Cibele Vieira Cunha Rudge

Níveis de substâncias tóxicas persistentes (PTS) em sangue de parturientes de sete áreas selecionadas do Estado de São Paulo - Brasil

Tese apresentada a Faculdade de Medicina de Botucatu- UNESP no programa de Pós-Graduação em Ginecologia, Obstetrícia e Mastologia, Área de concentração Obstetrícia e ao Institute of Community Medicine – University of Tromso, Noruega, para obtenção do título de doutor em co-tutela.

Orientadores: Iracema M. P. Calderon e Jon Øyvind Odland

UNESP
2010

Cibele Vieira Cunha Rudge

Levels of selected persistent organic pollutants in blood from delivering women in seven selected areas of São Paulo State, Brazil

Thesis presented to Botucatu Medical School – UNESP, to Gynaecology, Obstetric and Mastology Program; Obstetrics area, and to the Institute of Community Medicine – University of Tromso, Norway to obtain the double title PhD.

Supervisors: Iracema M. P. Calderon and Jon Øyvind Odland

UNESP
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Palavras chave: Brasil; Gestação; Pesticidas; Substancias tóxicas persistentes

Dedicatória

"Tenha bom animo e coragem: você vencerá todas as dificuldades!".

A vida apresenta-nos problemas às vezes difíceis, mas dificuldade superada é problema resolvido.

Jamais desanime: você há de vencer galhardamente todos os problemas que se apresentarem. Se o problema for complexo, divida-os em partes, e vença cada uma delas separadamente.

Não desanime jamais!"

Deus,

Pela oportunidade da vida, da minha família, da minha saúde, paz e alegria.

Mãe e pai,

Vocês me transmitiram os valores mais importantes: a bondade, o perdão, a honestidade, a persistência, a consideração e principalmente, a paciência. Vocês me mostraram que até os dias mais difíceis são superados com carinho, trabalho, luta constante! Obrigada por me ensinarem a aproveitar todas as oportunidades que a vida oferece, por me ensinarem a ouvir, a valorizar o próximo.

Mas vocês fizeram muito mais do que isso. Estiveram sempre ao meu lado, dando a mão para que eu tivesse a firmeza para seguir em frente, o colo para acalmar, os ouvidos para me ouvir (principalmente na Noruega), a mente para entender minhas idéias e propor soluções, a espiritualidade para me fazer entender a grandeza de tudo, e principalmente o útero, por me carregar, proteger, guiar e dar a grande oportunidade: ser sua Filha!

Pá e mã, vocês me ensinaram a substituir a preocupação em fé, a sensação de vazio por paz de espírito e o desespero em esperança.

Cunha,

Obrigada por me fazer acreditar que eu poderia crescer e conquistar o que quisesse desde que acreditasse em mim, como você sempre acreditou. Nem consigo dizer o quanto significa saber que você sempre está ao meu lado, me incentivando a realizar meus sonhos. Por ser companheiro na caminhada, guia nas encruzilhadas, alento no cansaço. Agradeço a compreensão e a maturidade por entender o quanto morar fora era importante para mim.

Felipe e Helena,

"Plante em torno de você as sementes do otimismo e bondade, para que possa colher amanhã os frutos do amor e felicidade". Agradeço a Deus a oportunidade por fazer parte da vida de vocês. Obrigada pela compreensão dos momentos de ausência.

Mara,

"Obrigada por ter me criado, substituindo muitas vezes o papel da minha mãe"

Raul, Vânia, Moema (in memorian) e Tati,

Obrigada pelo ombro amigo, por mostrarem que nossas dificuldades sempre são pequenas se comparadas a de outros. Vocês me deram confiança para enfrentar os desafios da vida com sorriso nos lábios.

Aos meus Avós (in memorian), Vó Lete, Tia Ruth e padrinhos,

Por terem mostrado sempre o valor do estudo, da honestidade, e da ética.

Jon Oyvind,

Pela visão de mundo e de futuro. Por iniciar, manter e engajar uma network mundial. Você é exemplo a ser seguido para a criação de um mundo plano, sem fronteiras. Você tem feito a diferença por mundo melhor.

Tia Cema,

Obrigada por aceitar esta missão. Admiração, respeito, gratidão é pouco para expressar nosso sentimento por você! Você foi capaz de carregar o desejo de unir gerações através do estudo. Mais do que admiração e respeito , sinto afeto e gratidão profunda.

Halina,

Por ser exemplo de como lidar com pessoas: ouvir ativamente, ter e expressar consideração, elogiar, reconhecer, estabelecer o padrão, deixar claras as expectativas, dar condições para manter o padrão estabelecido.

Tia Cida,

Obrigada por ser paciente comigo, por me dar atenção, me valorizar e incentivar.

Adriano, Helio e toda equipe do GAP,

Pela paciência, disponibilidade e capacidade: vocês fazem diferença na vida do pós-graduando: obrigada!

Gustavo, Geraldo, João Luiz, Corintio, Roseana, Nelson,

Por juntar nossas forças num trabalho em equipe, aproveitando as oportunidades com visão, ousadia e confiança."Há uma mão lá em cima que o ajudará a avançar. Não se importe caso o considerem louco em sua busca.Certamente os que o julgam,não teriam coragem de realizar suas façanhas"(Tiago Toricelli)

Professores do EPINOR,

Pela receptividade e por terem me proporcionado uma experiência indescritível.

Laila, Anita, Ellen, Anna-Sphia, Solrunn, Olga, Natalia, Linda, Trina, Isabel, Josepha e famílias,

Houve dias maravilhosos, dias de desanimo, mas enfim, esta jornada me revigorou. Vocês me ajudaram a exercitar a paciência, a tolerância, a diminuir a solidão, a dor, o medo, as dúvidas e a saudade. Com vocês conheci lugares que nunca havia ouvido falar, senti a bondade das pessoas, convivi com suas famílias e hoje agradeço a todas esperando um dia retribuir.

Iara Linhares,

Por ter me dado a mão num momento difícil, oferecendo sentimentos puros e grandiosos.

Pessoal da PGGOM, seção de PG e PROPG:

Pela simpatia, amizade, dedicação e presteza com que sempre me trataram.

Ao Skype,

Por ter permitido conviver com muitos amigos durante todo período que estive longe.

Amigos,

O carinho de vocês foi muito importante, principalmente nos dias mais difíceis!

Adnice,
Pela impressão da tese, e por me acalmar num momento de tensão, ensinando que as pequenas coisas da vida são simples e não custam nada.

CAPES,
Pela bolsa PDEE que permitiu a realização de um vôo imensurável. O Brasil precisa de vocês.

Sandra e Sun,
Por acreditarem no meu potencial, e entenderem a importância deste doutorado.

Artic Monitoring Assessment Programme (AMAP)
Pelo financiamento deste projeto.

Norwegian Institute of Air Research (NILU), em especial ao Torkjel, Linda e
Pela analise das amostras.

"Um homem precisa viajar. Por sua conta, não por meio de historias, imagens, livros ou televisão. Conhecer o frio para desfrutar o calor. E o oposto. Sentir a distância e o desabrigo para estar bem sob o próprio teto. Um homem precisa viajar para lugares que não conhece para quebrar essa arrogância que nos faz ver o mundo como o imaginamos, e não simplesmente com é ou pode ser..."(Airton Ortiz)

Acknoledgements

"Have animus and courage: you will overcome all obstacles! Life gives us some difficulties. However, once they are overcome, the trouble is solved.

Never get disheartened: you must surpass all the problems that you ever have. If a problem is complex, split it up, and conquer each one. Never get hopeless"

To God,

To the opportunity of my life, my family, health, peace and happiness.

My mother and my father,

You have taught me the most important values: humanness, forgiveness, honesty, persistency, consideration, and mainly, patience. You showed me that even the hardest days could be surpassed with kindness and hard work. Thanks for drill me to keep all the opportunities that life gives us, to educate me to hear and to value the other.

But, you did more. You have always been beside me, helping me feel steady, the lap to be calm, lending your ears (especially during the stay in Norway), the mind to comprehend my ideas and show me new horizons, the spirituality to understand the hugeness, and of course the uterus to carry, protect, guide and give the best opportunity: be your daughter!

Mom and Dad, you coached me to change worries by faith, emptiness for peace, and desperation in hope.

Cunha,
Thank you for making me believe that I could grow and conquest whatever I could, since I believe in my potential. I have no words to thank you for being always on my side, encouraging me to accomplish my dreams. For being my partner though the track, guide in the crossroads, and the breath in the tiredness. I do appreciate your maturity in understanding how important it was for me to live abroad.

Felipe e Helena,
Cultivate the seeds for optimism and charity, to harvest in the future the love and happiness. Thanks for being part of my life. Please apologize me for the absence periods.

Mara,
"For have always taking care of me, replacing my mother's position"

Raul, Vânia, Moema (in memorian) e Tati,
Thanks for the friendship, for showing me that our problems are usually minor ones when comparing with the others. You provide me the trust to fight the challenges, and always with a smile.

My grandparents (in memorian), Vó Lete, Tia Ruth e Godparents,
For teaching me important values, such as: study, honesty and ethics.

Jon Oyvind,

For your vision about the world and future. To star witht, for having maintained and engaged a network world. You are an example to be followed for creating a world plan, without borders. You have made the difference for a better world.

Tia Cema,

Thank you for accepting this mission. Admiration, respect, gratitude is little to express our feelings for you! You were able to load the desire to unite generations through the study. More than admiration and respect I feel deep affection and gratitudefor you.

Halina,

For being an example of how to deal with people: hearing activily ,having and expressing account, praising, recognizing, establishing a pattern, leaving clear the expectations and giving conditions to keep the established pattern.

Tia Cida,

Thank you for being patient with me, for giving me attention, appreciatiation and encouragement.

Adriano, Helio and the whole team of GAP,

Thanks for the patience, availability and capacity: you do make a difference in the life of graduate: Thank you!

Gustavo, Geraldo, João Luiz, Corintio, khokhoi, Roseana,

By joining our forces in teamwork, leveraging opportunities with vision, boldness and confidence. "There is a hand up there to help you go further. No matter you are considered insane in this search. Certainly, the ones who judge would not have the courage to perform these deeds "(Tiago Toricelli)

EPINOR, teachers

The responsiveness and for having provided an indescribable experience.

Laila, Anita, Ellen,Anna-Sophia, Solrunn, Olga, Natalia, Linda, Trina, Isabel,Josepha and families.

There were wonderful days, upsetting days, but this has reinvigorated my journey. You helped me to me exercise patience, tolerance, decrease solitude, pain, fear, doubts and homesick. With you I have known places that I had never heard before, I felt the goodness of people, I lived with their families and today I thank all of them hoping that one day I can give all their love in return.

Iara Linhares,

For having in my difficult moments, offered pure and great feelings.

The staff of PGGOM, PROPG and PG section

For the sympathy, friendship, dedication and preparedness that you have always addressed me.

The Skype,

For having allowed me to keep in touch with many friends throughout the period that I was away.

Friends,

Your love was very important, especially in the most difficult days!

Adnice,

For printing the thesis, and for cooling me in times of tension, teaching me that small things in life are simple and cost nothing.

CAPES,

For the PDEE scholarship that provided the accomplishment of a higher flight. Brazil needs you.

Sandra and Sun,

For believing in my potential and understanding the importance of this doctorate.

Artic Monitoring assessment programme (AMAP)

For sponsoring this project.

Norwegian Institute of Air research (NILU),

Specially Torkjel, Linda for reviewing the samples.

"A man needs to travel. By himself, not though histories, pictures, books or television. He has to know the cold to enjoy the heat. And as opposed. feel the distance and the lack of shelter to be well under the own roof. A man needs to travel to places that he doesn't know to break the arrogance that makes us see the world as we imagine, and not simply as it is or it can be ... "(Airton Ortiz)

Prefácio

DOUTORADO: fatos desta etapa da minha vida

Assim como uma vida, este doutorado nasceu do encontro despretensioso do professor norueguês Jon Odland, então em visita a Botucatu, com a Diretora da Faculdade de Medicina de Botucatu, Marilza V C Rudge, minha mãe. Um projeto foi delineado para identificar se os poluentes encontrados nas gestantes do hemisfério Norte eram os mesmos nas do Hemisfério Sul (Brasil e África do Sul). Buscava-se também a internacionalização do programa de PGGOM.

A idéia inicial seria apenas de me responsabilizar pelo "site" de Santos. Mas, muitas coisas aconteceram...

Bom, vamos a uma breve retrospectiva deste período...

Em março de 2007, o Jon ofereceu vaga para aluno de doutorado na Universidade de Tromso na Noruega, no EPINOR (Epidemiology of North). A proposta me agradou, apenas 15 dias na Noruega a cada 6 meses durante 2 anos, não poderia querer algo melhor! Sem nem perguntar onde ficava esta cidade, disse sim. Mas, aos poucos a realidade foi se moldando diferente, eram 2 meses, que depois se estenderam para 9 meses em Tromso...lém disso, a Pró-reitora (minha mãe...) inventou mais um complicador: transformar o doutorado do PGGOM em doutorado em co-tutela com dupla titulação. Estava eu no meio de uma coisa nova, tanto na Faculdade de Medicina de Botucatu como na University of Tromso.

Começavam os problemas e também as ajudas...

Somente apoio do marido e da família não seria suficiente. Alguém precisava se responsabilizar pelas coletas nos outros sites e armazenamento do material. Obrigada Gustavo, João Luiz, Geraldo, Rosiane, Coríntio e Nelson: ***vocês foram fundamentais para que esse projeto acontecesse.***

A Maria do Carmo (PROPG/UNESP) sempre me auxiliou no relacionamento com a CAPES para a bolsa PDEE. ***Obrigada pela sua capacidade!***

Chegada a Tromso...

Ingvill, aluna do Jon, foi me buscar no aeroporto e levou-me para jantar em casa. E logo ofereceu roupa de cama, toalha, etc... detalhes que nem imaginava ao sair do Brasil.:a ficha caiu., estava no Pólo Norte, ou seja: 69°39,N, 18°56,E!

Primeira disciplina do EPINOR: Epidemiologia/ Estatística. O curso começou com revisão "básica" com o Prof Bjorn Straume, mas eu nem sequer sabia que desvio padrão e "standard deviation" eram

a mesma palavra ! Imaginem meu desespero! Pior, os outros alunos discutiam qual software seria usado... Ao me estressar, olhei pela janela, e o que vi? NEVE! ***Minha primeira neve!!! Deletei aquela aula e fiquei admirando o silêncio da neve!!!***

Éramos 12 alunos da primeira turma do EPINOR, alguns da Noruega, outros da Rússia, das Ilhas Faraoes e até do Sri-Lanka: pessoas de culturas diferentes, com backgrounds diversos, e um objetivo em comum: fazer PhD. Tínhamos uma sala, e foi nesta sala que trocamos nossas experiências, fizemos nossos trabalhos e criamos um círculo de amizade. Comecei levando o café brasileiro, que fazia o maior sucesso, e aos poucos todos traziam coisas típicas e comemorávamos os aniversários.

*Comecei a ser convidada para reuniões festivas nas casas. A primeira foi a Laila, em janeiro (com muita neve, muito frio e escuro); logo depois a Anita me levou para esquiar. Trabalho duro durante a semana, e nos finais de semana comidas típicas (bacalhau fresco e com cabeça; lingua de bacalhau, fígado de bacalhau, rena, foca, baleia, etc), e claro caipirinha. Aos poucos fui conhecendo as famílias de cada um, acompanhei um pouco da vida deles, como a crisma do Joaquim (filho da Anita), a viagem anual com a família da Ellen, a ida ao circo com os filhos da Solrunn e Anna-Sophia, o lugar exótico e maravilhoso em que a mãe da Laila mora sózinha e a minha cama, que ficou lá para que a Laila tomasse conta, até hoje! Enfim, um g****rupo maravilhoso e amigo, que fez da minha estada na Noruega um momento especial que será lembrado por toda vida.***

O tempo foi passando e as aulas foram ficando claras, e eu começando a entender o mundo da epidemio/ estatística. ***Tia Cida, sem você para clarear minhas dúvidas, hoje eu estaria no Hospital Psiquiátrico! O Adriano com muita paciência me explicava conceitos básicos de epidemiologia pela internet. A estrutura e as pessoas do GAP fazem diferença na vida do pós-graduando: obrigada!***

Enquanto não tinha meus dados comecei a trabalhar com os da Halina, que tem um projeto semelhante ao nosso na África do Sul. ***Eu*** *fiz a análise estatística dos dados, mas minha curiosidade científica levou-me a delinear outro trabalho com os mesmos dados; esses são hoje os capítulos 1 e 2.* ***Considero Halina a madrinha deste doutorado; muito obrigada pelo apoio, ensinamentos, amizade, risadas e companheirismo.***

Recebi visitas inesquecíveis: o Cunha, meus pais, tio Wado e tia Cida: um sonho! Reencontrei amigos de infância: Fabio, Vanessa e Mariana: ***Obrigada pelo apoio de vocês!***

Participei da organização do International Conference on Circumpolar Health Research, em Tromso. Foi muito interessante ver as diferenças de problemas e de soluções. E claro também tive as

dificuldades de entender o idioma norueguês e, em especial, durante o congresso quando pedi para o patrocinador pagar sua própria inscrição! ***Até hoje sou lembrada por essa gafe!!!***

Fui recebida com muito carinho em Bodø pela família do Jon e tratada como filha. Essa interação resultou no intercâmbio de duas alunas na graduação da FMB-UNESP, a Maria (filha do Jon) e a Kristin que aqui ficaram 6 meses. ***Jon, sua família é maravilhosa. Obrigada por ter me acolhido como filha.***

Sou muito feliz em dizer que deixei muitos amigos na Noruega, e fiz muitos outros ao redor do mundo. E para terminar com chave de ouro este período, fiz curso de estatística aplicada ministrado por professores de Harvard! ***Olhem como voei muito mais alto do que um dia sequer imaginei!***

De volta ao Brasil aos 12 de julho de 2008...

Como é bom estar perto de todos, reencontrar os amigos, em especial a família. ***Vocês fazem muita falta!***

Ao voltar, finalizei os dois primeiros papers para publicação. Enquanto isso, a problemática com as análises laboratoriais das amostras do Brasil parecia insolúvel. Por fim a solução foi mandar o sangue para a Noruega. Não tão simples como parece, pois para envio das amostras até curso de certificação do IATA eu fiz!

O aceite do capitulo 2 desta tese me deu muita força e incentivo para continuar, pois foi considerado um dos 10 "hot articles" do Journal of Environmental Monitoring.

Em agosto/ 2009 na Irlanda, sob a coordenacão do Jon, apresentamos e discutimos os resultados deste projeto multicêntrico: Halina (África do Sul), Solrunn (Noruega, trabalhando com dados do Vietan) e Alexander (Rússia). ***Uma verdadeira network estava estabelecida. Jon, parabéns por esta realização****.*

Só ao final de 2009 recebi os resultados das análises do Brasil. Tive que correr contra o tempo, pois este minava mais rápido que uma ampulheta. Estava pronta a introdução para um paper sobre metais pesados, quando recebo e-mail com resultados sobre pesticidas...(capitulo 3). Quando questionei, ouvi do Jon: "agora eu tenho que ser o orientador, ***just do it****". Aprendi porque as crianças norueguesas são diferentes! Fiquei quieta, e fiz. Espero que gostem!*

Ah, Tia Cema acha que eu esqueceria de você? Minha querida orientadora, muito obrigada por tudo, desde suporte seja nos aspectos burocráticos, aos puxões de orelha, e por estar sempre ao meu lado para entender o que estava tentando dizer ou pensar. Você conseguiu clarear várias das minhas idéias.

Mas por último tenho que dizer que tudo isso só foi possível devido a uma pessoa muito especial: minha mãe! Mã, foi você quem organizou este projeto no Brasil, que me permitiu fazer parte dele, e até mesmo de concluí-lo. É bem verdade que quando as pessoas perguntavam quem tinha me mandado para um local tão longe e frio, tinha que dizer: "MINHA PRÓPRIA MÃE!!!".

Conclusão...

Hoje olho para trás e vejo que esta tese é a somatória da ajuda de muitas pessoas, com muito trabalho associado à dedicação e compreensão. Nunca pude imaginar que o resultado desta experiência seria tão positivo. Ganhos que não tem preço: conhecimento, amizades, cultura, abertura de horizontes, valorização de tudo o que temos e recebemos a cada dia.

Deixo meu estímulo para aqueles que puderem (ou melhor, que quiserem), lutem por este sonho, vale muito a pena.

Cibele

Preface

PhD: memories of this time of my life

As is often the case in life, this PhD was the result of an unpretentious meeting between the Norwegian Prof Jon Odland, visiting Botucatu, and Marilza Rudge , the Dean of Botucatu Medical School at that time, , and my mother. A project was designed to identify if the pollutants found in pregnant women in the Northern Hemisphere were the same as in the South (Brazil and South Africa). Indeed, the internationalization of our ObGyn graduate program was a secondary endpoint.

At that time, the plan was that I should be only responsible for the Santos site. However, a lot of things happened during this period…

Lets go through a brief retrospective...

In March 2007, Jon offered a position for a PhD student at the University of Tromso, Norway, at EPINOR(Epidemiology of North). From the beginning it seemed to a good proposal: 15 days, twice a year: what more could I wish? Even without questioning where this city was located, I just answered "yes, I want to go!". Bit by bit, the reality was being built in a quite different way. It should be 2 months instead of 15 days, and in the end there was 9 months in Tromso.

Furthermore, the graduate provost (my mother again) added a new complicating factor: upgrade a Brazilian PhD to a PhD in double title. So there I was, in the middle of a lot of new issues, not just for me, but also for Botucatu Medical School and also for the University of Tromso.

Problems starting to come up, as well as the assistances....

To have my family and husband's support would not be enough. I would need someone to be responsible for the collection of samples and the logistics. Thanks to Gustavo, João Luiz, Geraldo, Rosiane, Coríntio e Nelson: ***you all exerted a fundamental role to make this project a reality.***

Maria do Carmo (graduate program) was always helpful to me, especially concerning CAPES issues. ***Thank you for being so skilled a person!***

Arriving in Tromso...

Ingvill, Jon's student, picked me up at the airport, and just invited me to a dinner at her house. Promptly, she offered me bed linens, towels, etc… details that I had never thought I would need before leaving Brazil, than I realized…. I was at the North Pole, 69°39N, 18°56E!

The first course in EPINOR was in basics epidemiology and statistics. The course started with a "quick"overview of basic concepts, by Prof Bjorn Strume, but I did not even know that "desvio padrão"and "standard deviation" were the same! Can you imagine my desperation?! It can be worse; the other students were discussing which software should be used! Once when I felt hopeless, I looked through the window, and can you imagine what I saw? SNOW! ***My first SNOW! I just deleted the class and appreciated the silence of the snow!!!***

We were 12 students in the first EPINOR group, some from Norway, others from Russia, Faroes Island, and from Sri-Lanka: people with different cultures, backgrounds, with a common objective: to be a PhD. We shared a room (8501), and were in this room where we changed experiences, did our daily work, and started a friendship circle. I started bringing the famous Brazilian coffee, and the others brought typical foods, and we always celebrated each other's birthday.

I started to be invited for dinners and parties. The first was Laila, in January (with a lot snow, cold and darkness); after just a short while, Anita took me skiing. Hard work during the week days, and typical food on weekends (fresh cod, tong cod, cod liver, reindeer, seal, whale, etc..) and caipirinha of course! Suddenly I got to know and share all the students' families, the charisma of Joakim (Anita's son), the famous 17th of May, the annual travel with Ellen's family, the circus with Solrunn and Anna-Sophia, and the very special place where Laila's mother lived alone, and my bed, that Laila is taking care of until today! ***Thus, a so marvelous and friendly group that evolved from my stay in Norway. A unique experience that I'll never forget.***

The months were passing and the classes becoming more clear, I came to realize that I was understanding the Epidemiology/ Statistical world. ***Dear Cida, without you to clarify my doubts, today I might be in a Psychiatric Hospital! Adriano, your patience in explaining to me such basic concepts by internet, the structure and people from the Research Support Center made the difference in a PhD student's life: thank you very much!***

As long as I did not have my own data, I started working with Halina's data that was a similar project in South Africa. I did the statistical analysis from this data, and my scientific curiosity took me to design another paper with the same data; these are now chapters 1 and 2 from this thesis. ***I consider Halina as a Godmother of this thesis; thanks a lot for your support, teachings, friendship, laughs and communion.***

I received such unforgettable visits during my stay: Cunha, my parents, Wado and Cida Brasil: quite a dream! Reunited childhood friends: Vanessa, Fabio and Mariana: ***thank you for your assistance!***

I was a member of the organizing committee for the International Conference on Circumpolar Health Research, Tromso. It was very interesting to realize the differences, shared problems, and solving them. For sure, there were some difficulties with the language (names from Scandinavian countries are quite different!), that got me in trouble like the time I asked for the sponsor to pay for his own registration! ***Until now I am remembered for this gaffe!***

Jon's family very well received me in Bodo, and they treated me like a daughter. The result of this interaction was an exchange program with two medical students from Tromheim University to Botucatu (Maria- Jon's daughter and Kristin). ***Jon, you have a very special family. Thank you for having accepted me as daughter.***

I am very happy to say that I left many friends in Norway, and others from around the world. And to finish on a high this time of my life, I attended a course in modern methods in Biostatistics and Epidemiology, run by Harvard and Karolinska University! ***It is impressive how far I flew, I couldn't believe it!***

Back to Brazil on July, 12, 2008...

To be just close bodily to all, meet all my friends, and especially my family ***is absolutely priceless! I missed you a lot!***

In Brazil, I finalized the first paper, and submitted the second for publication. In the meanwhile, the troubles with lab analysis seemed never-ending. The solution was to send the blood to be analyzed in Norway. Not as simple as it seems. In order to realize it, I even took a IATA course.

The acceptance of the second chapter gave me strength to continue, since it was considered one of the 10 "hot articles" from the Journal of Environmental Monitoring.

In August/2009, in Dublin, under Jon's coordination, we presented and discussed the results from this multicenter study: Halina (South Africa), Solrunn (Norway, working data from Vietnam, and Alexander (Russia). A real network was established. ***Jon, congratulations for this conquest****.*

And only in November 2009, I received the results from the lab analysis. I needed to run as quickly as possible, since the time passed very fast. Faster than an hourglass. I was done with an introduction for a metal paper, when I received the results about pesticides…. (chapter 3). I questioned and received the reply from Jon in the same way: "Now I need to be the supervisor, ***just do it****". Than I understood the reason why the Norwegian kids are so different from ours! I just kept quiet, and did it. Hope you appreciate this effort!*

Oh, dear Iracema, do you think I would miss you? ***My dear supervisor, thank you for everything, from your support in bureaucratic issues to calling the shots, and also for being always on my side, understanding and clarifying my thoughts and ideas.***

And last but not least, I need to say that the feasibility of this project was only possible due to a very special person: MOM! Mom, you have organized the project, allowed me to share, and even to conclude it. But, when people in Norway asked me who sent me so far North, I needed to be honest: ***my own mother!***

Conclusion...

Looking backward, I would say that this thesis is a sum of many help, associated with hard work and comprehension. I could never imagine how positive such an experience could be. Gains that I consider priceless: knowledge, friendship, culture, opening new horizons, and appreciation of all we have and receive each day.

I hereby strongly encourage all that have this possibility (or better, really wish to have this opportunity) to fight for your dreams. It is worthwhile!

Cibele

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Levels of toxic and essential metals in maternal and umbilical cord blood from selected areas of South Africa—results of a pilot study†

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This pilot study uses concentrations of metals in maternal and cord blood at delivery, in seven selected geographical areas of South Africa, to determine prenatal environmental exposure to toxic metals. Samples of maternal and cord whole blood were analysed for levels of cadmium, mercury, lead, manganese, cobalt, copper, zinc, arsenic and selenium. Levels of some measured metals differed by site, indicating different environmental pollution levels in the regions selected for the study. Mercury levels were elevated in two coastal populations studied (Atlantic and Indian Ocean sites) with mothers from the Atlantic site having the highest median concentration of 1.78 μg/L ranging from 0.44 to 8.82 μg/L, which was found to be highly significant ($p < 0.001$) when compared to other sites, except the Indian Ocean site. The highest concentration of cadmium was measured in maternal blood from the Atlantic site with a median value of 0.25 μg/L (range 0.05–0.89 μg/L), and statistical significance of $p < 0.032$, when compared to all other sites studied, and $p < 0.001$ and $p < 0.004$ when compared to rural and industrial sites respectively, confounding factor for elevated cadmium levels was found to be cigarette smoking. Levels of lead were highest in the urban site, with a median value of 32.9 μg/L (range 16–81.5 μg/L), and statistically significant when compared with other sites ($p < 0.003$). Levels of selenium were highest in the Atlantic site reaching statistical significance ($p < 0.001$). All analysed metals were detected in umbilical cord blood samples and differed between sites, with mercury being highest in the Atlantic site ($p < 0.001$), lead being highest in the urban site ($p < 0.004$) and selenium in the Atlantic site ($p < 0.001$). To the best of our knowledge this pilot investigation is the first study performed in South Africa that measured multiple metals in delivering mothers and umbilical cord blood samples. These results will inform the selection of the geographical sites requiring further investigation in the main study.

Introduction

Human exposure to persistent toxic substances (PTS) in the living environment, which include toxic metals and persistent organic pollutants, can be from natural sources, anthropogenic from current or past industrial activities, and from living activities of the population. PTS have the ability to exert negative health effects that are often subtle, long-term, sometimes transgenerational and difficult to measure, even in epidemiological studies in large populations. Furthermore, the most vulnerable periods for toxic impact of pollutants on human development are the embryonic and foetal stages, followed by early childhood; most PTS are known to affect reproductive health and pregnancy outcomes, reduce disease defense mechanisms, impact on children's physical and mental development, and increase the risk of cancer.[1–3]

Several multidisciplinary international projects are currently investigating firstly, the sources and levels of PTS in people residing in different geographical regions and secondly, ascertaining the relationship between the levels of these compounds and health. For example, the Arctic Monitoring and Assessment Programme (AMAP) initiated in 1991 measured levels of multiple contaminants and studied possible health effects and birth outcomes of these in the indigenous and other populations living in the Arctic and other areas of the Northern Hemisphere.[4–6] Studies in Canada found elevated levels of methyl mercury not only in indigenous Dene and Inuit populations, but also in the general population residing in other areas of Canada.[7] Elevated levels of organochlorines and metals were also detected in human fluids such as breast milk, in populations residing in different areas within the polar region.[8–12]

At present no comprehensive data exist on the levels of contaminants in ecosystems and populations in the Southern Hemisphere. To date, in South Africa, research linking environmental exposures to human health outcomes in the general population has been scarce. A number of South African studies used animals as bio-indicators for environmental contamination; examples are vanadium levels in cattle, cadmium levels in terrestrial isopod (Porcellio laevis) and in the river crab, or the arsenic resistance in species of multi-host ticks.[13–15]

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† Part of this study was presented at the Symposium held at the 19th Conference of the International Society for Environmental Epidemiology, ISEE 2007 in Mexico City, Mexico, 5–9 September 2007.

A limited number of community based studies performed in South Africa indicate elevated environmental levels of metals that may have detrimental effects on public health. For example, environmental mercury pollution, fish contamination and health problems in the community residing in the vicinity of a non operational mercury processing plant have been reported.[16] Due to the pervasive lead contamination in the country, a high percentage of children residing in inner cities and informal settlements, as well as peri-urban and rural areas, was found to have unacceptably high blood lead levels.[17–20] Elevated levels of blood manganese have been found in school children in some areas of South Africa.[21,22]

Although a number of South African studies report on exposure to metals and possible health effects in occupationally exposed subjects, little is known about exposures of the communities residing in the vicinity of such operations.[23,24]

South Africa, the southernmost part of Africa in the Southern Hemisphere, and being both a developed and a developing country, is of particular importance to the global research in the science of environmental pollutants and human health outcomes.

Firstly, South Africa is rich in mineral deposits and economically important metals such as lead, manganese, platinum, chromium, vanadium and gold, are being mined extensively. The country is also a major producer of other metals such as aluminium, zinc and copper, from enriched deposits; thus the potential exists for emission of these metals into the environment. Though industries are constantly upgrading production technologies to meet global standards and to comply with environmental regulations, the use of outdated technologies in the past may have contributed to toxic metal contamination around certain industrial sites, particularly mining and smelting. South Africa is also experiencing a rise in informal mining (especially artisanal gold mining) and other uncontrolled informal industrial activities that often take place within the living environments of the communities.[25] Secondly, increased population migration and rapid urbanization, with its wide range of anthropogenic activities, may further contribute to environmental degradation and pollution. A high prevalence of infectious diseases (lung diseases, TB, HIV/AIDS) and endemic malaria present in parts of the country make South African and other populations living in the developing countries of the Southern Hemisphere more susceptible to the toxic effects of pollutants in the living environment.

Within this context and in response to the lack of comprehensive data on levels of PTS in populations residing in South Africa, a pilot project was designed and carried out under the auspices of AMAP, by the South African Medical Research Council and the University of Tromsø, Norway during 2005–2006.

Although both metals and organic persistent pollutants were measured in maternal and cord bloods in the pilot phase of the study, this paper reports on the metal results only. The results for the concurrently measured organic pollutants (polychlorinated biphenyl congeners (PCBs), pesticides and their metabolites, and perflourinated compounds) will be reported separately.

The present paper reports on the levels of cadmium (Cd), mercury (Hg), lead (Pb), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn), arsenic (As) and selenium (Se) found in maternal and umbilical cord bloods drawn from random samples of delivering women in seven selected regions of South Africa that differ in their degree of environmental pollution. Other parameters reported include socioeconomic factors of participants, self reported health status, life style, diet and birth outcomes. The manuscript that will assess in detail the placental permeability for metals measured in paired maternal-cord blood samples is in preparation.

Materials and methods

Selection of study sites

All seven study sites were purposely selected to include a range of different communities: rural, urban, industrial, fishing (situated on the Atlantic Ocean), mining, coastal endemic malaria (situated on the Indian Ocean) and inland endemic malaria. Selected sites differed in the type of environmental pollution and all had a provincial delivery hospital serving the particular community. The rural site is situated close to the Botswana border where no agricultural and industrial activities take place with no major roads or traffic in the area. The urban site is the large city of Johannesburg with extensive gold mining and other industrial activities in and surrounding areas and heavy traffic. The industrial site selected is a coal mining and stainless steel producing small town. The fishing site in the Western Cape is situated on the Atlantic Ocean is known for its fishing and fish processing industry. The mining site is a small town where extensive gold mining takes place. The coastal village on the Indian Ocean is only 8 km away from the Mozambique border and in the vicinity of the world heritage site of Kosi Bay, where only subsistence fishing is allowed. The inland site is a small town with very little industrial activity that is about 70 km away from the Indian Ocean coastal site, but also malaria endemic. The choice of two malaria endemic sites was necessary for the investigation on persistent organic pollutants which are also part of this project. Fig. 1 shows the geographical location of each study site within South Africa.

Recruitment of participants and informed consent

Ethics clearance certificate Protocol Number M040314 for the study was granted by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg, South Africa. In addition, informed written consent was obtained from each participant prior to commencement of the study.

Potential participants were recruited from women who presented for delivery at the hospital. Recruitment was done by the health worker on duty and trained research assistant who briefly explained the objectives of the study and distributed a detailed information sheet about the project, written in simple language. About 95% of potential participants approached agreed to participate. Women who volunteered to participate signed an informed consent form and agreed to donate blood and urine samples before delivery and cord blood samples post-partum and they agreed to answer a socioeconomic questionnaire by interview in the language of their choice and to grant the research team access to hospital records, post-partum.

Data collection

A socioeconomic questionnaire that also included dietary, life style and self reported health status questions was administered

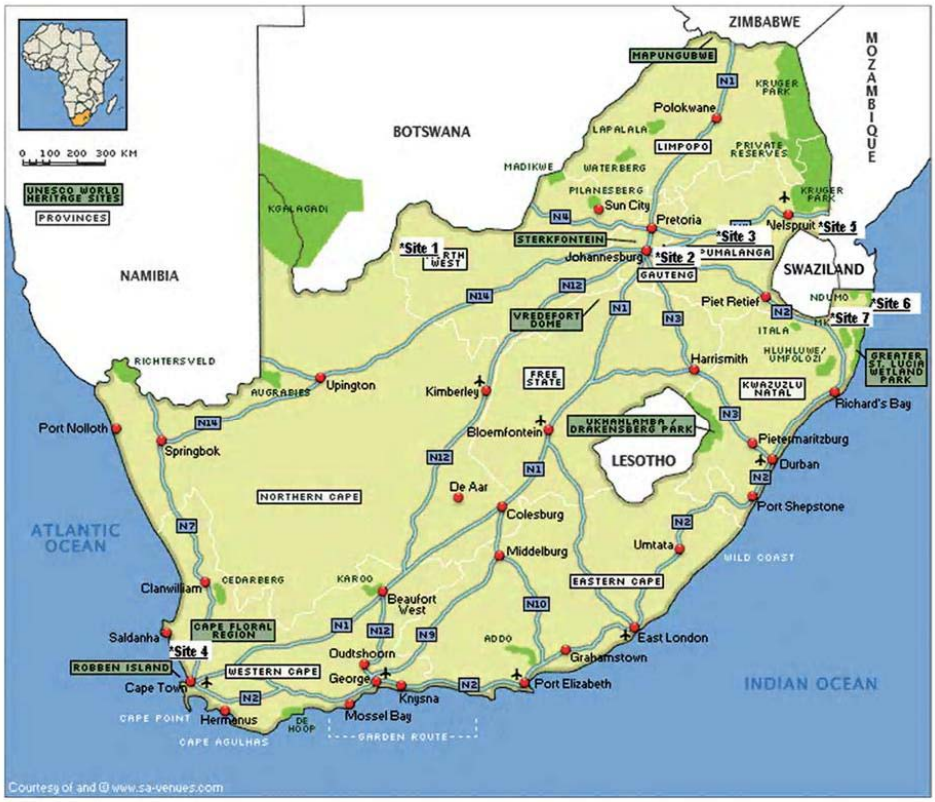

Fig. 1 Geographical positions of study sites within South Africa. Legend: Site 1 = Rural; Site 2 = Urban; Site 3 = Industrial; Site 4 = Atlantic Ocean; Site 5 = Mining; Site 6 = Indian Ocean; Site 7 = Inland malaria.

by trained research assistants in English or in a language of the participants' choice. After delivery, the researchers extracted records from patient hospital files that included date of delivery, weight and length of the baby, head circumference, Naegele term, Apgar score, gestational age, as well as noting any congenital malformations and birth complications as per comments of doctor or sister present at delivery.

Sampling procedures

For each mother, 30 ml of blood was drawn by venous puncture into 3 Vacutainer tubes before delivery, and umbilical cord blood was collected after delivery by a nursing sister, using the sterile Vacutainer disposable system. Metal-free vessels were used throughout and great care was taken to prevent contamination of samples during collection and fractionation. All samples were stored at −20 °C and shipped in a frozen state to the University of Tromsø, Norway, from where samples were transferred in a frozen state to the analytical laboratories. Measurements of metal content in whole blood were performed by the National Institute for Occupational Health (NIOH), Oslo, Norway.

Analytical methods

Samples of maternal and cord whole blood were analysed for levels of Cd, Hg, Pb, Mn, Co, Cu, Zn, As and Se. Mn, Cu, Zn and Se are considered to be essential metals, but are known to be toxic at elevated levels. Chemical analyses were performed using the inductively coupled plasma-mass spectrometry (ICP-MS) technique. The required contamination elimination procedures and validation of results by using certified standards were applied throughout the analyses.

Sample preparation

For the measurements of elements in whole blood, 1.5 mL of 65% ultrapure nitric acid (Chemscan Ltd., Elverum, Norway) was added to 1 mL of whole blood in a polypropylene digestion tube. The mixture was digested by heating the tube at 95 °C for 1 hour. The acid homogenization procedure using nitric acid was performed in covered tubes at ambient pressure, and is a well accepted and verified procedure for whole blood, with no losses of *e.g.* Se or Hg. These procedures have been carefully studied and are used extensively at NIOH, as well as by many other international laboratories.

The digest was cooled to room temperature and 200 μL of an internal standard solution containing ^{72}Ge for ^{75}As and ^{77,78,82}Se, ^{115}In for ^{114}Cd, ^{204}Tl for ^{206,207,208}Pb and ^{200,201,202}Hg, ^{60}Ni for ^{55}Mn, ^{59}Co, ^{63,65}Cu and ^{64,66,68}Zn was added and diluted to a final volume of 10 mL with ultrapure water.

Instrumental measurements

The digested blood was analysed by using an Element 2 mass spectrometer (Thermo Electron, Bremen, Germany) calibrated with the whole blood matched standard solutions. The instrument was programmed to determine Cd by use of the $^{114}Cd^+$ ion with automatic mass correction caused by the $^{114}Sn^+$ ionic interference. Since the molybdenum (Mo) concentration in whole blood is around 1ng/mL or lower, any mass interference at $^{114}Cd^+$ from the $^{98}Mo^{16}O^+$ was not considered to contribute to the overall signal. The following mass resolutions were used; low for Cd, Hg, Pb, medium for Mn, Cu, Zn and high for As and Se. The detection limits (three times standard deviation of all blank samples) for metals in whole blood were as follows: As: 0.09 μg/L, Cd: 0.01 μg/L, Co: 0.07 μg/L, Cu: 1 μg/L, Hg: 0,1 μg/L, Mn: 0.3 μg/L, Pb: 0.1 μg/L, Se: 1 μg/L, Zn: 20 μg/L. One aliquot of each blood sample was analysed in triplicate. Seronorm ™ Trace Elements (Sero Ltd., Billingstad, Norway) human whole blood quality control materials were used for quality assurance of all element measurements; after every ten blood samples analysed, a quality control sample at two different concentration levels was also analysed.

The NIOH laboratory participates in the Wadsworth Center-New York State Department of Health Proficiency (USA) trace element testing schemes for whole blood and urine, with consistently acceptable results and no indication of any systematic biases.

Statistical analysis

Statistical analyses were conducted using the statistical STATA package, version 10 (Stata10 2007).[26]

Descriptive statistics were calculated for metals, including median, first and third quartiles. Comparisons between different sites were made using Kruskal Wallis test and Dunn test for multiple comparisons. A p value of less than 0.05 indicated a significant difference.

Results

The pilot study took place in seven selected sites during 2005–2006, and the analytical work was completed by mid 2007. In the tables that follow, study sites are referred to according to their characteristics and presented in a particular order: rural, urban, industrial, Atlantic Ocean, mining, Indian Ocean malaria and inland malaria. In total, 96 women participated in the pilot study, 12 women each at five sites, with 20 and 16 women at rural and urban sites respectively.

Socioeconomic and housing characteristics

Socioeconomic and housing characteristics for participants at each site are summarized in Table 1 and Table 2. Questionnaire data confirmed a similar socioeconomic status of participants at

Table 1 Socioeconomic characteristics of participants by site

Statistics	Rural N = 20	Urban N = 16	Industrial N = 12	Atlantic Ocean N = 12	Mining N = 12	Indian Ocean malaria N = 12	Inland malaria N = 12
Population group % B = African Black, C = Coloured, W = White	B 100	B 94, C 6	B 92, W 8	B 27, C 73	B 100	B 100	B 100
Marital status (%) M = married, S = single *L/T* = living together, D = divorced	M 25, S 75	M 29, S 17, *L/T* 54	M 9, S 75, *L/T* 16	M 20, S 40, *L/T* 30, D 10	M 20, S 40, *L/T* 40	S 100	S 100
Home language (%) E = English, S = Sotho, Z = Zulu, X = Xosa, A = Afrikaans, T = Tswane, O = Other	S 50, T 50	E 27, X 7, S 7	E 11, Z 67, O = 23	E 18, A 55, X27	S 90, Z 10	Z 100	Z 100
Educational status (mean years) W = women, P = partner/husband	W 9.6, P 7.5	W 11.4, P 11	W 10.5, P 11.7	W 9.6, P 10.8	W 8.5, P 10	W 8.3, P 11.3	W 9.8, P 9.6
Mean monthly income Rand (1US$ = 7.2 R)	943 (445)	4166 (4440)	No data	3286 (2276)	2250(1838)	No data	968 (547)
Number of persons (%) employed per household	1 (55%), 2 (27%), 3 (9%)	1 (54%), 2 (38%), 3 (7%)	1 (78%), 2 (22%), 3 (9%)	1 (28%), 2 (57%), 4 (14%)	1 (85%), 3 (14%)	1 (33%), 2 (67%)	1 (100%)

Table 2 Housing characteristics of participants by site

Statistics	Rural N = 20	Urban N = 16	Industrial N = 12	Atlantic Ocean N = 12	Mining N = 12	Indian Ocean malaria N = 12	Inland malaria N = 12
Ownership %	95	19	92	80	60	100	92
Rooms	3.5	2.3	3.5	1.8	1.9	4.5	2.8
Occupancy mean (SD[a])							
Males > 15 y	1.6 (1.3)	1.4 (0.87)	1.7 (1.2)	1.4 (1)	1.4 (0.7)	2.4(1)	2.1(1.5)
Females > 15 y	1.5 (1)	1.3 (1)	2.8 (1)	2 (0.9)	1.7 (0.7)	3.4(0.81)	2.6(1.4)
Children<15 y	4 (2)	1.6 (1)	2.4 (0.8)	2.5 (1.8)	2.4 (0.8)	2.5(1.8)	4.6(1.8)
Electrified %	60	100	83	91	40	9	0
Water source %							
Tap indoor	10	88	75	55	20	0	0
Tap outdoor	70	12	25	45	80	45	42
Borehole	20					27	17
River						27	42
Somebody smoking in household %	55	18	50	73	30	22	33

[a] SD = standard deviation.

all sites. Most of the women were unemployed and relied mainly on social grants or financial support of their partner and other family members. Although cigarette smoking among residents in participants' households was reported (highest 73% in fishing Atlantic Ocean and 55% in rural sites), 56% of actual study participants from the fishing Atlantic site admitted to smoking cigarettes before and during pregnancy. Similarly, alcohol intake was reported only at the Atlantic site by 14% of women, but no participants reported the use of drugs.

The participants were also asked how they rated air quality in the vicinity of their residence and the majority (92%) at the industrial site and 50% at the mining site reported air to be highly polluted (Table 3). The most satisfied with air quality were participants from the Atlantic fishing site. Overall, participants reported to be healthy. Their dietary intake as evaluated from dietary questionnaire appeared to be sufficient and adequate, both before and during pregnancy. Attendance at antenatal clinics by participants before delivery varied from never (mining and coastal malaria) to an average of five times reported in the industrial site.

Maternal age, weight, height and parity by site

Table 4 reports on maternal age, weight, height and parity. The youngest delivering woman was 14 years old; the oldest was 41 years old. The number of children the women already had ranged from none to 6.

Short overview of birth outcomes by site

Details of birth outcomes by site are presented in Table 5. Overall birth weight of babies ranged from 1900 to 3900g, and length from 40 to 56 cm, and gestational age varied between 26 and 41 weeks. 8.3% of the total number of babies born in the study

Table 3 Self reported information on air quality, health, diet and clinic visits by site

Statistics	Rural N = 20	Urban N = 16	Industrial N = 12	Atlantic Ocean N = 12	Mining N = 12	Indian Ocean malaria N = 12	Inland malaria N = 12
Air quality							
G = good, B = bad, D/K = do not know (%)	G 35, B 35, D/K 30	G 70, B 18, D/K 12	G 0, B 92, D/K 8	G 82, B 9, D/K 10	G 40, B 50, D/K 10	G 55, B 36, D/K 9	G 42, B 42, D/K 18
Health status good (%)	75	47	83	100	60	100	83
Diet before/ during pregnancy as evaluated by researchers	Sufficient	Sufficient	Sufficient	Sufficient	Sufficient	Sufficient	Sufficient
Fresh fish intake during pregnancy Weekly %	0	69	33	33	57	18	71
Clinic visits prior to delivery	4	4	5	2	—	—	2

Table 4 Maternal age, weight, height and parity by site

Statistics	Rural N = 20	Urban N = 16	Industrial N = 12	Atlantic Ocean N = 12	Mining N = 12	Indian Ocean malaria N = 12	Inland malaria N = 12
Maternal mean age years (SD)	26 (6)	28 (4)	23 (6)	25 (5)	25 (8)	25(7)	22 (6)
range	16–38	21–36	16–34	20–23	14–38	17–41	17–35
Maternal mean weight (SD) Kg	67 (13)	83 (14)	79 (12)	67 (12)	75 (16)	70 (6)	69 (6)
Maternal mean height (SD) m	1.56 (0.03)	1.59 (0.06)	no data	1.68 (0.09)	no data	1.61 (0.08)	1.53 (0.07)
Parity mean (SD)	2.5 (1.6)	1 (0.5)	1.5 (0.7)	2.4 (1.8)	2 (1)	2 (2)	1 (1)

Table 5 Birth outcomes by site

Statistics	Rural N = 20	Urban N = 16	Industrial N = 12	Atlantic Ocean N = 12	Mining N = 12	Indian Ocean malaria N = 12	Inland malaria N = 12
Birth weight (g) mean (SD)	3126(379)	3170 (351)	2900 (608)	3012 (588)	3184 (444)	3238 (468)	3116 (473)
range	2260–3800	2660–3800	1900–3800	1900–3900	2090–3600	2500–4150	2200–3900
Birth length (cm) mean (SD)	49 (3)	49 (5)	49 (3)	50 (4)	51(2)	50 (3)	49 (4)
Head circumference (cm) mean (SD)	34 (1)	35 (1)	33 (3)	34 (2)	35 (2)	35 (1)	34 (2)
Gestation age (weeks) mean (SD)	40 (1)	39(2)	37 (5)	40 (1)	40 (1)	40 (1)	39 (2)
Gender: girls %	44.4	54.5	44.4	27.2	40	66.8	41.6

sample had low birth weight (LBW), below 2500g, that was directly associated with gestation age and preterm delivery due to a medical emergency. The difference in mean gestational age between the lowest (37.2 weeks at the industrial site) and the highest (39.7 at the coastal malaria Indian site) was found to be statistically significant ($p < 0.04$). Gender ratio differed by site with the lowest number of girls born at the fishing Atlantic site and highest at the coastal malaria Indian site, but did not reach statistical significance. Due to the limited number of participants in the pilot study, the statistical interpretation of gender ratio must be done with caution.

Maternal blood levels of Cd, Hg, Pb, Mn, Co, Cu, Zn, As and Se

Concentrations of the different metals in maternal blood by site are summarised in Table 6. Concentrations of Cd in maternal blood were found to be highest in the Atlantic site where the median concentration was 0.25 μg/L, range between 0.05 and 0.89 μg/L. These concentrations were also significantly higher compared to all other locations, reaching statistical significance of $p < 0.032$ overall. Notably, concentrations of Hg were also highest in mothers from the Atlantic site with a median of 1.78 μg/L, range 0.44–8.82 μg/L and overall highly significant ($p < 0.001$), except when comparing the two coastal populations at the fishing Atlantic and the coastal malaria Indian sites ($p > 0.11$). The Hg levels in the coastal malaria Indian Ocean site were found to be higher than all other sites, except the Atlantic site but were not statistically significant ($p > 0.05$). Median Pb levels were found to be highest in the urban site (32.9 μg/L; range 16.3–81.5 μg/L), significantly higher when compared to any of the other study sites ($p < 0.003$). The Mn levels were found to be similar in all study areas, with no significant differences. Concentrations of Co were found to have similar median levels, with some outliers at relatively high concentrations in the mining area. The Cu levels in whole blood were found to be higher in the inland malaria site compared to all other areas, $p < 0.032$. Levels of Zn were very similar in all sites, except for some real outliers on both high and low concentrations in the Atlantic site. The highest median concentration of As was found to be 0.74 μg/L with range 0.17–2.46 μg/L in inland malaria site, significantly different when compared to other sites ($p < 0.005$). The Se concentrations demonstrate the same pattern as the Hg levels, with highest concentrations in the Atlantic and the coastal malaria Indian Ocean sites, with significant differences between the Atlantic and all the other sites ($p < 0.001$). In addition, the coastal malaria Indian Ocean site shows a significant difference when compared to industrial and mining sites ($p < 0.001$).

Umbilical cord blood levels of Cd, Hg, Pb, Mn, Co, Cu, Zn, As and Se

Umbilical cord blood results for all sites except rural are summarised in Table 7. All toxic metals measured were detected in umbilical cord blood. Median cord blood levels of Cd were found to be low in all sites, and highest in the urban site (0.04 μg/L), with statistical significance between sites ($p < 0.001$). The median levels of Hg were found to be lowest in urban and industrial sites (0.5 μg/L) and highest in the Atlantic site (4.6 μg/L); this result was highly significant ($p < 0.001$). The lowest median levels of Pb were measured in the inland malaria site (9 μg/L), while the highest were recorded for the urban site (23.9 μg/L); with statistical differences between the sites ($p < 0.004$). Levels of Mn in cord blood ranged from lowest (19.7 μg/L) in the urban and highest (36.6 μg/L) in the mining site; lowest levels of Co were measured in the fishing Atlantic site (0.17 μg/L) and the highest in the industrial site (0.38 μg/L), with statistical significance between sites ($p < 0.013$). No statistical differences were observed between sites for Zn and Cu in cord bloods. The lowest levels of As were measured in the urban and industrial site and the highest in the inland malaria site (0.79 μg/L), followed by the Atlantic site with no significant differences between sites. The median concentration of Se was highest in the fishing Atlantic site measuring 159 μg/L and overall highly significant ($p < 0.001$).

Discussion

Women's exposure to metals during their lifetime and during pregnancy, combined with metabolic alterations in status of essential metals such as zinc, copper, iron and selenium during this period, may mobilize metals from body stores and affect the function of placenta and foetal development.

The present pilot study found significant statistical differences in maternal blood levels between different geographical sites for cadmium, mercury, lead, copper, arsenic and selenium. When comparing umbilical cord blood metal levels between the sites, significant differences were found for cadmium, mercury, lead, cobalt and selenium. Essential metal levels measured (copper, zinc and selenium) were within normal concentrations, probably due to adequate diet and to the intake of vitamin and iron supplements, which are routinely prescribed to all pregnant women attending antenatal clinics in South Africa. It is important to mention that in South Africa staple foods are fortified with micronutrients, including folic acid, which is crucial for healthy foetal development.

As for cadmium, the median levels of this toxic metal in maternal blood and cord bloods were highest in smokers.

Table 6 Concentration of Cd, Hg, Pb, Mn, Co, Cu, Zn, As and Se in maternal blood (μg/L) by site[a]

Metals μg/L	Statistics	Rural N = 20	Urban N = 16	Industrial N = 12	Atlantic Ocean N = 12	Mining N = 12	Indian Ocean malaria N = 12	Inland malaria N = 12
Cd [b]	Median	0.10	0.15	0.10	0.25	0.13	0.16	0.12
	Range	0.05–0.25	0.06–0.48	0.05–0.50	0.05–0.89	0.06–0.39	0.06–0.46	0.04–0.26
	IQR	0.07–0.16	0.10–0.22	0.06–0.17	0.20–0.52	0.10–0.16	0.10–0.20	0.10–0.22
Hg [c]	Median	0.40	0.30	0.30	1.78	0.61	0.93	0.89
	Range	0.18–0.82	0.19–3	0.13–0.99	0.44–8.82	0.28–1.25	0.25–3.18	0.22–1.25
	IQR	0.30–0.57	0.30–0.70	0.22–0.50	0.64–2.91	0.39–0.91	0.61–1.33	0.50–1.13
Pb [b]	Median	20.9	32.9	20.7	23.7	26.4	21.9	11.5
	Range	7.4–50.3	16.3–81.5	11–32.3	10.6–38.9	6.1–161.5	8.8–29.4	6.3–49.4
	IRQ	15.2–30.4	22.5–37.8	15.7–23.6	18.1–34.2	12.5–35.2	14.7–27	9.8–19.6
Mn	Median	16.4	17.7	12.7	16	17	17.9	16.8
	Range	8.3–25.2	8.8–30.6	7.9–63.5	9.4–26.6	9.2– 36.1	8.8–24.9	12.7–22.3
	IQR	12–22	13–22.5	9.5–22.7	10.4–20.8	14.5–22.3	13.6–23.1	14.4–20.6
Co	Median	0.62	0.46	0.64	0.63	0.54	0.78	0.65
	Range	0.15–1.75	0.22–2.68	0.14–1.67	0.45–0.9	0.21–15.32	0.24–2.41	0.3–6.10
	IQR	0.34–1.05	0.26–0.94	0.28–1.36	0.54–0.85	0.36–2.41	0.27–1.16	0.4–0.96
Cu[b]	Median (SD)	1639	1600	1787	1693	1825	1784	1913
	Range	1349–1938	1329–2035	1200–2040	1279–2271	1506–2173	1500–2336	1329–2418
	IQR	1568–1687	1520–1788	1480–1964	1455–1889	1561–1992	1641–2333	1640–1913
Zn	Median	5934	5849	6098	6296	6989	6286	6465
	Range	4201–7628	3745–8075	4708–9103	2995–11349	5078–9788	4282–8719	4275–8401
	IQR	5385–6596	5100–6750	5698–6934	5415–6858	5695–8521	4503–7627	5434–7561
As[b]	Median	0.37	0.43	0.33	0.66	0.73	0.59	0.74
	Range	0.08–0.67	0.08–1.65	0.17– 0.96	0.29–2.84	0.35–3.12	0.31–1.73	0.17– 2.46
	IQR	0.25–0.56	0.16–0.88	0.22–0.53	0.36–1.51	0.39–1.37	0.37–0.95	0.34–0.94
Se [c]	Median	101	100	85	131	89	122	105
	Range	84–125	82–153	63–101	117–203	69–108	84–192	85–163
	IQR	94–108	92–111	79–95	118–151	76–101	101–151	89–144

[a] IQR = inter-quartile range (25–75). [b] Statistically significant differences ($p < 0.05$). [c] $p < 0.001$.

Table 7 Concentration of Cd, Hg, Pb, Mn, Co, Cu, Zn, As, Se in umbilical cord blood (μg/L) by site[a]

Metals μg/L	Statistics	Urban N = 16	Industrial N = 8	Atlantic Ocean N = 12	Mining N = 12	Indian Ocean malaria N = 12	Inland malaria N = 7
Cd [c]	Median	0.04	0.01	0.03	0.01	0.01	0.01
	Range	0.01–0.1	0–0.01	0.01–0.32	0–0.13	0.01–0.02	0–0.1
	IQR	0.02–0.1	0.01–0.01	0.02–0.05	0.01–0.02	0.01–0.02	0.01–0.02
Hg [c]	Median	0.5	0.5	4.6	0.7	1.7	1.5
	Range	0.1–5.4	0.2–1.1	1–9.7	0.5–2.3	0.5–6.4	0.3–2.4
	IQR	0.3–2.0	0.3–0.8	1.4–6.3	0.6–1.3	1.1–3.3	1.0–2.2
Pb[b]	Median	24	15	15	21	14	9
	Range	15–87	10–21	7.7–37	1.4–95	5.6–28	5–14
	IQR	18–28	11–17	11–22	8–34	10–19	5–12
Mn	Median	19.7	34.5	33	36.6	35.9	34.6
	Range	7.2–69	29.7–53.6	16.8–80.7	11–59	20–59	19–61
	IQR	15.8–53.2	30.4–39.9	27.2–45.2	27–45.2	27.7–47	31.1–44.4
Co[b]	Median	0.34	0.38	0.17	0.33	0.23	0.33
	Range	0.07–0.85	0.16–0.97	0.41–0.31	0.11–9.4	0.07–0.66	0.17–1.47
	IQR	0.17–0.42	0.27–0.85	0.08–0.19	0.19–1.39	0.16–0.47	0.21–0.4
Cu	Median	641	618	668	637	668	724
	Range	384–1165	532–698	543–931	511–1413	578–870	588–842
	IQR	487–699	551–682	611–702	526–727	641–710	653–842
Zn	Median	2338	2237	2708	2285	2717	2499
	Range	1558–4738	1684–3637	2357–4018	1926–2883	1969–4685	2190–3493
	IQR	1797–4441	1909–2752	2545–3615	2070–2511	2158–3131	2293–2715
As	Median	0.41	0.37	0.57	0.49	0.48	0.79
	Range	0.09–1.26	0.2–0.89	0.13–2.44	0.16–2.84	0.13–1.21	0.09–1.95
	IQR	0.26–0.90	0.22–0.49	0.35–1.3	0.3–1.0	0.34–0.73	0.16–0.82
Se[c]	Median	99	89	159	98	141	131
	Range	79–182.	65–96	128–202	50–113	83–188	103–170
	IQR	83–106	83–93	134–187	83–104	116–158	111–166

[a] Note—no cord blood results for rural site available IQR = inter-quartile range (25–75). [b] Statistically significant differences ($p < 0.05$). [c] $p < 0.001$.

However, lower cadmium levels measured in the respective cord bloods may indicate a limited transfer from mother to foetus. This may be of clinical significance, as cadmium has been shown to cause fetotoxic and embrogenic effects in animal studies and has been found to accumulate in the placenta of smoking mothers.[27,28] Animal experiments have also shown that the protective capacity of the placenta against exposure to Cd is at its strongest during the last trimester of pregnancy, combined with the alteration in the deposition and transportation of zinc and copper.[29,30] During pregnancy these alterations are thought to be mediated by metallothioneins (MT) that bind cadmium to decrease its toxicity.[31] When compared with other studies in the Arctic region, levels of cadmium in South African non-smoking delivering women were found to be very similar.[32,33]

Mercury is a toxic metal that has been identified as a priority contaminant in the circumpolar north (AMAP 2003) and was detected in more than 97% of maternal blood and cord blood samples in the recent study.[33] Concentrations of mercury in umbilical cord blood in our study were found to be almost double when compared to maternal levels at delivery at all sites. The mercury level ratio of cord blood to maternal blood was 2.58 and 1.82 in participants from Atlantic and Indian Ocean coastal populations, respectively. This is of concern as recent studies confirm loss of IQ in children prenatally exposed to mercury, even at very low concentrations.[34] A number of studies have found a direct correlation between blood mercury levels and frequent consumption of fish and other mercury contaminated traditional foods. This is the case for general populations and not only pregnant women. In our study sample, it was found that fish consumption was very low, even in participants residing in coastal areas, where levels of mercury were found to be highest when compared with other sites. In South Africa, other environmental sources such as informal gold mining in the region and the effect of global climate change may be main contributors of increased blood mercury levels. Globally it is estimated that mercury levels will continue to increase for at least the next decade.[35]

The neurotoxic effects of lead, particularly to foetus and children, are well documented.[36] Lead has an ability to freely cross the placental barrier but generally its concentrations in cord blood are slightly lower than in maternal blood. In the present study the highest median levels in both compartments were found in participants from the urban area of Johannesburg. Recent research suggests that there is no safe level of lead and the action level in children should be reduced from the current 100 μg/L to 20 μg/L.[37] Overall levels of lead in maternal and cord blood at delivery were found to be similar to those other industrialised countries in the north, but relatively lower than levels reported in rural, industrial and urban regions of China.[33,38]

Although no data on levels of lead in pregnant South African women exist, lead levels in South African school children have been extensively studied over time.[19,20] A study performed in 2002 found that about 35% of 7 year old schoolchildren in urban areas and approximately 6% in rural areas were found to have blood lead levels equal or above action levels of 100 μg/L. Main confounding factors were the proximity to busy roads and the use of lead in paint in old dwelling and schools, and informal industrial activities and pica. It should be noted that lead was removed from South African petrol only in the beginning of 2007 and the ban on the addition of lead to paint is still to be legislated. Other

developing countries also reported elevated blood levels of lead in young children: rural children in Philippines; children residing in industrial areas of Mumbai and Delhi in India; Karachi in Pakistan and Jakarta.[39–42] At this stage, the possibility of further exposure to lead after birth, especially in socioeconomically disadvantaged populations, not only in South Africa but also in other developing countries, cannot be ruled out.[43]

In contrast to cadmium, mercury and lead discussed above, manganese is an essential trace metal and both deficiency and excess of manganese are toxic to humans. Like lead and mercury, manganese is a neurotoxic metal able to easily cross the blood-brain barrier. In the present study, we found both median levels of manganese in blood samples from delivering women and in cord blood to be above what is considered to be a normal levels (above 14 μg/L) for all sites.[44] It is widely believed that blood manganese levels in pregnant women and neonates are higher than the normally accepted levels for populations due to a compromised processing mechanism, which requires further investigation.[45] A South African study of schoolchildren showed that manganese blood levels were elevated in 14% of children living in Johannesburg (where manganese containing the methylcyclopentadienyl manganese tricarbonyl compound (MMT) was introduced to petrol to replace lead, 24 months before the survey) and low in other sites.[21,22]

Concentrations of arsenic were found to be similar at all sites but highest in the inland malaria site. Unconfirmed sources of arsenic may be from the local water as there is no known mining activity at this particular site. Concentrations of cobalt and selenium were found to be within normal limits at all sites.

In summary, this pilot study is the first study performed in South Africa that concurrently measured a broad spectrum of metals in delivering mothers and their newborns in different geographical areas. This study confirmed the ability of toxic metals to permeate the placental barrier (manuscript in preparation). An obvious limitation is the small number of subjects studied in this pilot phase. The results emphasize the need for a main study aimed at indentifying risk factors associated with prenatal exposure to metals and other persistent pollutants in key South African settings, thus contributing to national and international databases and scientific knowledge.

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Capítulo II

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The placenta as a barrier for toxic and essential elements in paired maternal and cord blood samples of South African delivering women

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Environmental toxicants such as metals may be detrimental to foetus and infant development and health because of their physiological immaturity, opportunistic and differential exposures, and a longer lifetime over which disease, initiated during pregnancy and in early life, can develop. The placental mechanisms responsible for regulation of absorption and excretion of elements during pregnancy are not fully understood. The aim of this paper is to assess the correlation for selected toxic and essential elements in paired whole blood samples of delivering women and cord blood, as well as to evaluate the placental permeability for selected elements. Regression analyses used to assess this correlation in 62-paired samples of maternal and cord whole blood of delivering women show that the concentrations of mercury, lead, cobalt, arsenic and selenium in maternal and cord blood differed statistically. Lead, cobalt, arsenic and selenium appear to pass the placental barrier by a diffusion mechanism. It was also found that the mercury levels in cord blood were almost double those of the mother, suggesting that the foetus may act as a filter for the maternal mercury levels during pregnancy. Transplacental transfer for arsenic and cobalt was 80% and 45%, respectively, suggesting that the placenta modulates the rate of transfer for these elements. Cadmium, manganese, copper and zinc levels did not show statistically significant correlations between two compartments (maternal *versus* cord whole blood). The study confirms that most of the toxic metals measured have an ability to cross the placental barrier.

Introduction

The developing foetus is particularly vulnerable to the toxic effects of metals. The placenta is an organ of transfer between mother and foetus through which all necessary nutrients are delivered. It also functions as a detoxifying barrier to prevent the passage of toxic substances to the foetus, including elements.[1] If the latter is accomplished by binding of the element ions to the placenta, it may interfere with placental function, in particular with the transport of essential trace elements required for foetal growth and development.[2,3]

The transfer of all substances from foetus to mother and from mother to foetus depends primarily on the processes that permit or facilitate the transport of these substances, through the syncytiotrophoblast of the intact chorionic placenta. Transfer of substances from mother to foetus is accomplished first by transfer from the intervillous space into the syncytiotrophoblast. This process of transfer supplies the foetus with oxygen as well as nutrients and provides for elimination of metabolic waste products. Thus the chorionic villi and the intervillous space, together, function as lung, gastrointestinal tract and kidney for the foetus. Although this histological "barrier" separates the blood in the maternal and foetal circulations, it does not behave in an uniform manner as if it were a physical barrier. Throughout pregnancy, the syncytiotrophoblast actively or passively permits, facilitates, and adjusts the amount and rate of transfer of a wide range of substances to the foetus.[4]

To understand these mechanisms and determine the effectiveness of the human placenta as an organ of transfer, a number of factors must be considered, including substance concentration in the maternal plasma, the type of carrier protein and the area available for exchange. Furthermore, the physical properties of the tissue barrier and mode of transfer might influence the amount of substance metabolized by the placenta during the transfer. The area for exchange across the foetal capillaries in the placenta will determine the concentration of these substances in foetal blood. The rate of transfer is also guided by the presence of specific binding or carrier proteins and other ligands in maternal and/or the foetal circulations. Finally, the rate of the foetal blood flow through the villous capillaries will actively affect substance transfer.[4]

Even though the mechanisms of metal toxicity are mostly not known, it has been proposed that toxic element bioavailability, and hence toxicity, depends on the physiological form of the element before it enters the body to be transported within biological fluids and tissues. For example, studies with aluminium provide a very good example of how a detrimental metal ion can make use of the endogenous ligands, in its absorption, transport and availability to finally exert its toxic effects on target organs.

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Successful competition for the binding sites on different ligands, normally available to carry metal ions, which have similar properties, will be the target for such an interaction.[5] Another example of this interaction is the metal binding protein metalloprotein-1(MT) which is expressed in the human syncytiotrophoblast and able to bind or sequester a host of metals including zinc, copper, lead and cadmium.[4] The interaction of toxic elements with essential trace elements after environmental or occupational exposure plays an integral role in its toxicity. The toxicity of these elements may be increased or lessened by the metabolic status of the essential trace elements, which can be perturbed either through inborn genetic defects, or upon exposure to various environmental influences.[6,7]

It has been shown that 'chronic low-level exposure to lead during early development in children can result in behavioral alterations in the absence of overt toxicity'.[8] This perturbation of the function of the central nervous system may be due to deficiencies in essential elements, resulting from lead-induced impairment of mineral availability and/or an increased sensitivity to lead in the absence of adequate levels of essential trace metals such as zinc. Zinc is also thought to reduce cadmium-induced ultra-structural alteration of the liver.[9,10] Interactions between toxic and essential elements have been reported; it is well documented for lead and iron in anaemia due to iron deficiency. Similar interactions have been reported for mercury and iron, arsenic and selenium, iodine and selenium, cadmium and zinc, aluminium with copper and zinc.[11–14] Furthermore, zinc, copper and selenium are essential for reproductive processes.[15]

Therefore, the ability of the placenta to transfer nutrients and toxicants alike is of concern in relation to foetal development and health.[16] In most cases, the same processes that aid transport of nutrients through the placenta, may also act as the pathways for toxic elements, especially if these have chemical similarities with the nutrient metabolites, or simply because of passive diffusion. It is also understood that in some cases, the placenta acts as a barrier by preferentially concentrating and retaining specific toxicants, thus acting as a detoxicant and thereby reducing, to some degree, the toxic effect on the foetus.[17]

The placental mechanisms responsible for the regulation of elemental concentrations, both in the pregnant woman and the developing foetus, are not fully understood and need further investigation. Scientific evidence shows that foetus and infants are more affected than adults by a variety of environmental toxicants due to their physiological immaturity, opportunistic and differential exposures, and a longer lifetime over which disease, initiated during pregnancy and in early life, can develop.[18] A number of studies, by Odland *et al.*, performed under the umbrella of the Arctic Monitoring and Assessment Programme (AMAP) project in the Arctic region, measured 16 elements (both essential and toxic) in maternal and cord blood and in placental tissues, in different populations residing in the Arctic. These studies quantified the ability of elements to transfer through the placenta and evaluated/predicted their potential to impact on foetal development.[19–21] These investigations showed maternal age and body mass index (BMI) to be positive predictors of birth weight. They identified cigarette smoking and lead exposure as negative determinants in terms of established evidence and recognized confounders, including maternal genetic factors, socio-economic status, socio-political change, life-style issues, prenatal care and nutrition.[22] An important finding of these studies was the evidence that the placental concentrations of toxic elements may serve as an index of exposure and of nutritional intake and status for selected essential micro-elements.[23] The authors concluded that inter-element relationships and groupings of the measured elements may constitute a scientific base for the use of placental composition in environmental monitoring and epidemiological studies.[19]

These and other similar data originate predominantly from investigations carried out in the Northern Hemisphere. In contrast, in the Southern Hemisphere there has been limited research to assess levels of toxicants, to estimate human exposure and risks, and to evaluate spatial and temporal trends. Furthermore, women and children (who are the largest segment of these societies) are particularly affected, and considered to be most vulnerable. The confounding factors mentioned previously differ in the Southern Hemisphere, and the detrimental effects of toxicants may be further potentiated by the climatic, socio-economic and political conditions, rapid urbanization, poor health status and nutrition, high rate of unemployment and high prevalence of disease (TB, HIV/Aids, malaria) among populations in this region.

This paper reports on the correlation between mother and cord blood levels of toxic and essential elements, in paired samples of blood of delivering women in South Africa, as an indicator of placental barrier, and possible exposure during pregnancy.

The findings reported here form part of the pilot study titled "Levels of Persistent Toxic Substances (PTS) in maternal and umbilical cord blood from selected areas of South Africa"

Materials and methods

The results reported in this paper were obtained from sixty-two pairs of randomly selected mothers and newborns recruited from patients presenting for delivery at hospitals in South Africa. Whole blood was drawn by venous puncture from the mother before delivery, using the sterile Vacutainer disposable system; umbilical cord blood was collected post-partum by a nursing sister using a syringe, and stored at −20 °C until analysed. Contamination-free vessels and procedures were used throughout. Samples of maternal and cord whole blood were digested and analyzed for total concentrations of cadmium (Cd), mercury (Hg), lead (Pb), manganese (Mn), cobalt (Co), copper (Cu), zinc (Zn), arsenic (As) and selenium (Se) by inductively coupled plasma–mass spectrometry (ICP-MS). Seronorm™ Trace Elements (Sero LTD., Billingstad, Norway) human whole blood quality control materials were used for quality assurance of all element measurements; after every ten blood samples analyzed, a quality control sample at two different concentration levels was also analyzed. For the purpose of this paper, the researchers extracted delivery records from the patient hospital files; these included date of the delivery, weight and length of the baby, caput, Naegele term, Apgar score, gestational age, as well as congenital malformations, birth complications and outcomes as per comments of doctor or sister present at delivery. Details of data collection and analytical procedures are described elsewhere.[24]

Ethical considerations

Ethical approval of the study protocol was obtained from the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg (Clearance Certificate Protocol Number M040314). Informed written consent was obtained from each participant prior to commencement of the study.

Statistical analysis

Statistical analyses were conducted using the statistical STATA package, version 10 (Stata10 2007).[25] Descriptive statistics were calculated for birth outcome characteristics and element levels. A linear regression model was used to correlate levels of measured elements between paired maternal and cord whole blood samples.

Results

The study found that the mean age of the sixty-two delivering women included in this paper was 24.7 (SD 6.2) years. Only 8.7% of women were nulliparous and the majority (52.3%) was primiparous (range 1 to 6). Eleven percent of women reported to have had spontaneous abortion previously. Only three mothers in the group reported smoking during pregnancy (less than 10 cigarettes per day).

Details of birth outcomes are summarized in Table 1. The mean gestational age was 38.9 (SD 2.3) weeks. From sixty-two deliveries, 54 (87%) were vaginal and 8 (13%) were by Caesarean section. The mean birth weight was found to be 3104 (SD 490) g and the mean length of the babies was 49.5 (SD 3.6) cm with the mean head circumference of 34 (SD 2) cm. The gender split of the newborns was as follows: 29 (47%) were female and 33 (53%) were male. The Apgar score was normal in most cases with only two cases of Apgar less than 7 for one minute. Only one case of congenital malformation (clubfoot) was observed at birth.

The concentrations and descriptive statistics for each metal measured in paired maternal and umbilical cord bloods are shown in Table 2 and Table 3 respectively. In a few cases, the concentration of elements measured was equal or below the detection limit (LOD) of the method, and for statistical purposes, these concentrations were set at 0.5 × LOD.

Table 1 Characteristics of birth outcomes ($n = 62$)

Birth outcomes	Mean (SD)	%
Maternal age/years	24.7 (6.2)	
Gestational age/weeks	38.9 (2.4)	
Delivery methods:		
Vaginal	54	87
Caesarean Section	8	13
Birth weight/g	3104 (490)	
Birth length/cm	49.5 (3.6)	
Head circumference/cm	34 (1.9)	
Baby sex (n)	62	
Female	29	47
Male	33	53
Apgar score <7		
1 min	2	
5 min	—	

Table 2 Concentrations of Cd, Hg, Pb, Mn, Co, Cu, Zn, Se, As in maternal blood/μg L^{-1}

Metals/μg L^{-1}	Median $n = 62$	Range	IQR[a]
Cd	0.15	0.04–0.89	0.10–0.20
Hg	0.65	0.1–8.8	0.34–1.20
Pb	23	6–161	14.6–31.0
Mn	16.8	8.7–63.5	13.8–21.6
Co	0.60	0.21–15.3	0.41–0.98
Cu	1730	1200–2420	1520–1900
Zn	6290	3000–11400	5400–7130
As	0.57	0.08–3.12	0.32–0.91
Se	104	63–203	86–131

[a] IQR = inter-quartile range (25–75).

Table 3 Concentrations of Cd, Hg, Pb, Mn, Co, Cu, Zn, Se, As in cord blood/μg L^{-1}

Metals/μg L^{-1}	Median $n = 62$	Range	IQR[a]
Cd	0.02	0–0.32	0.01–0.21
Hg	1.2	0.1–9.7	0.56–2.16
Pb	15.4	1.4–95.1	10.2–23.4
Mn	34.9	7.2–80.7	27.3–45.3
Co	0.27	0.03–9.4	0.17–0.41
Cu	657	384–1410	590–704
Zn	2548	1560–4738	2160–2920
As	0.46	0.04–2.84	0.32–0.83
Se	111	50–202	93–145

[a] IQR = inter-quartile range (25–75).

Fig. 1–5 indicate the results of regression analyses used to assess the linear correlation between levels of mercury, lead, cobalt, arsenic and selenium in paired maternal blood (right hand side) and cord blood (left hand side).

The median Cd level in maternal (CdM) and cord blood (CdCB) was found to be 0.15 μg L^{-1} and 0.02 μg L^{-1} respectively. No correlation between maternal and cord blood for cadmium concentrations was found (r-sq = 2.4%, $p = 0.35$).

The median Hg concentration for maternal blood (HgM) was found to be 0.65 μg L^{-1} as compared to the median mercury cord blood level (HgCB) of 1.22 μg L^{-1}. This correlation was highly significant (r-sq 76.9%, $p < 0.0001$); see Fig. 1.

The median Pb concentration for maternal blood (PbM) was found to be 23 μg L^{-1} and the median Pb cord blood concentration (PbCB) was 15 μg L^{-1}. This correlation was also highly significant (r-sq = 83.3%, $p < 0.0001$); see Fig. 2.

The median Mn concentration for maternal blood (MnM) was found to be 16.8 μg L^{-1} with the median manganese cord blood concentration (MnCB) of 34.9 μg L^{-1}. However, there was no correlation between maternal and cord blood manganese levels (r-sq = 3.9%, $p = 0.13$).

The median Co concentration for maternal blood (CoM) was found to be 0.60 μg L^{-1}; the median cobalt cord blood concentration (CoCB) was 0.27 μg L^{-1} with a highly significant correlation between maternal and cord blood (r-sq = 95.8%, $p < 0.0001$); see Fig. 3.

The median Cu concentration for maternal blood (CuM) was found to be 1730 μg L^{-1} as compared to the median cord blood

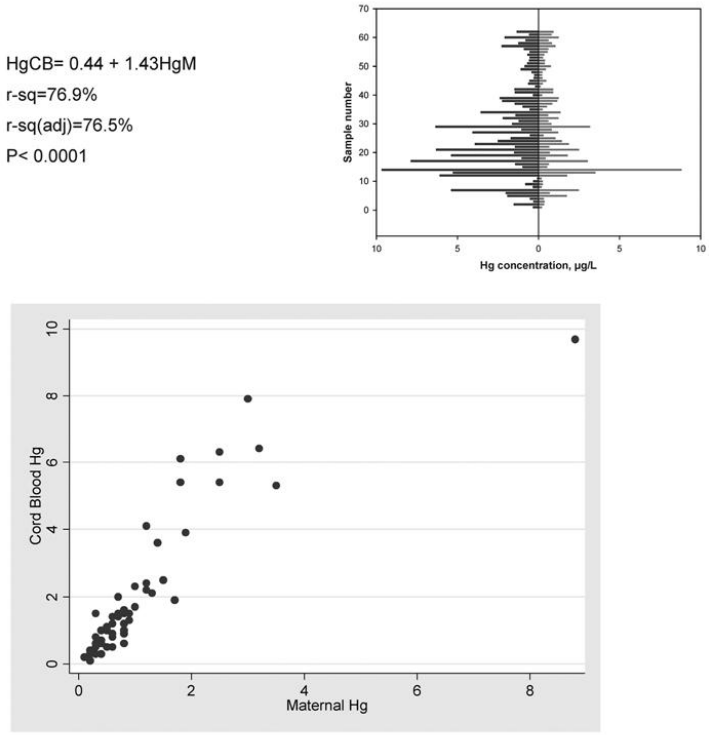


Fig. 1 Mercury concentration: correlation between maternal and cord blood. Left cord blood (HgCB); right maternal blood (HgM).

concentration (CuCB) of 657 μg L^{-1}. There was no correlation between maternal and cord blood for copper levels (r-sq = 0.0%, $p = 0.96$).

The median Zn concentration for maternal blood (ZnM) was found to be 6830 μg L^{-1} and the median cord blood concentration (ZnCB) was 2550 μg L^{-1}. There was no correlation between maternal and cord blood for zinc levels (r-sq = 0.0%, $p = 0.99$).

The median As concentration for maternal blood (AsM) was found to be 0.57 μg L^{-1} and the median As cord blood concentration (AsCB) was 0.46 μg L^{-1}. There was a high correlation between maternal and cord blood arsenic levels (r-sq = 85.7%, $p < 0.0001$); see Fig. 4.

The median Se concentration for maternal (SeM) and cord blood (SeCB) was found to be 104 μg L^{-1} and 111 μg L^{-1}, respectively, with significant correlation between selenium in maternal and cord blood (r-sq = 60.3%, $p < 0.0001$); see Fig. 5.

Linear equations for each element are shown in each of the figures.

When comparing concentration of elements in cord blood in different maternal age groups, highest median mercury cord blood concentration of 1.4 μg L^{-1} was in the 20–25 year age group; and the highest median lead cord blood concentration of 23 μg L^{-1} was found in the group older than 30 years of age; these results were not statistically significant. The study found significant correlation between selenium and mercury in maternal blood, in the 20–25 year age group ($p < 0.001$).

Discussion

This study quantified the levels of toxic and essential elements in sixty-two paired samples of maternal and cord blood, and assessed the statistical significance of the correlation for metals between each individual mother and child pair. This allowed for the assessment of the detoxifying ability of the placenta, and/or prediction of placental barrier for each element.

Regression analyses showed that the correlation between maternal and cord blood for mercury, lead, cobalt, arsenic and selenium was highly significant indicating that the foetal body burden reflects the maternal exposure.[26] However, no correlation was found for cadmium, manganese, copper or zinc.

The study found different levels of total mercury in maternal and cord bloods, with a median of 0.65 μg L^{-1} and 1.2 μg L^{-1}, respectively. These levels indicate the absorbed dose as well as the amount available systemically. This is of concern, as the methyl mercury fraction (usually >98% of total mercury) binds to haemoglobin and, in particular, has a high affinity for foetal haemoglobin; hence cord blood mercury in its methylated form passes easily through the placenta.[27,28] This specific mechanism

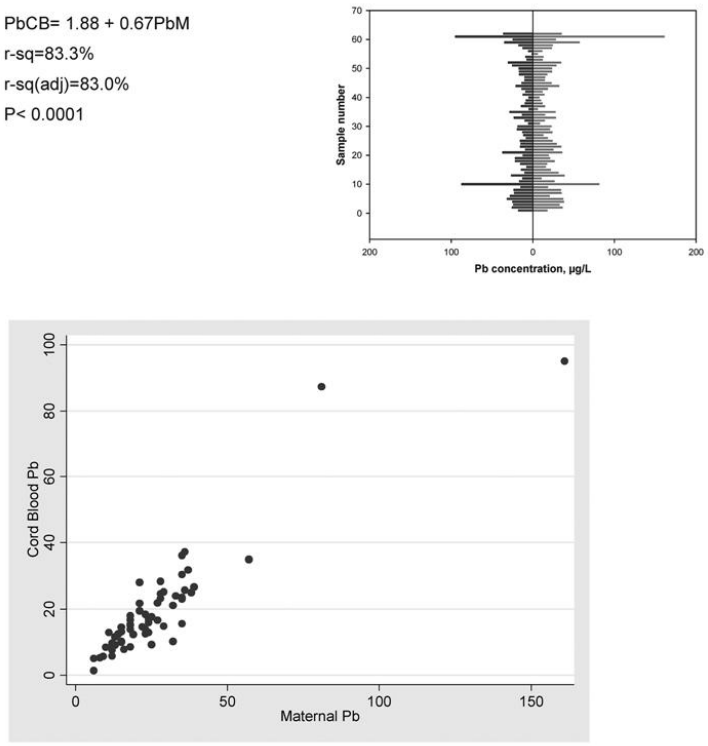


Fig. 2 Lead concentration: correlation between maternal and cord blood. Left cord blood (PbCB); right maternal blood (PbM).

results in a higher concentration of mercury in cord blood, when compared with maternal blood, as shown in this and other studies.[29,30] In our study, 7.5% of cord bloods had mercury concentrations above 5.8 μg L^{-1}, a level associated with loss of IQ.[31] Follow-up studies of a cohort of children, by Faroe Island researchers, found significant dose-related, adverse associations between pre-natal exposure to mercury and intellectual performance indicators such as memory, attenuation, language, and visual–spatial perception.[32] Furthermore, a recent study by Jedrychowski *et al.* suggests the cut-off value for mercury for newborns to be 0.90 μg L^{-1}.[33] In the present study, 25% of cord bloods exceeded this suggested concentration. The ratio between cord and maternal blood was calculated to be 1.85, similar to the value of 1.7 reported by Vahter *et al.*[34] These findings are in agreement with other studies and confirm that the placenta does not act as a protective barrier against the transfer of mercury, from mother to foetus.[35,26]

Speciation of mercury in maternal and cord blood and placentas by Ask *et al.* show that methyl mercury species concentrations are higher in the umbilical cord blood than in maternal blood, and the methyl mercury concentrations in placentas are twice of those in maternal blood, indicating retention of methyl mercury in placenta tissue.[36] The majority of studies associates elevated concentrations of mercury with consumption of contaminated fish and marine products.[37] In our study, dietary intake of fish was found to be low. When comparing our findings with others in the Southern Hemisphere, mercury levels in maternal blood were comparable to those reported in women with low fish consumption from the Amazon, Brazil.[38] This higher foetal cord blood concentration, compared to maternal blood, provides an example of transport and sequestration of this metal. It may indicate that mercury is transported actively from maternal to foetal plasma by a facilitated diffusion mechanism.[4]

Our study found a significant correlation for lead between maternal and the corresponding cord bloods, although low in both, confirming that lead easily crosses the placental barrier. Nevertheless, these findings should be interpreted with caution, as recent research suggests that there is no safe level of lead in foetus and young children, and even low concentrations can have negative neurotoxic effects.[39–42] Other studies have also shown a significant correlation between maternal and cord blood lead at higher concentrations.[43–46] In our study, only one case of elevated lead level (above the action level of 100 μg L^{-1}) was found in a maternal and cord blood pair −161 μg L^{-1} and 95 μg L^{-1}, respectively. The lead concentrations found in our study were lower that those reported in other developing countries.

Another toxic element that showed significant correlation between maternal and cord blood was arsenic. In the South

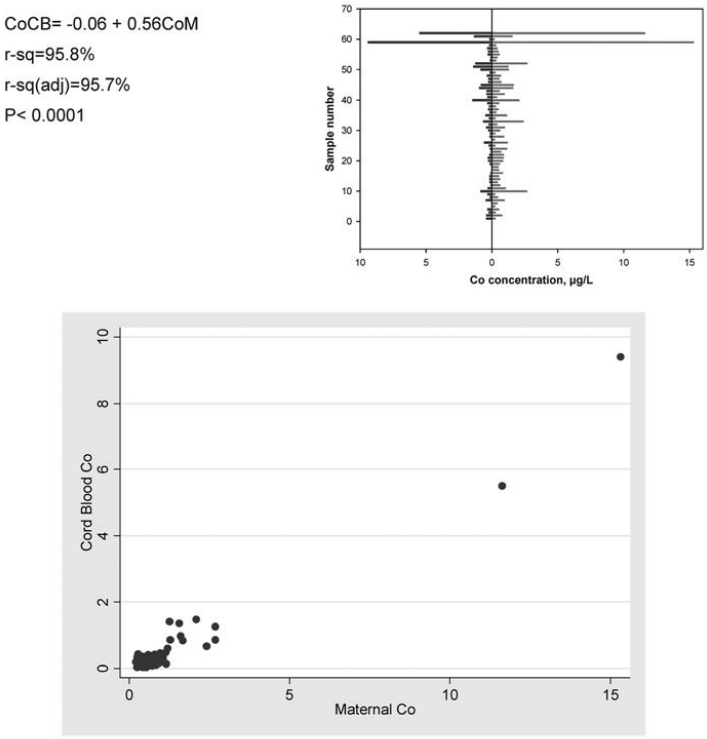


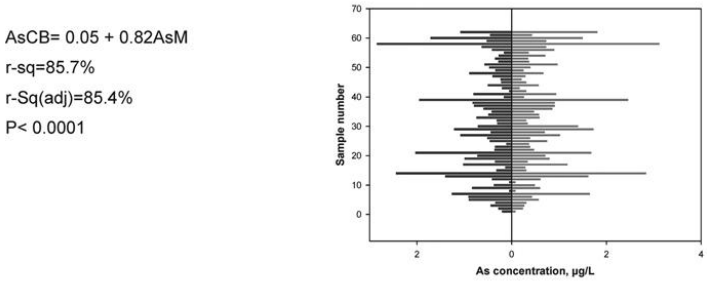
Fig. 3 Cobalt concentration: correlation between maternal and cord blood. Left cord blood (CoCB); right maternal blood (CoM).

African context, the sources of arsenic are likely to be mining activities, agriculture and food. More research is needed to confirm this. Studies elsewhere, suggest that most of the total arsenic measured in food is largely in its organic form (especially in fish products) and high levels of inorganic arsenic are not commonly found. The differences in cadmium concentration between maternal and cord blood were not statistically significant and much lower than in most of the reported studies, probably due to the low prevalence of smoking. Other studies also reported no significant correlation for cadmium between maternal and cord blood cadmium concentration.[46]

Of the elements measured, manganese, cobalt, copper, selenium and zinc are essential elements, but toxic at high concentrations. The main source of these elements is food. However, nutritional status can be both a confounder and an effect modifier of the association between elements and toxicity. In the case of manganese, both deficiency and excess may have detrimental health effects. As with mercury and lead, the brain is the critical target organ for manganese. Our study found no significant statistical correlation between manganese levels in paired maternal and cord blood, but mean concentrations of manganese in both compartments were found to be higher than the upper limit of 14 µg L^{-1}, (ATSDR, 2000).[47] At present, the risk of manganese—induced neurotoxicity during pre- and postnatal brain development, is not fully researched or understood.[48] The association between manganese uptake during pregnancy and early psychomotor development of children was reported.[49] Animal experimentation also show that neonatal rodents are at increased risk for manganese-induced neurotoxicity.[50,51] In contrast, Takeda *et al.* showed that the higher manganese concentrations are needed during foetal rat brain development, suggesting that a sufficient manganese supply is critical.[52]

Copper is an essential trace element for the enzyme systems of catalase, superoxide dismutase and cytochrome oxidase, and its deficiency can lead to a variety of nutritional and vascular disorders. The newborn is dependent on stored copper, which may not be adequate in premature infants. The ratio of newborn to adult liver copper levels is about 4 : 15.[53] This study found the levels of copper to be on average lower in cord blood, with no significant correlation between maternal and cord blood. Interestingly, our results showed higher concentrations of copper in maternal whole blood when compared to those reported by Al-Saleh *et al.*, but a similar ratio between cord and maternal blood levels. Similarly, no correlation was found for zinc between maternal and cord concentrations.[54]

Cobalt and selenium show a high correlation between maternal and cord blood and both were found to be within normal levels. As with manganese, selenium excess and deficiency may be detrimental to health, with deficiency being reported to impair foetal development in animals.[55] The levels of



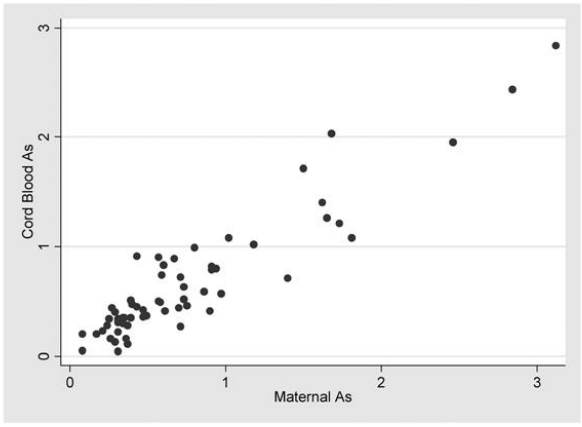


Fig. 4 Arsenic concentration: correlation between maternal and cord blood. Left cord blood (AsCB); right maternal blood (AsM).

selenium in our study were very similar to those reported by Al-Saleh in the maternal population of Kuwait.[54] Furthermore, selenium is an essential element and is thought to lower mercury toxicity, but its underlying mechanism is not fully understood. Our study found a correlation between selenium and mercury levels in maternal blood in spite of low fish consumption in the study population. Similar findings have been reported by other researchers, suggesting that this interaction may be independent of fish consumption.[56,57] Thus, more research is needed to assess the role of selenium in mercury toxicity.

Conclusions

The study confirms that most of the toxic metals have an ability to cross the placental barrier. All subjects in this group were from lower socioeconomic circumstances and none reported previous or current occupational exposures to chemicals, which seems to indicate that the contaminants present in water, food, soil, and air are a primary source of toxins. A possible placental threshold mechanism for cadmium could not be demonstrated in our study. The mercury and manganese levels in cord blood were almost double those of the mothers, indicating high placental permeability between both compartments. The almost free passage of lead from mother to foetus is of clinical relevance, as lead is always toxic, irrespective of concentration.[38]

A limitation of this study is that the levels of copper and zinc were measured in whole blood, thus not allowing for direct clinical comparison with other studies that measured these elements in serum or plasma. Another constraint was the small sample size of the study population, hence the findings cannot be considered representative of the South African pregnant population. Our findings highlight the need for a more comprehensive study that will address these issues.

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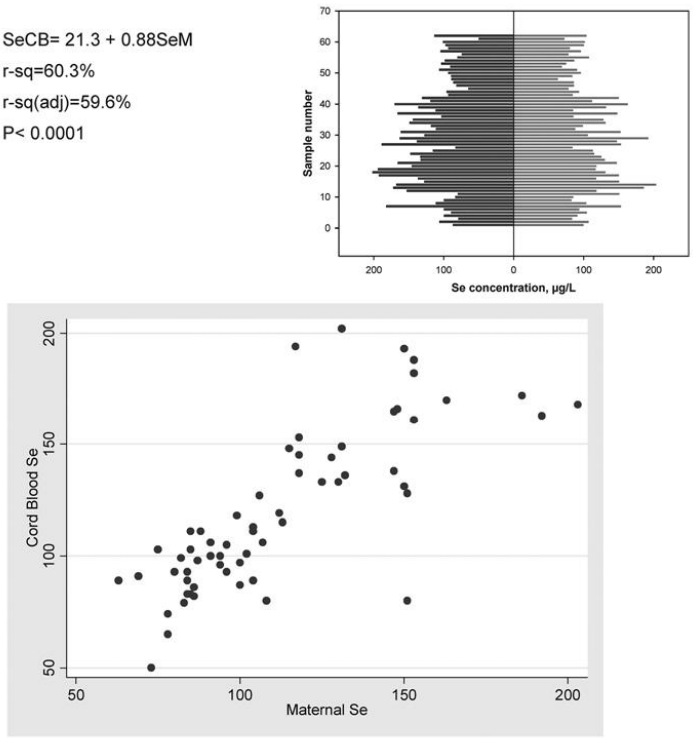

Fig. 5 Selenium concentration: correlation between maternal and cord blood. Left cord blood (SeCB); right maternal blood (SeM).

biological samples. We thank Natalya Romanova for statistical advice.

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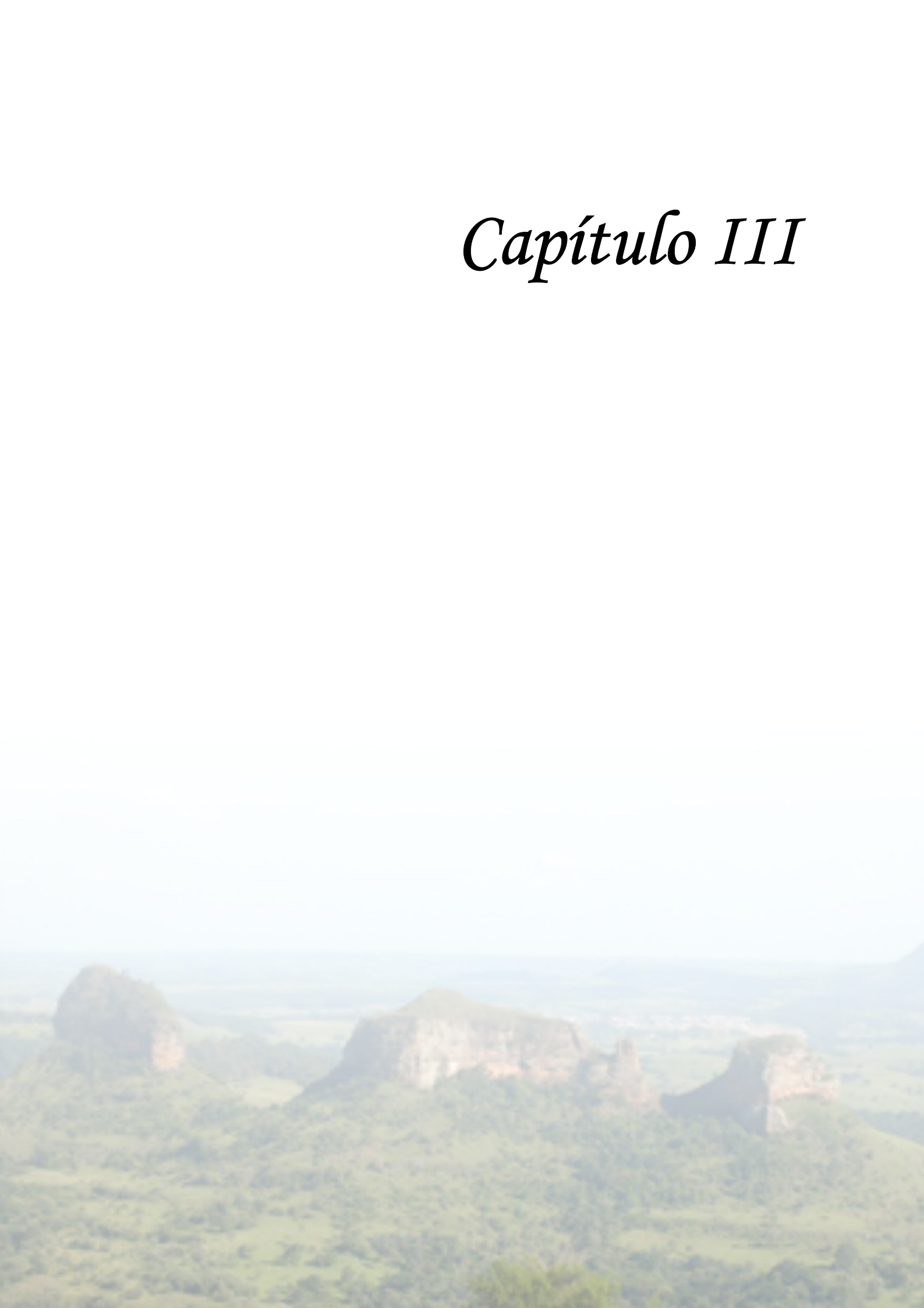
Capítulo III



Levels of selected persistent organic pollutants in blood from delivering women in seven selected areas of São Paulo State, Brazil

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ABSTRACT

Persistent organic pollutants (POPs) present in the living environment are thought to have detrimental health effects on the population, with pregnant women and the developing foetus being at highest risk. We report on the levels of selected POPs in maternal blood of 160 delivering women residing in different regions within the São Paulo State, Brazil. Overall levels of measured compounds were found to be low with only PCBs 118, 138, 153 congeners, *p-p*'-DDE metabolite, β-HCH, γ-HCH, HCB, oxy-chlordane and *t*-nonachlor pesticides having levels above LOD in more than 70% of the samples, thus comparisons between sites were performed for those compounds only. Statistical differences between sites were only observed for PCB 118 congener, with the highest concentration measured in the industrial site – Campinas (4.7 ng/g lipids). The p,p'-DDE metabolite was detected in all participants, with the median for all sites being 58.2 ng/g lipids, and large regional differences were evident. The highest levels of *p,p'*-DDE were measured in the rural 2 site – Ribeirão Preto with a median of 123 ng/g lipids that was significantly higher if compared with the urban 2 site (São Paulo City). The median concentration of ß-HCH for all sites was 6.31 ng/g lipids with the significantly highest concentration found in the rural 1 site – Botucatu (18.20 ng/g lipids). Oxy-chlordane was detected in all samples, with the highest concentration measured in the rural 2 site - Ribeirão Preto (3.6 ng/g lipids), which was found to be significantly higher than in all other sites The *t*-nonachlor compound was detected in 99% of samples with the highest concentration being 1.17 ng/g lipids, also in the rural 2 site- Ribeirão Preto. The level found in the rural 2 site was significantly higher than the level measured in the urban 3 site (São Paulo City). Although overall POPs concentrations in whole blood were low in the majority of delivering women, some significant statistical differences between geographical sites were found, with delivering women from the rural areas presenting the highest concentrations of pesticides in their blood.

Key words: Pesticides exposure, delivering women, human blood, São Paulo State, Brazil

1. Introduction

Worldwide, concern is growing about the presence of persistent organic pollutants (POPs) in the living environment and their possible negative impacts both on the environment and on human health. These man-made chemicals are highly resistant to biodegradation and have high affinities for bioaccumulation and biomagnifications, both in the environment and in living organisms, including humans. A number of human studies have shown POP exposure effects on neurological development; thyroid, estrogen, immune function and cancer promotion. [1-7]

The most vulnerable periods for toxic impact of environmental pollutants on human development are the embryonic and fetal stages.[8-10] Pregnant and nursing women pass these pollutants to their babies both transplacentally and via lactation, and evaluating the maternal contamination is an indirect measurement of the exposure of the fetus to external contaminants.[11, 12] One of the most significant concerns regarding health effects is the harmful influence of PCBs and PCDDs/PCDFs on future generations, stemming from prenatal and/or postnatal exposure.[12, 13]

Furthermore, women and children are most vulnerable as they bear a disproportionate burden from environmental pollution and degradation. Hence they are exposed to particulate pollution and other environmental toxins in the air, food and water of rural and urban areas. [14]

In most of the developing countries including Brazil, the rural population is most affected by exposure to insecticides, fungicides and herbicides, as agriculture is a main source of employment, particularly for women.[15,16] Reports of acute toxic exposure to POPs in adults, including a few studies on the reproductive effects of pesticides, have been published in Brazil.[17-19] Additionally, human data obtained by the Unified Health System's database ("DATASUS"), from 1994 to 2004 in the State of Paraná, Brazil, showed a significant decline in male birth rates in municipalities with high levels of pesticides in the environment.[20]

Brazilian agriculture and the use of agrotoxics

Pesticides have been widely used in agriculture in Brazil since the 1940s. In the 1950s, organophosphate insecticides were introduced in Brazil to substitute organochlorines that were found to have a long environmental persistence.[21] According to the Brazilian Agricultural and Livestock Confederation Report of 2008, agribusiness generated 28% of the Brazilian GNP (Gross National Product), and in 2003, sales of agrotoxics amounted to 375.000 tons of commercial product, which is equivalent to 182 400 tons of the active ingredient.[22] Over the years, the use of agrotoxic substances has increased significantly, and currently Brazil is considered to be one of the biggest consumers worldwide, with sales increasing by 160% between 1991 and 1998.

Historically, DDT was manufactured in Brazil between 1962 and 1982, with the total production estimated to be about 73 481 tons. In 1985 DDT was banned for agricultural use, and in 1998 it was banned for public health use. In 2009 all DDT products still in circulation were removed and currently DDT is prohibited for production, trade and use.[23] In contrast, since 1995 Brazil has markedly increased the use of organophosphates with the average annual use between 1998-2005 being 60 935 tons. [21]

It is estimated that in the agricultural sector, about 12 millions rural employees are exposed daily to toxic substances. Although personal protective equipment (PPE) is provided, in many cases employees ignore and/or fail to use the PPE correctly, leading to poor compliance. As a result, farm workers are considered to be the most exposed population and the main target for adverse effects.[24]

The National Agency of Sanitary Vigilance (ANVISA) in Brazil reported in 2002 that because of Brazil's very high usage of agrotoxics, new legislation was implemented.[21] The reverse logistic legislation compels the producers to collect the packaging of products after

they have been used, thus preventing inappropriate disposal of agrotoxics that could result in secondary pollution to rivers and soils.[21] This legislation has an important public and environmental health benefit, if it is applied strictly.

In response to the lack of comprehensive data on levels of persistent toxic substances (PTS) in pregnant women from Brazil, a pilot project was designed and carried out by the Univ. Estadual Paulista (UNESP) and the University of Tromsø, Norway, under the auspices of the Arctic Monitoring and Assessment Programme (AMAP) during the period 2007-2008. The study measured levels of selected POPs in maternal whole blood of delivering women. This paper reports on the levels of PCBs congeners and pesticides (DDTs metabolites, α-HCH, β-HCH, γ-HCH, HCB, *t*-chlordane, *c*-chlordane, *oxy*-chlordane, *t*-nonachlor and *c*-nonachlor) in maternal whole blood of delivering women in seven selected regions of São Paulo State in Brazil. The seven study sites differ in their degree of environmental pollution and were selected for this very reason.

Socio-economic and self-reported health and lifestyle factors as well as birth outcomes, are also reported. Based on these findings, a longitudinal main study will be designed to investigate sources of exposure and reproductive outcomes in various parts of Brazil.

2. Materials and Methods

2.1. Study sites and population

Agricultural and industrial activities were considered when choosing the sites.The study took place in seven sites of São Paulo State situated in the south-eastern region of Brazil (Figure 1). São Paulo State is the most developed state in Brazil with an area of 248 808 km^2 and a population of approximately 40 million inhabitants.

Two rural sites (Botucatu and Ribeirão Preto), one industrial site (Campinas), one coastal site (Santos) and three metropolitan urban sites (Univ. Federal of São Paulo, UNIFESP, Vila Nova Cachoeirinha and Hospital and Maternity Leonor Mendes de Barros, HMMLMB) were selected. Two rural sites are located inland of São Paulo State (Botucatu and Ribeirão Preto) and are approximately 230 km from São Paulo city. Their economy is based mainly on agriculture, derived mostly from sugarcane, and the population is exposed to sugarcane activities, mainly burning and pesticide application. Three urban sites were selected in different areas of São Paulo city (UNIFESP, HMMLMB, Vila Nova Cachoeirinha). The urban site of São Paulo city was selected because of its large population, high-density living, heavy traffic volume and excessive air pollution. The coastal site, the city of Santos situated on the Atlantic Ocean and approximately 70km from São Paulo city was selected mainly because its estuary is polluted by industrial and urban sewage discharges and by harbour activities. Additionally, it is an endemic dengue area where the spraying of insecticides was reintroduced in 2000. The industrial site (Campinas) is a large city with heavy traffic and is home to a variety of industries, including petrochemicals.

Furthermore, a national and international network was established, with the national group consisting of seven centres and a national coordinator from the bio repository centre (Botucatu).

This pilot study had a total of 160 participants; 20 delivering women from each of the seven sites, except for one rural site (Botucatu) where 40 delivering women participated. All study sites are public maternal hospitals. Enrollment criteria for this project included women who were admitted to the delivery rooms at seven maternity hospitals during 2007 and 2008 and had resided in the specific study site for at least one year prior to the pregnancy. Women who volunteered to participate in the study signed an informed consent form and agreed to donate blood and answer a socio-economic questionnaire and allow access to their post-delivery records. Pesticide exposure was defined as a history of exposure to spraying during pregnancy. In urban areas, domestic use of insecticides was assumed to be a mode of exposure, whereas in rural area agricultural sites, spraying was thought to be the predominant source of exposure. The questionnaire was based on prior studies of women and newborns, translated and adapted for the Brazilian population.[25]

2.2. Sampling procedures

From each mother, approximately 10 ml of blood was drawn by venous puncture into Vacutainer tubes (blue cap trace metal free tubes containing heparin) using the sterile Vacutainer disposable system. The samples were stored at -4°C until the samples were shipped in a frozen state to the University of Tromsø, in Norway, for analyses.

2.3 Sample preparation

The whole blood samples were extracted according to a liquid-liquid extraction method published by Sandanger *et al.*[26] In short, an internal standard mixture (containing fifteen 13C labelled PCBs and eleven of the chlorinated pesticides) was added to 2 ml of blood before mixing with 2 ml of ethanol and 2 ml of deionised water saturated with ammonium sulfate, and extracted twice with 10 ml of n-hexane in a small glass tube. The volume of the extract was reduced to 0.5 ml using the Rapidvap (Labconco Corp., Kansas City, MO), before clean up and fractionation on a florisil column as described previously[25].

2.4. Instrumentation: Gas chromatography

Whole blood samples were analysed for the following compounds; PCB 99, 101, 118, 138, 153, 156, 163, 170, 180, 183, 187, 194 and α-HCH, β-HCH, γ-HCH, HCB, *t*-chlordane, *c*-chlordane, *oxy*-chlordane, *t*-nonachlor and *c*-nonachlor at the Norwegian Institute for Air Research (NILU) laboratory in the Polar Environmental Center, Tromsø, Norway.

The extracts were analysed using an Agilent 7890A gas chromatograph (GC) equipped with a 5975c mass spectrometer (Agilent Technologies, Böblingen, Germany). The GC was fitted with a 30 m DB5-MS column (0.25 mm id and 0.25 μm film thickness; J&W, Folsom, USA). Helium (6.0 quality, Hydrogas, Porsgrunn, Norway) was used as carrier gas at a flow rate of 1 mL/min. Two μL of the sample extract were injected in splitless mode using a split / splitless injector (injector and auto sampler - Agilent 7683 Series, Agilent Technologies, Böblingen, Germany). The GC temperature programme for chromatographic separation was done using an initial temperature of 70°C with a hold time of 2 min, the temperature was then ramped at 15°C/min to 180°C, followed by a temperature ramp of 5°C/min to 280°C with a hold time of 5 min. The electron capture negative ionization (NCI)

mode was used for identification and quantification of the pesticides, while the electron impact (EI) mode was used for determination and quantification of PCBs and DDTs. In both cases, the selected ion-monitoring (SIM) mode was used and the different compounds were identified from their SIM masses, isotopic ratio and retention times. Peaks with differences in isotopic ratio greater than 20%, compared with the quantification standard, were rejected and not quantified. For every 10 samples, a blank was analyzed to assess laboratory-derived (i.e., inadvertent) sample contamination. A standard reference material from the AMAP ringtests was also included in each 10 samples batch. The LODs were calculated using the signal to noise calculations in real samples.

Lipids were determined gravimetrically by evaporating the extract to dryness and weighing the extract when stable weight was achieved.

2.5 Quality assurance and control

NILU participates in the AMAP Human Health Ringtest for plasma samples. The laboratory has participated in the programme from the outset and has performed well to date. Ringtest performance and the regular analysis of certified reference materials clearly indicate that the uncertainty associated with the calculated concentrations is well within +/- 20 %, which is considered the best performance, according to the AMAP ringtest protocol. The high number of labeled internal standards also ensures the quality of the analysis.

As for the determination of lipids, the enzymatic method is considered to be the more precise measurement. In this study only whole blood samples were available; hence, lipids had to be determined gravimetrically. As shown in the work by Sandanger *et al*. 2004, the gravimetric determination is expected to yield results that are 10 – 20 % lower than the enzymatic method.[27] This needs to be taken into consideration when lipid weight levels are being compared to other data.

2.6. Statistical analysis

Descriptive analyses preceded formal hypothesis testing. Due to a non-normal data distribution, the median and first and third quartiles are reported for each substance measured after lipid adjustment. In cases where concentrations of POPs were equal to or below the LOD of the instrument, the concentrations were set at 0.5X LOD, for statistical purposes.

Only compounds with levels above LOD in more than 70% of samples (PCB 118, PCB153, PCB 138, p,p'-DDE, β-HCH, γ-HCH, *oxy*-chlordane, *t*-nonachlor) were evaluated further, statistically . As most of the results do not present a normal distribution, the Kruskal-Wallis test, for comparison between groups with regard to independent V variables, and the Dunn test were applied for multiple comparisons. The criteria of significance was set to p=0.05. The STATA software package, version 10.0 was used to perform the statistical calculations.[28]

2.7. Ethical considerations

The study protocol was submitted and fully approval by the Brazilian National Research Ethical Council (CONEP-Brazil; protocol number 12388). The study subjects were women admitted for delivery at institutional hospitals in each of the seven study sites. Potential participants received plain-language information about the study and those who agreed to participate signed a written consent form, which stated that participation was voluntary, confidentiality was assured and participants could withdraw from the study at any time. Subjects were also informed that if results where cause for concern, they would be referred to an appropriate care medical facility. Ninety eight percent of potential study participants approached agreed to participate.

3. Results

The study took place during 2007 and 2008, and the analytical measurements were completed at the end of 2009. In the tables that follow, study sites are referred to according to their characteristics, and presented in a particular order: Rural 1 (Botucatu); Rural 2 (Ribeirão Preto); São Paulo metropolis is divided into: Urban 1 (UNIFESP), Urban 2 (Vila Nova Cachoeirinha), Urban 3 (HMMLMB); Industrial (Campinas) and Coastal (Santos).

Socio-economic, demographic and lifestyle characteristics

Socio-economic, demographic and lifestyle characteristics for participants at each study site are summarized in Table 1. The subsets did not differ from the overall cohort in terms of demographic variables. The majority of participants were married or lived with their partners, owned their own homes (median 3 rooms / house) and reported a median monthly income of about US$ 480.The majority of the subjects classified themselves as being from the Caucasian race. Less than half of the women were employed. The pesticides used at home varied according to the site, the most prevalent usage being in the three urban sites (São Paulo city). The domestic use of pesticides (yes / no) was reported by 84 women, representing 53% of the study participants. Almost 44% of the women reported that at least one person smoked at home. The majority (90%) of the participants reported to be in a good state of health; the study subjects who reported being most satisfied with their living environment were from the industrial site.

Maternal age, weight, height, parity and neonatal outcomes by site

Table 2 reports on maternal age, weight, height, parity and neonatal outcomes by site. The mean maternal age of all delivering women was 26.3 years; the youngest delivering woman was 14 years old and the oldest was 43 years old. The parity ranged from 1 to 6 with a

median of 2. Overall 36.5% of deliveries were by Caesarian section. The mean maternal weight on the last prenatal care visit was 74.9 kg, varying from 45 to 114 kg. The mean maternal height was 1.59m, ranging from 1.45m to 1.73 m.

Overall birth weight for newborns ranged from 995 to 4460g, and length from 29 to 58 cm. The mean gestational age at delivery was 38.2 weeks, varying from 28 to 42 weeks. Gender ratio differed between sites, ranging from 28.6 to 71% frequency for girls.

Maternal blood levels of contaminants

The concentrations of the different compounds measured in maternal whole blood, adjusted for lipids, are summarized in Table 3. The overall median, first and third quartiles, LOD value for each compound and the percentage of detected cases higher than LOD are presented. Levels were found to be low overall and of the 23 compounds measured, 14 (61%) showed concentrations well below the LOD of the instrument.

For statistical purposes only, Table 4 reports results from sites where the concentrations of specific compounds were above LOD, in more than 70% of blood samples. The values found to be lower than LOD were adjusted using 0.5 X LOD. Only nine compounds were evaluated further and these are PCB 118, PCB 138, PCB 153, *p,p'*-DDE, β -HCH, γ-HCH, HCB, *oxy*-chlordane, *t*-nonachlor.

The overall median concentration of PCB 118 was 3.9 ng/g lipids and it was present in all sites, with the highest concentration found in the industrial site (4.6ng/g lipids) followed by the rural 1 site (3.7ng/g lipids). The lowest concentration was found in rural 2 site (1.5 ng/g lipids) with rural 2 and ocean sites having highly significant lower levels than the industrial site ($p<0.006$). The median of PCB 138 and PCB 153 for all sites was 3.6 ng/g lipids and 4.5 ng/g lipids, respectively, with no significant differences between sites for both congeners.

The median concentration of *p,p'*-DDE for all sites was 58.2ng/g lipids and *p,p'*-DDE was present in all samples, with the highest concentration found in the rural 2 site (123 ng/g lipids) followed by the rural 1 site (82.9 ng/g lipids). The lowest concentration was found in urban 2 site (27.4 ng/g lipids). The level in rural 2 site was significantly higher than in urban 2 site ($p<0.017$).

The median concentration of ß-HCH for all sites was 6.31 ng/g lipids; it was detected in 76.9% of the samples, with the highest concentration being in the rural 1 site (18.2 ng/g lipids) followed by the rural 2 site (6.57 ng/g lipids).. The lowest concentration was found in urban 2 site (1.61 ng/g lipids). The levels in rural 1 site were significantly higher than those in urban 2, urban 3 and ocean sites ($p<0.0001$).

The median concentration of γ-HCH for all sites was 0.67 ng/g lipids, and for HCB it was 8.37 ng/g lipids, with no significant differences between sites.

Oxy-chlordane was present in all of the samples, with the highest concentration found in the rural 2 site (3.59 ng/g lipids) followed by the rural 1 site (1.71 ng/g lipids). The lowest concentration was measured in urban 3 site (0.96 ng/g lipids). The level found in rural 2 site was significantly higher than all other sites, with a value of 3.59 ng/g lipids and a high significance ($p<0.0001$).

T-nonachlor was present in 99% of the samples, with the highest concentration found in the rural 2 site (1.17 ng/g lipids) followed by the rural 1 site (0.75 ng/g lipids). The lowest concentration was found in the urban 3 site (0.42 ng/g lipids). The level found in rural 2 site was significantly higher than that of the urban 3 site ($p<0.003$).

4. Discussion and conclusions

This pilot study quantified the levels of selected POPs in whole blood of delivering women from seven selected regions of São Paulo State, Brazil. Overall, the study found low levels of PCB congeners, but levels in our study were higher than those reported in the plasma of delivering women in South Africa and Vietnam. In contrast, our study found lower levels than those reported recently from Japan.[29-31]

In our study, only PCB 118, 138 and 153 congeners had more than 70% of the samples with concentration higher than LOD of instrument and only PCB 118 in the industrial site was found to be significantly higher. Nevertheless, our findings have shown that PCBs are present in the São Paulo State environment in low concentrations. Indeed, the low maternal levels found may well be a direct consequence of the introduction and implementation of Brazilian legislation as early as 1981, prohibiting the manufacture, marketing, use and disposal of PCBs in Brazil.[32] It is also important to highlight that São Paulo State is at the same latitude as South Africa, where PCBs were also found to be low.[29] These study findings are comparable to those published for non-industrialised developing countries such as Tanzania and India,[33, 34] and in contrast with those reported from industrialized countries. [33, 35]

The environmentally persistent *p,p'*-DDE metabolite was detected in 100% of whole blood samples in our study, even if at low concentrations. Another study performed in 2003 in Rio de Janeiro, Brazil, found *p,p'*-DDE in the breast milk of 97% of participating breastfeeding mothers; the concentrations ranged from 0.16 to 8 ppb. The results from the present study in São Paulo State indicate that DDT contaminations are comparable to those in Rio de Janeiro State.[36] However, the concentrations of *pp'*-DDE evident in delivering women

from São Paulo State are lower than those found in Mexican and South African delivering women, in areas where DDT is used to control malaria. In contrast, levels of *pp'*-DDE were found to be much higher in delivering women in recent similar studies in Vietnam.[29, 31, 37]

As expected the rural areas are more contaminated with pesticides, especially with *p,p'*-DDE, oxy-chlordane, *t*-nonachlor and *c*-nonachlor) than urban sites In our study, higher levels of pesticides were found in delivering women from the rural areas; exposure to these pollutants may be from recent and past activities. In Brazil, it is common practice to use municipal solid waste as compost to enrich the soil for agricultural use; it is very possible that this custom has led to increased levels of pollutants in soil, groundwater and in plants, with a concomitant exposure to humans. [38]

Even though our study found generally low levels of POPs in delivering women, it is well known that chronic exposure to low levels of POPs, over an extended period of time, can lead to cumulative adverse health effects in exposed populations.[39]

To the best of our knowledge, this pilot study is the first Brazilian investigation that concurrently measured a wide spectrum of POPs in whole blood of delivering women, in selected areas of São Paulo State, Brazil. The findings may be useful as baseline data for future research, particularly regarding temporal trends of exposure to POPs. In conclusion, our study found low concentrations of most measured POPs, and as there are no similar data for other parts of Brazil, it is recommended that the study should be extended to other regions, for a more comprehensive overall investigation.

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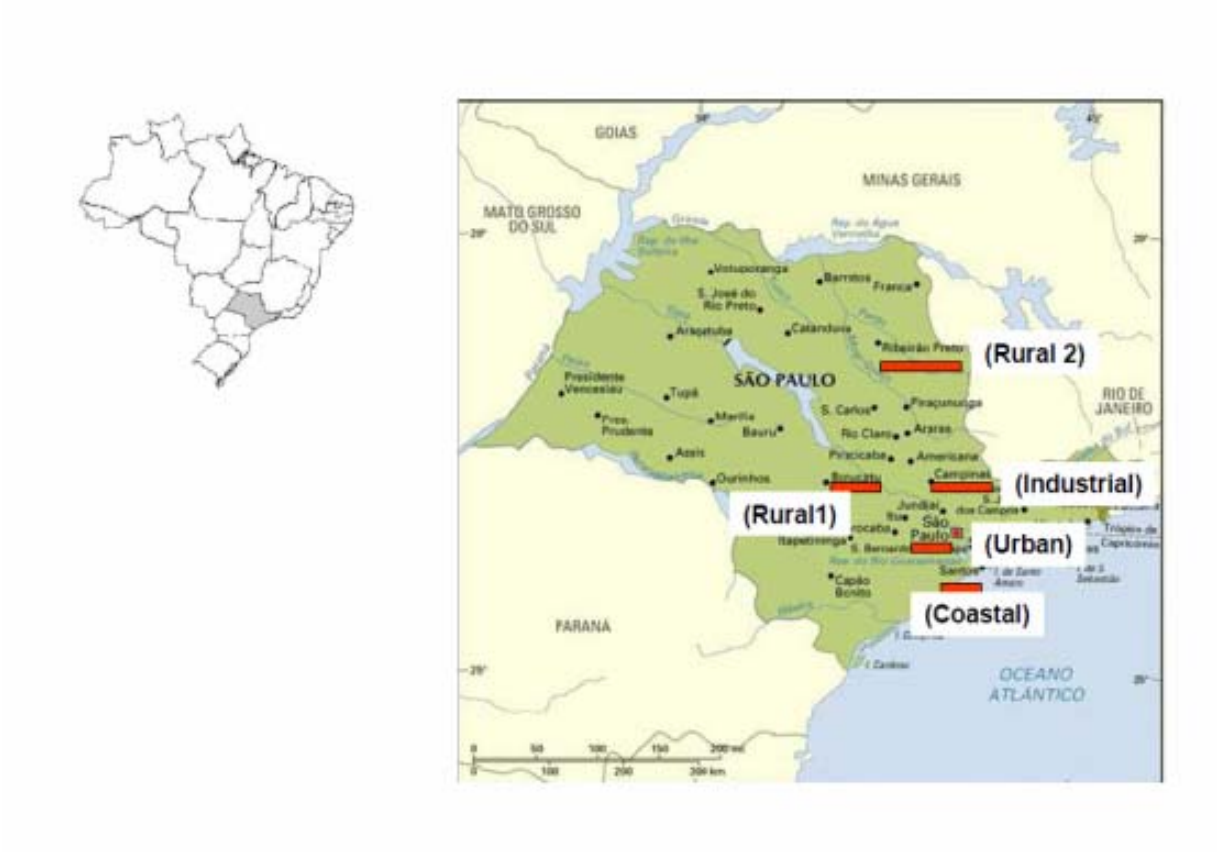

Figure 1 – Geographical locations of study sites within Brazil (The three urban sites are located in São Paulo city)

Table 1: Socio-demographic characteristics and life habits of participants by site.

Statistics	**Rural 1 (n=40)**	**Rural 2 (n=20)**	**Urban 1 (n=20)**	**Urban 2 (n=20)**	**Urban 3 (n=20)**	**Industrial (n=20)**	**Ocean (n=20)**	**Total (n=160)**
Population group (%) B=Black W= White C= Coloured	B=10 C=30 W=60	B=5 C=33 W=62	B=10 C=45 W=45	B=29 C=33 W=38	B=15 C=15 W=70	C=15 W=85	B=10 C=20 W=70	B=11.1 C=27.8 W=61.1
Do you consider yourself health (% YES)	80	100	65	90	95	100	90	87.7
Marital status M= married/ living together (%)	85	90	85	80	65	65	80	77.8
Home ownership %	62.5	52.4	60	61.9	75	75	65	63.6
Rooms	3.2	3.0	2.7	2.1	2.1	2.8	2.5	2.7
Income/ monthly-median (US$)	388	388	555	600	388	417	611	494
Employed? %	40	20	40	40	20	30	50	36.5
Somebody smoking In household%	57.5	47.6	60	56.7	40	25	30	43.8
Pesticide use at home%	50	50	65	75	65	35	30	52.5
Environmental pollution in neighborhood? (%yes)	32.5	76.2	45	52.4	55	25	55	47

Table 2: Maternal age, weight, height, parity and birth outcomes by site

Statistics	**Rural 1 (n=40)**	**Rural 2 (n=20)**	**Urban 1 (n=20)**	**Urban 2 (n=20)**	**Urban 3 (n=20)**	**Industrial (n=20)**	**Ocean (n=20)**	**All (n=160)**
Maternal age	28.7	25.5	29.4	22.9	25.4	24	26.1	26.3
mean (SD)	6.4	7.1	7.6	4.5	6.3	6.5	6.2	6.7
Maternal weight (kg)								
Mean	77.3	75.9	72.6	72.8	71.8	77.5	73.9	74.9
SD	12.9	18.3	16.1	13.6	15.6	18.1	14.2	15.2
Maternal height (m)								
Mean	1.56	1.60	1.62	1.60	1.63	1.58	1.62	160
SD	25.9	7.5	6.1	7.2	7.3	11.4	7.6	14.8
Parity (median (range)	2 (1-3)	2 (1-4)	3 (2-4)	2 (1-3)	2 (1-6)	2 (1- 3)	2 (1-3)	2(1-6)
Birth weight (g)								
mean	3023	2770	3138	3259	3200	2928	3069	3050
SD	446	711	683	397	339	567	505	535
Birth length (cm)								
Mean	47.1	47.6	47.5	48.0	48.8	47.4	47.5	47.6
SD	4.7	3.4	3.0	2.8	2.1	4.8	2.6	3.6
Head circumference (cm)								
Mean	35.0	33.9	34.2	35.1	34.3	34.9	34.5	34.6
SD	3.6	2.3	1.6	1.4	1.3	2.8	1.6	2.4
Gestational age (w)								
mean	38.3	37.0	38.0	39.2	38.6	37.8	38.2	38.2
SD	2.1	3.7	1.6	1.4	1.5	2.2	2.1	2.2
Gender: girls (%)	35	28.6	55	71	35	65	60	48.4
Cesarean section (%)	45	30	50	25	10	50	45	35.6

Table 3: Overall levels of measured POPs in maternal whole blood at delivery (pg/mL) for all samples.

Compounds	Total Samples PCBs detected	Overall Median (all samples)	Overall 1st quartile (all samples)	Overall 3rd quartile (all samples)	Overall Median (>LOD)	Overall 1st quartile (>LOD)	Overall 3rd quartile (>LOD)	LOD	% detected >LOD*
PCB 99	16/147	4.46	2.32	8.25	14.96	14.27	17.48	11.91	10.9
PCB 101	28/147	6.01	3.90	9.51	14.33	12.81	16.71	11.90	19.1
PCB118	117/147	14.93	10.13	19.31	16.36	13.47	21.00	9.5	79.6
PCB138	112/147	12.83	9.55	20.35	15.77	12.11	22.88	9.67	76.2
PCB163	123/147	3.38	1.94	5.15	12.10	9.54	14.31	9.06	11.5
PCB153	123/147	17.74	112.08	28.33	20.41	15.19	33.10	11.16	83.7
PCB156	0/147	3.12	1.87	4.63				41.07	0
PCB170	24/147	6.03	2.89	9.30	15.96	14.75	20.81	12.18	16.3
PCB 180	92/147	14.19	8.93	20.14	19.58	15.68	24.60	11.03	62.6
PCB187	19/147	4.10	2.04	7.83	13.77	12.09	18.23	10.81	12.9
PCB183	1/37	1.23	0.84	2.59	5.76	5.76	5.76	4.96	2.70
PCB194	0/37	1.86	1.22	2.24				5.00	0
p,p'-DDE	147/147	280.14	113.12	860.24	280.14	113.12	860.24	11.30	100
p,p-DDT	13/64	17.64	10.99	35.65	85.19	65.96	228.48	41.36	20.3
α-HCH	17/147	1.0	0.63	1.62	3.40	3.15	7.40	3.0	11.6
β-HCH	113/147	28.64	12.32	77.42	48.70	24.23	108.09	11.0	76.9
γ-HCH	108/128	2.8	2.15	4.11	3.20	2.55	4.49	2.0	84.4
HCB	147/147	35.5	31.4	42.52	35.58	31.39	42.69	2.0	100
t-chlordane	54/128	0.4	0.3	0.5	0.55	0.50	0.65	0.5	41.9
c-chlordane	10/141	0.2	0.15	0.3	0.60	0.60	0.70	0.5	7.0
oxy-chlordane	134/134	5.4	3.99	11.2	5.70	4.00	11.35	2.1	100
t-nonachlor	138/139	2.7	1.59	5.65	2.75	1.65	5.65	0.5	99.3
c-nonachlor	85/147	0.5	0.3	0.99	0.75	0.55	1.25	0.4	57.8

*LOD= limits of detection

Table 4: Comparison of PCB congeners and other pesticides levels in whole blood of delivering women by site (ng/g lipids).

Statistics	All sites	Rural 1 (n=40)	Rural 2 (n=20)	Urban 1 (n=16)	Urban 2 (n=11)	Urban 3 (n=20)	Industrial (n=20)	Ocean (n=20)	p
PCB118									
median	3.31	3.71	1.49	3.26	3.26	3.30	4.64	2.34	
1st quartile	2.11	2.83	1.15	2.02	3.01	2.78	3.60	1.11	0.006*
3rd quartile	4.74	4.74	3.88	4.51	3.97	4.32	6.68	3.09	
IIIQ	2.62	1.91	2.73	2.49	0.96	1.54	3.04	1.98	
PCB138									
median	3.12	3.45	2.98	2.97	2.89	1.95	3.08	3.04	
1st quartile	1.78	2.35	1.34	1.95	2.34	1.14	1.45	2.49	0.197
3rd quartile	4.64	4.93	5.12	4.42	3.38	3.52	4.29	5.02	
IIIQ	2.86	2.57	3.78	2.47	1.04	2.38	2.84	2.52	
PCB153									
median	3.95	4.05	3.29	3.95	4.05	3.66	5.87	4.09	
1st quartile	2.61	3.04	1.77	2.69	1.64	3.07	2.68	2.89	0.395
3rd quartile	6.66	7.23	5.49	6.30	4.94	4.18	8.63	6.20	
IIIQ	4.05	4.19	3.72	3.61	3.30	1.12	5.94	3.30	
p,p'-DDE									
median	58.20	82.87	123.05	65.74	27.44	32.07	42.37	51.62	
1st quartile	30.45	43.65	43.22	31.76	17.54	12.10	32.07	18.53	0.017**
3rd quartile	201.91	222.41	231.72	180.08	97.60	96.00	165.10	140.5	
IIIQ	171.46	178.76	188.5	148.32	80.06	83.91	133.03	121.96	
β-HCH									
median	6.31	18.20	6.57	6.31	1.61	3.03	5.45	3.90	
1st quartile	2.04	7.86	3.85	2.30	1.06	1.30	4.10	1.25	0.0001***
3rd quartile	17.44	33.39	11.54	16.25	4.35	6.68	15.34	8.47	
IIIQ	15.41	25.53	7.68	13.95	3.29	5.37	11.24	7.22	
γ-HCH									
median	0.67	0.66	0.78	0.66	0.29	0.57	0.85	0.71	
1st quartile	0.45	0.51	0.61	0.45	0.20	0.30	0.67	0.41	0.06
3rd quartile	0.97	1.02	0.85	0.95	0.61	0.85	1.47	1.03	
IIIQ	0.52	0.51	0.23	0.50	0.41	0.54	0.80	0.63	
HCB									
median	8.37	8.40	7.66	8.19	7.95	8.21	10.17	7.79	
1st quartile	7.17	7.38	7.00	7.07	6.95	6.67	8.16	7.11	0.26
3rd quartile	10.48	11.27	9.36	10.07	9.51	9.38	12.51	8.88	
IIIQ	3.30	3.89	2.36	3.01	2.76	2.71	4.35	1.76	
oxy-chlordane									
median	1.34	1.71	3.59	1.34	1.00	0.96	1.34	1.06	
1st quartile	0.93	1.17	2.21	0.92	0.79	0.72	0.92	0.86	<0.0001****
3rd quartile	2.45	4.23	7.80	2.38	1.57	1.27	1.86	1.47	
IIIQ	1.52	3.05	5.59	1.46	0.78	0.55	0.94	0.61	
t-nonachlor									
median	0.64	0.75	1.17	0.63	0.43	0.42	0.64	0.54	
1st quartile	0.39	0.47	0.67	0.39	0.30	0.31	0.36	0.39	0.003*****
3rd quartile	1.18	1.36	3.08	1.13	0.59	0.61	0.78	0.85	
IIIQ	0.79	0.89	2.41	0.74	0.29	0.30	0.43	0.46	

* Industrial > Rural 2; Industrial > Ocean
** Rural 2> Urban 2
***Rural 1> Urban 2, Urban 3, Ocean
**** Rural 2 > all sites
*****Rural 2 > urban 3

Median, first and third quartiles and interval inter quartile (IIQ) are presented in ng/g lipid for all compounds. Kruskal-Wallistest for independent samples and Dunn test for multiple comparisons.

Capítulo IV

A ser submetido a revista

Níveis de substâncias tóxicas persistentes (PTS) no sangue de parturientes de sete áreas selecionadas do Estado de São Paulo-Brasil

Levels of selected persistent organic pollutants (POPs) in blood from delivering women in seven selected areas of São Paulo State, Brazil

Key words: pesticides, persistent toxic substances, pregnancy, Brazil

Palavras chaves: pesticidas, substâncias tóxico - persistentes gestação, Brasil.

RESUMO

OBJETIVO: Determinar substâncias tóxicas persistentes (PTS) no sangue de 160 parturientes em áreas do Estado de São Paulo-Brasil. **SUJEITOS E MÉTODOS**: Foram analisados os PCBs (99, 101, 118, 138, 153, 156, 163, 170, 180, 183, 187, 194) e pesticidas organoclorados (α-HCH, β-HCH, γ-HCH, HCB, *p,p'*-DDE, *p,p'*-DDT, *t*-chlordane, *c*-chlordane, *oxy*-chlordane, *t*-nonachlor and *c*-nonachlor) em sete áreas; duas rurais (Botucatu e Ribeirão Preto), uma industrial (Campinas), uma litorânea (Santos) e três urbanas em São Paulo (UNIFESP, Vila Nova Cachoeirinha e Leonor Mendes de Barros). Valores abaixo do limite de detecção (LOD) foram substituídos por 0.5XLOD. Teste de Kruskall Wallis e Dumm compararam as áreas com níveis de PTS acima do LOD. O projeto foi aprovado pelo CEP da FMB. **RESULTADOS**: Os valores das PTS (ng/g lipideo) foram baixos. PCBs 118, 138, 153, *p-p*'-DDE e pesticidas β-HCH, γ- CH, HCB, oxy-chlordane e *t*-nonachlor tiveram níveis acima do LOD em mais de 70% das amostras. PCB 118 foi significativamente diferente nas áreas, com concentração maior na industrial (Campinas; 4.64). O organoclorado *p-p*'-DDE na zona rural de Ribeirão Preto; (123.05) foi maior que na urbana de Vila Nova Cachoeirinha. A mediana do ß-HCH para as áreas foi 6.31, significativamente maior na zona rural de Botucatu (18.20). A maior concentração de oxy-chlordane (3.59) e de *t*-nanochlor (1.17) foi na zona rural de Ribeirão Preto ($p<0.05$), diferentes também da área urbana (Leonor Mendes de Barros; $p<0.05$). **CONCLUSAO:** As concentrações das PTS foram baixas com diferenças entre as áreas; os maiores valores estavam nas parturientes da zona rural.

Aluno: Cibele Vieira Cunha Rudge

Orientadores: Iracema Mattos Paranhos Calderon

Jon Øyvind Odland

Tese de doutorado em co-tutela

#Programa de Pós-graduação em Ginecologia, Obstetrícia e Mastologia- Faculdade de Medicina de Botucatu-Unesp

#Epidemiology of North-EPINOR, Institute of Community Medicine, University of Tromsø, Tromsø, Norway

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Data da defesa: 18/02/2010

Anexo

Anexo 1 – Parecer do Comitê de Ética em Pesquisa.

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Ilustríssima Senhora
Profª Drª Iracema de Mattos P. Calderon
Departamento de Ginecologia e Obstetrícia da
Faculdade de Medicina do Campus de Botucatu

De ordem do Senhor Coordenador do CEP, informo que o Projeto de Pesquisa **"Estudo piloto dos níveis de substâncias tóxicas persistentes (PTS) no sangue de parturientes de sete áreas selecionadas do Estado de São Paulo - Brasil"**, foi aprovado por este CEP em 03/10/1995 e pela Comissão Nacional de Ética em Pesquisa (CONEP) aos 13/12/1995, através do Parecer 1895/2005.

Informo ainda, que consta na documentação deste CEP que o referido estudo foi desenvolvido pela Doutora Cibele Vieira Cunha Rudge, orientada por Vossa Senhoria, sob coordenação internacional da Professora Titular Marilza Vieira Cunha Rudge.

Atenciosamente,

Alberto Santos Capelluppi
Secretário do CEP

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