostcode	varchar[10]	Recruit	
Telephone	varchar[25]	Recruit	
DateCompleted	Date	Recruit	
AlternativeName	varchar[100]	Recruit	
AlternativeAddress1	varchar[50]	Recruit	
AlternativeAddress2	varchar[50]	Recruit	
AlternativeAddress3	varchar[50]	Recruit	
AlternativeAddress4	varchar[50]	Recruit	
AlternativeAddressPostcode	varchar[10]	Recruit	
AlternativeTelephone	varchar[25]	Recruit	
HearProj	int	Recruit	coded
Pregnancy			
WeeksPregnant	? int	Recruit	How stored, range possible
FirstChild	int	Recruit	1 = yes
NumberExpecting	int	Recruit	? allow possibly twins etc
PlanFeed	int	Recruit	Coded 1=Breast, 2 = Bottle, 3 = Mixed ++?
PlanFeedLength	int	Recruit	tba
Participation			
Participate	int	Recruit	1 = yes
ReasonNotParticipate	varchar/coded	Recruit	
SignedConsent	int	Recruit	1 = yes
Personal			
DateBirth	Date	Recruit	
PartnerStatus	int	Recruit	Coded
NumberOlderSibs	int	Recruit	
CountryBirth	int	Recruit	Coded
PlaceBirth	varchar[50]	Recruit	
EthnicGroup	int	Recruit	Coded
ChFEthnicGroup	int	Recruit	Coded
Religion	int	Recruit	Coded
ChReligion	int	Recruit	Coded
Car	int	Recruit	1 = yes
AgeEducation	int	Recruit	? tba

Column Name	Datatype	Source	Comment
HighEducation	int	Recruit	Coded
Residences			
HouseArea1yr	int	Recruit	Coded
HouseArea5yr	int	Recruit	Coded
HouseAreaChild	int	Recruit	Coded
HouseMoves10yr	int	Recruit	
Occupations			
NumberJobs	int	Recruit	
JobGMother	int	Recruit	coded
JobGFather	int	Recruit	coded
AgeChJob	int	Recruit	? as a date of months etc

Table.MotherDietRecruit

This is at present split from Mothers personal table

Column Name	Datatype	Source	Comment
StudyID	int ?		tba
LowFat	int	Recruit	1=yes
Vegan	int	Recruit	1=yes
Vege	int	Recruit	1=yes
MostlyVege	int	Recruit	1=yes
OnlyOrganic	int	Recruit	1=yes
MainlyOrganic	int	Recruit	1=yes
OccOrganic	int	Recruit	1=yes
Balanced	int	Recruit	1=yes
Fried	int	Recruit	Coded
Dairy	int	Recruit	Coded
Meat	int	Recruit	Coded
OilyFish	int	Recruit	Coded
EverDieted	int	Recruit	1=yes
Alcohol	int	Recruit	Coded
AlcoholB4Preg	int	Recruit	Coded
AlcoholDesc	int	Recruit	Coded
AlcoholDescB4Preg	int	Recruit	Coded
NowSmoke	int	Recruit	1=yes
EverSmoke	int	Recruit	1=yes
WhenStopSmoke	int	Recruit	? in years, day etc or a date
NumberCigs	int	Recruit	

Table.MotherOcc

This is at present split from Mothers personal table - a one to many relationship

Column Name	Datatype	Source	Comment
StudyID	int ?		tba
JobID	AutoNumber		
JobTime	int	Recruit	number of years ago 0/1/5 etc
JobCode	int	Recruit	coded
IndustryCode	int	Recruit	coded

Table:MilkForm

This is a one to many relationship linked to Table.Mother

Column Name	Datatype	Source	Comment
StudyID	int ?		tba
SampleID	AutoNumber		
ChildAge	int	BagForm 1	in weeks
TimeExpress	? time	BagForm 1	
DateExpress	date	BagForm 1	
WhichBreast	int		coded
PointInFeed	int		coded
KitExpress	int		coded
KitSample	int		coded
MilkStore	int		coded
MilkTransport	int		coded
MilkTransportTime	time		
MilkTransportDate	date		
ChemPID	int		a code assigned to all labs ?
ChemPArriveTime	time		
ChemPArriveDate	date		
ChemPALiqDate	date		
ChemPFrozDate	date		
FormSentDate	date		

Table:MilkDrugs

This is a one to many relationship linked to Table.MilkForm

Column Name	Datatype	Source	Comment
StudyID	int ?		linked
SampleID	int		linked
DrugID	AutoNumber		
DrugCode	int	BagForm 1	coded
DrugName	varchar[200]	BagForm 1	free text allowed?

Table:Aliquot

This is a one to many relationship linked to Table.MilkForm

Column Name	Datatype	Source	Comment
StudyID	int ?		linked
SampleID	int		linked
ChemPID	int		lined
AliquotID	AutoNumber		
VolumeOriginal	int		tba
VolumeLeft	int		
TimesFrozen	int		1 at start
this may not be known			
FreezerID			from local laboratory storage system
RackID			
ColumnID			
RowID			
Results			
The results from the tests will go here			

Appendix A22

The stability and/or leaching of contaminants in breast milk during storage - a laboratory-based study

Introduction

Four separate laboratory-based protocols were conceived for evaluating various aspects of the breast milk collection and storage process, to determine whether the method of collection, handling, processing and storage of breast milk introduced any extrinsic contamination not present at the point at which a breast milk sample was collected by the donor mother. By the same process, it was also possible to determine whether contaminants in the milk leached or degraded over time, and whether this was affected by the nature of the storage container or the temperature of storage. By this means it was hoped to verify that milk held in long-term storage (as part of an archive) would be representative of the milk as collected and donated by participants, containing only intrinsic contaminants at their original level, and free from extrinsic contamination introduced by the milk collection and storage methodology.

Short-term introduction of extrinsic contamination was evaluated by a laboratory-protocol, the results of which were reported in Section 6.3, Table 6.2. As a result of this, one of the two types of hand-operated breast pump tested was found to leach unacceptable levels of one particular phthalate (DEHP) into milk, which increase over the period of exposure between milk and pump. This pump design was therefore rejected as a potential device for collecting breast milk samples, with the one remaining hand pump and breast shells used for milk sample collection.

This Appendix relates to testing the long-term stability of breast milk samples during storage at either -20°C or -70°C , and/or whether any contaminants leached into the breast milk from the storage container, glass or plastic, or were lost through absorption by the container. If there were losses of known contaminants from the stored breast milk, this protocol was not devised to determine whether this was by degradation of the contaminant or by absorption into the material of the container.

The protocol below, approved prior to the main study, describes the optimal study design proposed for its conduct as. The protocol was implemented by the Central Science Laboratory, York, to our original design with analytical work devolved between four separate teams. In practice, there were several departures from the original protocol, depending upon the analyte in question; these procedural variations are itemised following the main protocol. Certain unforeseen departures are likely to have influenced the effectiveness of the design and our ability to interpret the results as predicted.

Disclaimer: Planned departures from the protocol, likely to have benefited the conduct of the laboratory study, were taken under guidance from colleagues at CSL. Unplanned departures from the original protocol, which are likely to have had an adverse impact on the outcome of the protocol, were outside the direct control of the principal investigators. Accordingly, the authors of the present report (MW, MJR, AWMH) do not accept liability for any such departures from the original protocol or for unforeseen omissions.

Protocol for the laboratory study as originally proposed:

PROTOCOL A - GENERAL PURPOSE LEACHING AND STABILITY PROTOCOL: EVALUATION OF COLLECTION AND STORAGE PROCEDURES AS POTENTIAL SOURCES OF CONTAMINATION AND/OR DEGRADATION

Procedure:

1. A pooled human breast milk standard (PBMS) shall be compiled from milk obtained from as small a number of women as possible (1-5). Ideally, this milk should be freshly expressed, but may also include defrosted, pooled milk from several mothers (amount approximately 1,500 ml)⁵. Mothers generally express their milk by use of an electric breast pump (although it may also be hand expressed) and store it in glass bottles (recycled bottles previously containing distilled water). Such milk will therefore contain unspecified levels of both intrinsic (from the mother) and extrinsic contamination (from the collection process).
2. The pooled sample will be split twice into two portions; approximately 200 ml will be left in its natural form (natural PBMS), while to the remaining amount (1.3L) will be added measurable amounts of five chemical classes: phthalates, PCBs, organochlorine pesticides, dioxins and heavy metals (enhanced PBMS).
3. The portions will be further split, with one half being aliquoted into glass containers, the other into plastic containers (type to be advised by CSL, York). Half the containers of both types will be stored at -20°C and half stored at -70°C; storage at -70°C will ensure negligible degradation of contaminants in the sample.
4. Samples from both the natural PBMS and the enhanced PBMS will be extracted and assayed without being stored. These assays (Assay point 1) will constitute the baseline measurements or reference values for the six main contaminant groups against which all other sampling procedures and storage conditions will be evaluated.
5. Milk will remain in storage at -20°C & -70°C with subsets thawed and assayed for *phthalates* at intervals of 1 month, 2 & 9 months and one year, corresponding to intervals after initial freezing of 1 month, 3 months, 1 year and 2 years. Milk samples will be assayed for the remaining four classes of contaminant (excepting dioxins) at 1 and 2 years only.

The analytical costs for dioxins/furans encompass reporting of levels of ortho- and non-ortho forms of PCBs. These costs are relatively high and require greater volumes of milk for analysis. So, given the greater stability of these compounds, one limb of the study (storage at -70°C in glass containers) was excluded at the outset. The primary comparisons for contaminants other than dioxins remained as: (i) storage at -20°C versus storage at -70°C in plastic containers, and (ii) storage at -20°C in plastic containers versus storage at -20°C in glass containers.

Procedural amendments to planned protocol:

1. It was later concluded that dioxins, PCBs, OCs and metals would be likely to be present at sufficient concentrations for there to be no need to add these to the pooled 'standard'; this original decision was reversed therefore. In contrast, naturally occurring levels of phthalates and phytoestrogens could not be relied upon, so it was planned to add these to the 'standard' – i.e. it was 'spiked' with known amounts of these compounds in advance.

⁵ Milk donated by mothers who have expressed breast milk for their baby on a Special Care Baby Unit, but who no longer require this milk for their baby's nutrition, can act as this source, although collections will be relatively unstructured. There are, however, no ethical dilemmas associated with collecting such milk, as long as mothers give their fully informed consent.

2. The protocol proposed was devised to be as economical as possible in its demand for milk, but when re-evaluated by CSL a greater volume of milk was perceived to be required for its conduct. Accordingly, the volume supplied was increased from the 1.5 litres originally specified to 3 litres. Approximately 1.75L was obtained from a single source, which had been held in storage at -20°C for approximately 9 months. This was made up to 3 litres with milk from a further 3-4 mothers who had expressed for their baby while resident on the Special Care Baby Unit of St James's Hospital, Leeds, and was donated for this protocol when no longer required by women for their baby. In all cases the storage times for this milk was much less, as all had been collected in the preceding three months.

3. The reliance on intrinsic levels of stable contaminants meant that although the milk had been frozen for some while, this was not regarded as critical to the effective conduct of the protocol. Similarly, the retrospective view, gained from pooling for dioxins in the main study, was that there is 'regression-towards-the-mean' in the overall contaminant level of pooled milk. On the basis of this, it would not appear vital for milk to come from "as few women as possible".

4. The pool of milk collected was passed to CSL on November 17th 2001. Owing to some initial confusion, the protocol was not initiated until the following year, the dates for which the protocol was initiated in respect of each analyte was: Phthalates – Mar 23rd 2002; Metals – May 27th 2002; PCBs – Aug 9th-10th 2002; dioxins/furans – Aug 9th-10th 2002. This meant that the pooled milk was stored at -20°C for a further 4-9 months before the protocol was initiated.

5. The stability protocol was not initiated for phytoestrogens as evaluation of this contaminant was removed as directed by the funding agencies, when other data became available suggesting that phytoestrogens do not pass into breast milk from the mother's diet in substantial amounts.

6. At the point at which the protocol was initiated for dioxins/furans, which place a heavy volume demand for analysis, there was no longer sufficient milk in the pooled human breast milk standard (PBMS) for initiation of this arm of the study. At that point, the team at CSL drew together residual milk from previous studies, to make the requisite amount for analysis. This milk was stated as being "several years old before the stability trial started." "Some of the milk had been frozen, pooled and thawed several times previously." As a result of this the samples used for analysis, were said to be "of poor condition, with fats starting to separate". In the view of CSL, this is likely to have had an adverse bearing on the results of this protocol in respect of dioxins. In contrast, the analyses undertaken on pooled milk in the main study are not likely to have been compromised in this way, so those analyses can be regarded as intrinsically more reliable.

7. The protocol stated that following an initial analysis for contaminants at time t0, the milk should again be tested, for contaminants other than phthalates, at 12 months (t12) and 24 months (t24) after initial compilation and storage. In practice, the initial screen at time t0 for dioxins was not undertaken because of an oversight. The milk was first tested at 10½ months (t10.5) and again at 15 months (t15), so the analysis times do not correspond to the protocol or those used for other analytes.

8. Because of initial concerns that phthalates were declining during storage, milk stored in glass for future analysis of phthalates was re-assigned to other aspects of the phthalate protocol. No results are available, therefore, for phthalates following storage in glass at different temperatures. In view of the findings of Calafat et al (2004)⁶, milk stored in glass is unlikely to

⁶ Calafat AM, Slakman AR, Silva MJ, Herbert AR & Needham LL. Automated solid phase extraction and quantitative analysis of human milk for 13 phthalate metabolites. *J of Chromatography B*. 2004; **805**:49-56.

have yielded different results from storage in plastic, as the material from which the collection tube was sourced was not implicated in the degradation of phthalates.

Results - Metals

The results for metals are shown in figures A22.1-4, with the results for glass shown on the left and for plastic to the right. The t12 values for -20°C and for -70°C appear to the right of the t0 value. Metals are plotted separately where the scale of measurement does not coincide: units for the vertical axes are µg/kg of whole milk.

Figure A22.1

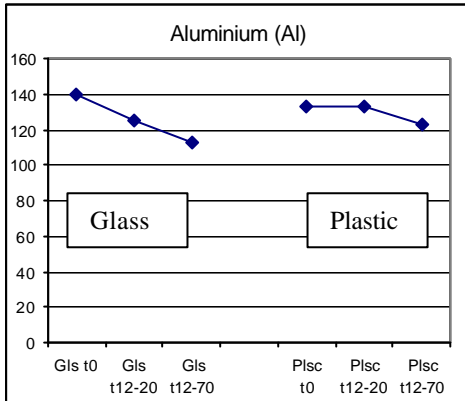


Figure A22.2

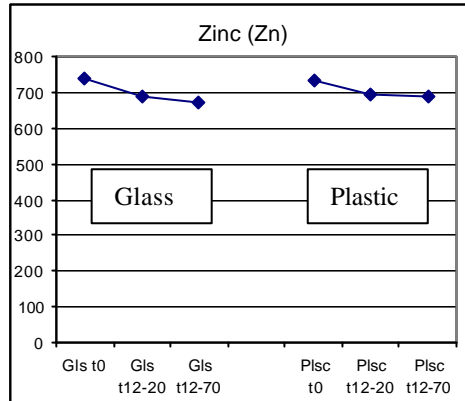


Figure A22.3

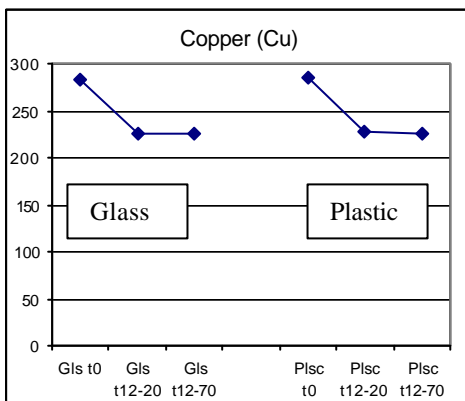


Figure A22.4

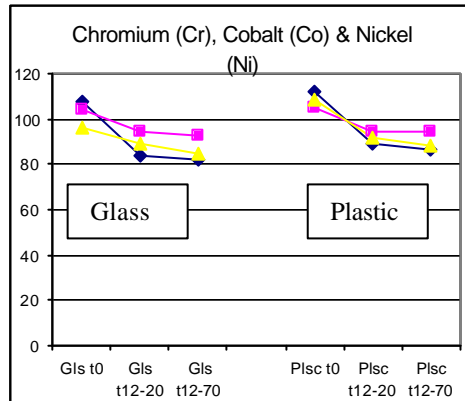


Figure A22.5

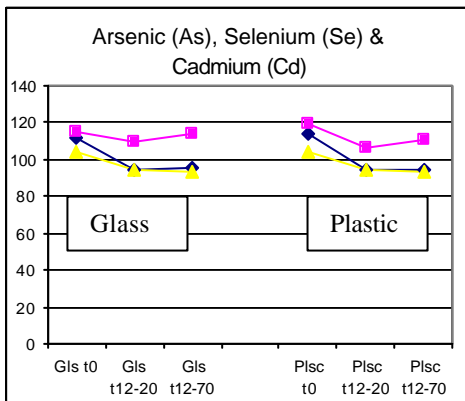
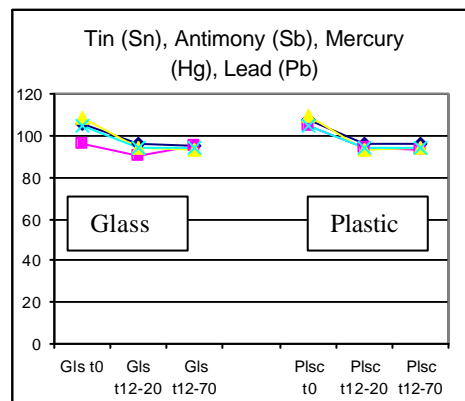


Figure A22.6

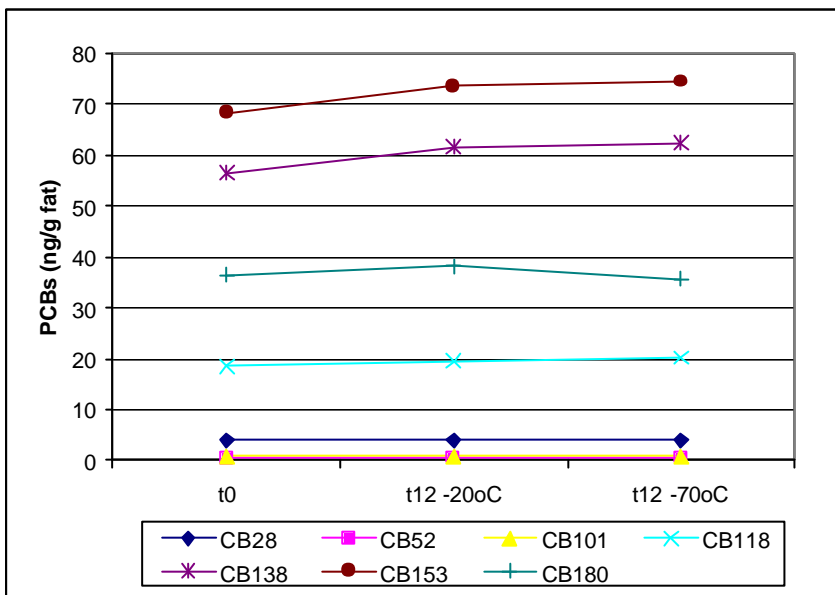


On average, there is an apparent 11-13% fall in levels after 12 months in storage, although there are no clear or consistent differences between storage in glass or in plastic, and in storage at -20°C or -70°C. Accordingly, the decline after 12 months storage is likely to be accounted by analytical variation, and not because of a genuine decline in contaminants during storage. Similarly, there is no evidence of metals leaching from the glass into milk during storage.

Results - PCBs / OCs

The change in levels of PCBs after 12 months storage at -20°C and -70°C, relative to their levels at time t0 are shown in figure A22.7.

Figure A22.7 Change in levels of PCBs in milk after storage for 12 months



The percentage shifts for each PCB are shown in Table A22.1. These appear quite large for certain PCBs (e.g. PCB52 & PCB101) which are only recorded at low levels, but in absolute terms the change is negligible. For PCBs recorded at higher levels the changes vary between -2% to +11% and, overall, the change in Sum PCBs is 7.3% at -20°C and +7.1% at -70°C. The difference as a function of storage is negligible, suggesting that, once again, this is not a true shift in concentration over time, but is more likely to be due to analytical variability between runs separated over time.

Table A22.3 Levels of 7 congeners (ng/g fat) analysed, with percentage change in level from start of storage.

	t0	t12 @ -20oC	% Diff	t12 @ -70oC	% Diff
CB28	3.89	3.98	2.40	4.05	4.29
CB52	0.43	0.57	32.81	0.51	19.53
CB101	0.90	0.82	-8.55	0.79	-12.27
CB118	18.48	19.62	6.17	20.21	9.38
CB138	56.44	61.67	9.27	62.41	10.57
CB153	68.33	73.52	7.60	74.49	9.01
CB180	36.49	38.14	4.51	35.67	-2.25
SUM	184.96	198.53	7.34	198.13	7.12

PCBs are expressed in units of ng/g fat, and the fat concentration of the milk is more likely to have changed over time as a function of storage (than the PCBs within the fat); so, analytical variability in fat measurement is likely to be greater than in measurement of PCBs. So, to all intents and purposes, it would appear that PCBs are resistant to degradation in storage, and so can be regarded as stable, being unaffected by storage temperature within the range -20 to -70°C .

The picture described for PCBs is essentially the same for OCs, although as these are reported in parts per million (ppm) (rather than in parts per billion (ppb) for PCBs) there is much less sensitivity within the measurements for detecting small changes over time.

Results - Dioxins/furans

No measurements for dioxins were made at time t_0 , which means that changes over time can only be evaluated between 10.5 and 15 months at -70°C , when milk was removed from storage for analysis. The comparison between -20°C and -70°C at 15 months should indicate the differential change in level since the start of the protocol.

Figure A22.7

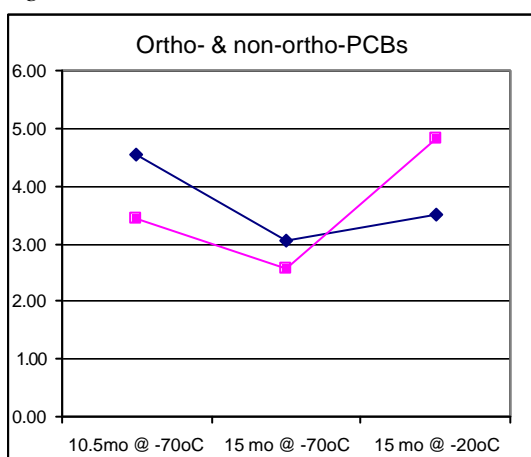
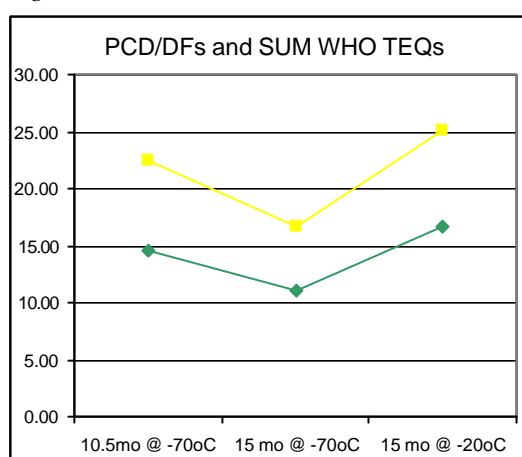


Figure A22.8



Figures 22.7 & 22.8 show the change in dioxins, and PCBs with dioxin-like activity; the units on the vertical axes in figures 22.7 & 22.8 are ng TEQ/kg fat.

It is apparent from these results that there is marked variability in the levels of all three classes of compounds measured (dioxins/furans, ortho-PCBs & non-ortho-PCBs), which are not clearly related to storage time or to temperature. Levels at 15 months fall between -33% (ortho-PCBs at -70°C) and +41% (non-ortho PCBs @ -20°C) of their respective values at 10.5 months. These changes are of an unexpected magnitude (up to 41% of previous value) over the short timescale involved (4.5 months) and in an inexplicable direction (i.e. less of a change at -20°C , compared to -70°C).

The probable explanation for these results, suggested by CSL, is that the substrate used for this study was compiled from residual milk left over from several previous studies; much of it had been frozen and thawed several times, and the fat was observed as 'beginning to separate'. As a consequence, the milk was of a poor quality, and liable to have given suspect results.

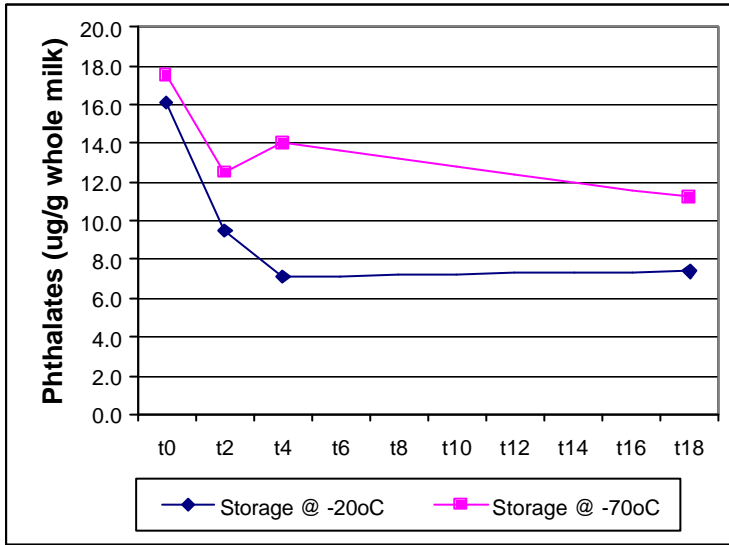
We have no reason to believe that the assays conducted on milk samples from the main study will have been subject to the same problems of milk quality. Milk samples were frozen and thawed once, then pooled immediately prior to analysis. The quality control data from the main study analyses indicate that they were of entirely acceptable quality.

The evidence above suggests, however, that this protocol should be repeated, showing strict adherence to the protocol defined for it, and using as its substrate a batch of milk of uniform quality (as originally specified), which has not undergone repeated processing prior to analysis.

Results - Phthalates

Of the phthalates measured, certain ones showed a decline over time, while other showed little change; so, in the figure below (figure A22.9) data are summed across phthalates to show the general trend.

Figure A22.9: Change in sum of phthalates from 0 to 18 months storage at -20°C and at -70°C.



There is a marked decline from 0 to 2 months at both storage temperatures, although it is greater at -20°C. There is an apparent rise between 2 and 4 months at -70°C which must either be attributed to analytical variability, or to some other factor (see below).

In figures A22.10 to A22.16 below the changes are shown for each phthalate individually; the data at 2 and 4 months have been averaged and plotted at 3 months to remove unwanted variability (units for the vertical axis are µg/g whole milk).

Figure A22.10 – DMP

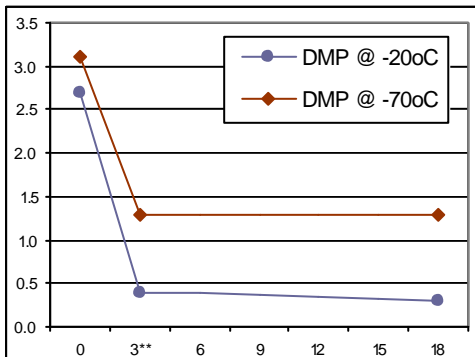


Figure A22.11 - DEP

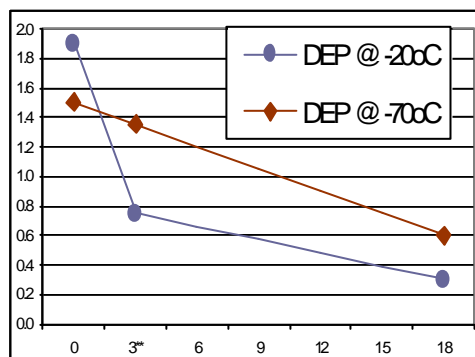


Figure A22.11 – DBP

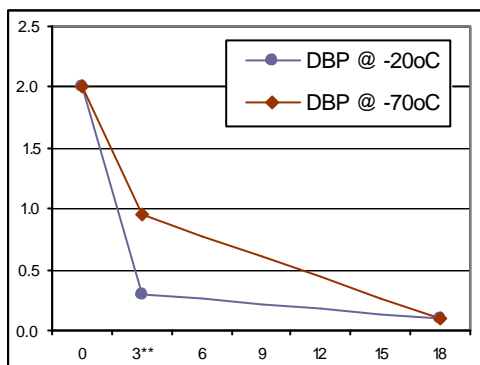


Figure A22.12 - BBP

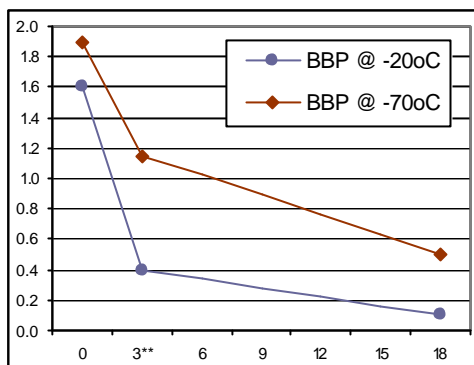


Figure A22.14 – DCHP

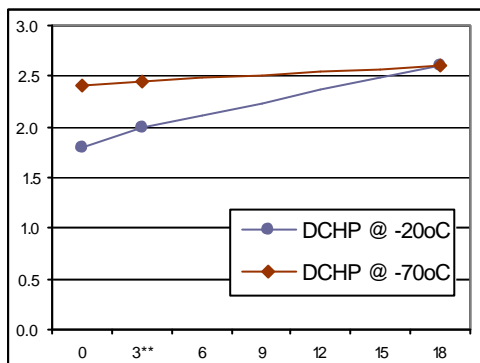


Figure A22.15 - DOP

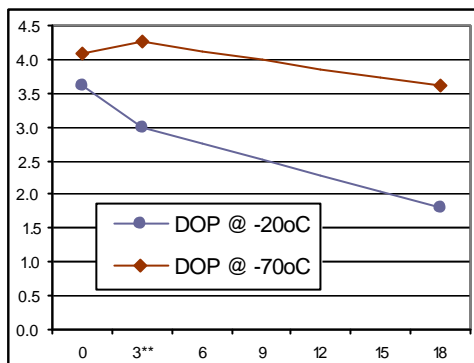
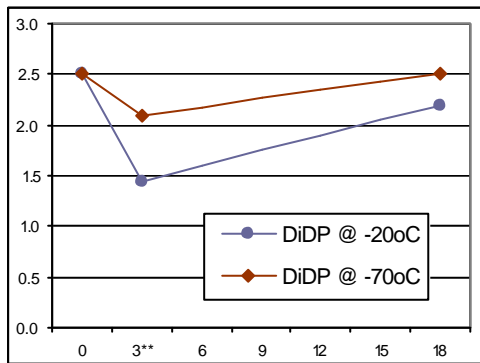


Figure A22.16 – DiDP



For four of the seven phthalates measured (DMP, DBP, BBP & DEP) there is a marked decline over the first 3 months in storage, which is greater at -20°C than at -70°C. There is a less marked decline for DOP, while DCHP & DiDP show no decline; there is even the suggestion of a small increase in DCHP over 18 months storage, which might indicate migration into the milk from the storage tube.

The unpredictable nature of some of these results, including the apparent dip in DiDP levels at 3 months followed by a recovery to 18 months, suggest either analytical variability, or that there were differences in the way the samples were handled/ processed at different times points.

These changes in phthalate levels during storage are discussed at greater length below. Initially, it was assumed that the changes represent loss of analyte during storage, although it is not possible (on the basis of this protocol design) to discriminate between degradation of phthalates within the

milk, or their absorption into the body of the container. What is more probable, however, is that changes attributed to 'analytical variability' may in fact be caused by hydrolysis of di-esters of phthalic acid to mono-esters. It has recently been reported by Calafat et al (2004) that when milk is held at room temperature, there is quite rapid loss of the di-esters from the milk as they are hydrolysed to mono-esters by the action of naturally-occurring esterases.

Between the points of analysis used in this protocol (0, 2, 4 & 18 months), there will have been differences in the way the milk was handled and/or processed prior to analysis. These are likely to have had a differential impact on the rate of hydrolysis; for example, frozen milk may have been defrosted over 2-3 hours on the day of analysis, or left out overnight to thaw prior to analysis the next day – such procedures were unregulated by the study protocol. Whatever impact this process had on intrinsic levels of phthalates in the milk, there is also a clear differential effect of storage temperature. This effect of storage temperature is in the direction predicted, signifying that storage temperature does affect the time course of any decay in phthalates during storage. At any given point of analysis, milk stored at the two different temperatures will be handled and processed in the same way (although milk held at -70°C would take slightly longer to thaw relative to milk stored at -70°C), so its impact will be minimised at any one time point.

Summary of results on the stability of the contaminants tested during long-term storage:

In short, these results indicate that for stable contaminants (e.g. PCBs, OCs, dioxins/furans, metals) the change in concentration during storage is within the analytical variability of the assay method. In other words, over the timescale being considered (several years) there are likely to be changes in the analytical methodology (reagents, equipment, personnel turnover) which are likely to lead to small differences in reproducibility over time. These, however, are likely to be of the same order of magnitude as the change in contaminant level during storage.

One contaminant, in contrast, proved much less stable during processing and storage – phthalates. The probable causes of the instability indicate that several other factors are likely to affect our ability to measure them reliably, some of which were not evaluated during the course of this study.

Two novel facts about the stability of phthalates came to light during the SUREmilk study, one from the protocol to evaluate phthalate stability during storage which suggested there was time- and temperature-dependent degradation of phthalates during storage, and the second from a published report of another study (Calafat et al 2004).

First, as shown above, levels of several phthalates (notably DMP, DBP, BBP, DEP, DiDP & DOP) declined markedly during storage, approaching the level of detection in between two to four months at -20°C (DBP, DMP, & BBP), with a slower decline at -70°C . Other phthalates (DOP, DCHP, DiDP) showed little to no decline over the 18 months for which this protocol was conducted, irrespective of storage temperature.

Separately, it has been shown that di-esters, which are normally metabolised within the body to the mono-esters, are similarly broken down in human milk at room temperature as a result of the action of esterases present in the milk (Calafat et al 2004). So, diethyl phthalates, as measured in this study, are likely to have been converted to mono-ethyl phthalates at times when the temperature of the milk sample was unregulated.

There were several steps in the process of milk sample collection for the main study, sample transport, storage, and preparation for analysis which were unregulated, with the result that the

temperature was not controlled. Following collection, breast milk samples were kept liquid at around 4°C in the domestic fridge of the donor; this process might have lasted anywhere from 1-24hr before the sample was shipped to the receiving laboratory. Samples in transit were unregulated in temperature, so that hydrolysis of phthalates might have taken place at a variable rate for indeterminate periods of time. On arrival at the processing laboratory, samples were chilled until the next working day when they could be processed. This could be on the same day, or might be as long as four days when a weekend intervened (longer for bank holiday weekends, staff leave or illness). Samples were then held in storage at -70°C for up to 3 months prior to transport to the analytical laboratory, followed by a further delay of up to 3 months (while held at -20°C) prior to their analysis: further degradation of phthalates is likely to have occurred during this time, as no procedures were adopted to block the process. Finally, following storage, samples were defrosted prior to analysis – this might have taken place over a period as short as 2-3 hours, or it may have been overnight.

All of these were variable and uncontrolled for the milk samples collected for the regional archive. While they might be controlled more effectively in future studies, some of these may prove difficult to verify in practice (e.g. time in donor's fridge prior to despatch, temperature during despatch). Accordingly, a more effective method would be to add a chemical reagent to the milk (e.g. acidification) at the point of collection to halt any degradation of candidate contaminants.

Interpretation of the current data relating to phthalates

The evidence from the laboratory protocol to evaluate the stability of phthalates during storage indicates that no confidence can be attached to the levels reported to be present in the milk samples collected for the main study, forming the regional archive. The generally low incidence of results above the limit of detection for cohorts 1 and 2 (which had the shortest storage times) probably suggest that original levels if present, were unlikely to be high (below the level at which milk was 'spiked' in the storage trial). The fact, however, that samples donated by the same mother may or may not have shown detectable levels, may simply indicate that variation in the holding, transit, processing or defrosting times of separate samples contributed to variable degrees of degradation of any phthalates within the donated milk. The unpredictable appearance of phthalates in milk of the same mother cannot therefore be taken as an indication that exposure was intermittent.

It is recommended that if phthalates are to remain a potential contaminant of interest in breast milk, then further laboratory-based protocols need to be established and run in a highly structured manner, to determine potential losses of analyte at each stage. There are likely to be other non-persistent, unstable contaminants for which archive samples are likely to be tested at a future stage. Alternative procedures must be developed, piloted and tested for each unstable contaminant for which archived milk might be tested. At the present time, it may be stated with some confidence that the proposed protocol for a national archive will operate effectively for stable contaminants (e.g. OCs, PCBs, dioxins & metals), but that this state would not apply in respect of certain other analytes (e.g. phthalates).

It is recommended that the starting point for the study of any novel contaminant would be a laboratory-based protocol to compare analyte levels in freshly expressed milk (without storage, freezing or de-frosting), compared to levels in that same milk after being held for variable periods at ambient temperatures, perhaps even frozen and stored. An important step to achieving this would be the facility for enabling breastfeeding mothers to travel to the analytical laboratory and express milk for analysis on site. The expressed milk might be split, with one fresh portion being analysed with minimum delay, the second half, might be transported home by the mother, then returned to the processing laboratory by a more traditional route; the milk being re-tested once it

has passed through routine sample handling and processing steps. This would provide a simple and pragmatic test of several areas of ambiguity, while rather more focused, controlled laboratory studies would be necessary to determine the absolute constraints on sample handling, storage and processing.