UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA TOXICOLÓGICA

EFEITOS DE COMPOSTOS ORGÂNICOS DE SELÊNIO NA TOXICIDADE INDUZIDA POR METILMERCÚRIO: ESTUDOS *IN VITRO*.

TESE DE DOUTORADO

Daiane Francine Meinerz

Santa Maria, RS, Brasil
2014

EFEITOS DE COMPOSTOS ORGÂNICOS DE SELÊNIO NA TOXICIDADE INDUZIDA POR METILMERCÚRIO: ESTUDOS *IN VITRO*.

Daiane Francine Meinerz

Tese apresentada ao programa de Pós-graduação em Ciências Biológicas: Bioquímica Toxicológica da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de
Doutora em Bioquímica Toxicológica

Orientador: Prof. Dr. João Batista Teixeira da Rocha
Co-orientador: Prof. Dr. Jeferson Luis Franco

Santa Maria, RS, Brasil
2014

Ficha catalográfica elaborada através do Programa de Geração Automática da Biblioteca Central da UFSM, com os dados fornecidos pelo(a) autor(a).

Meinerz, Daiane Francine
Efeitos de compostos orgânicos de selênio na toxicidade induzida por metilmercúrio: estudos in vitro. / Daiane Francine Meinerz.-2014.
154 p.; 30cm

Orientador: João Batista Teixeira da Rocha
Coorientador: Jeferson Luis Franco
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Bioquímica Toxicológica, RS, 2014

1. Metilmercúrio 2. Estresse oxidativo 3. Selenoenzimas 4. Compostos orgânicos de selênio I. da Rocha, João Batista Teixeira II. Franco, Jeferson Luis III. Título.

Universidade Federal de Santa Maria
Centro de Ciências Naturais e Exatas
Programa de Pós-Graduação em Ciências Biológicas:
Bioquímica Toxicológica

A comissão Examinadora, abaixo assinada, aprova a Tese de Doutorado:

EFEITOS DE COMPOSTOS ORGÂNICOS DE SELÊNIO NA TOXICIDADE INDUZIDA POR METILMERCÚRIO: ESTUDOS *IN VITRO*

elaborada por
Daiane Francine Meinerz

como requisito parcial para obtenção de grau de
Doutora em Ciências Biológicas: Bioquímica Toxicológica

COMISSÃO EXAMINADORA:

João Batista Teixeira da Rocha, Dr. (UFSM)
(Presidente/Orientador)

Cristiane Lenz Dalla Corte, Drª (Unochapecó)

Roselei Fachinetto, Drª (UFSM)

Denis Broock Rosemberg, Dr. (UFSM)

Marcos Martins Braga Dr. (UFSM)

Santa Maria, 23 de outubro de 2014

AGRADECIMENTOS

Agradeço inicialmente aos meus pais, José e Marlene, pelo exemplo, amor e dedicação. A vocês que sempre me apoiaram em todas as minhas decisões e jamais mediram esforços para me ajudar. Também a minha irmãzinha querida, Daniele, que é minha conselheira e companheira para todas as horas. Está sempre disposta a me ajudar no que for preciso. Simplesmente melhor irmã do mundo.

Ao meu noivo Maurício por todo carinho, apoio e amor e por estar sempre ao meu lado mesmo nas horas mais difíceis. Também à sua família, pelo apoio e presença em minha vida.

Ao meu orientador João Batista Teixeira da Rocha por me ensinar que dedicação e esforço valem a pena. Obrigada por toda compreensão que tens tido comigo. Admiro-te pela sabedoria, dedicação e competência.

Ao meu co-orientador Jeferson L. Franco pela amizade e paciência. Obrigada por estar sempre disposto a me ajudar e pela dedicação ao meu trabalho.

Aos colegas de laboratório em especial à Emily, ao Diones, ao Douglas e a Bruna que sempre foram grandes parceiros na pesquisa e na vida. Obrigada pela amizade e companheirismo.

Aos colegas e amigos que fiz em minha estada em Portugal durante o Doutorado Sanduíche, e em especial a Professora Cristina Carvalho, por sua ajuda e compreensão.

Também a todos os demais colegas do laboratório do professor João e da professora Nilda pelo companheirismo, ajuda e conhecimento compartilhado.

Aos membros da banca por aceitarem o convite para avaliar este trabalho.

Aos professores do curso de pós-graduação em Ciências Biológicas: Bioquímica Toxicológica pelos ensinamentos.

A Capes e a CNPq pela bolsa de estudos e pelos recursos financeiros concedidos.

E a Universidade Federal de Santa Maria e ao PPGBTOX pela possibilidade de realização deste curso.

“Nunca deixe que lhe digam que não vale a pena
acreditar no sonho que se tem.”
Renato Russo

“A persistência é o menor caminho do êxito”
Charles Chaplin

“A ciência nunca resolve um problema sem criar pelo menos outros dez.”
George Bernard Shaw

RESUMO

Tese de Doutorado
Programa de Pós Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria

EFEITOS DE COMPOSTOS ORGÂNICOS DE SELÊNIO NA TOXICIDADE INDUZIDA POR METILMERCÚRIO: ESTUDOS IN VITRO.

AUTORA: Daiane Francine Meinerz
ORIENTADOR: João Batista Teixeira da Rocha
CO-ORIENTADOR: Jeferson Luis Franco
LOCAL E DATA DA DEFESA: Santa Maria, 23 de outubro de 2014.

O metilmercúrio (MeHg) é um conhecido poluente ambiental que afeta uma variedade de funções celulares, sendo o estresse oxidativo um dos mecanismos propostos para explicar sua toxicidade. A inibição de selenoenzimas (Glutationa Peroxidase (GPx) e Tiorredoxina Redutase (TrxR)) tem sido demonstrada como um importante fator relacionado ao estresse oxidativo induzido por MeHg. Por outro lado, compostos sintéticos de selênio apresentam importante ação antioxidante, atribuída principalmente a atividade mimética a GPx e ao fato de serem substratos para TrxR, podendo inclusive exercer proteção contra a toxicidade do MeHg. Considerando que o mecanismo exato pelo qual o MeHg exerce toxicidade ainda deve ser melhor investigado e que não existem terapias eficazes para a toxicidade deste organometal, este estudo avaliou os diferentes mecanismos envolvidos na toxicidade induzida por MeHg, bem como, um possível papel protetor de compostos orgânicos de selênio (ebselen, disseleno de difenila $(PhSe)_2$ e análogos (3'3-disseleneto de ditrifuorometildifenil (DFD), p-metoxi-disseleneto de difenila (MD) and p-cloro-disseleneto de difenila (CLD)) frente aos efeitos tóxicos deste organometal em diferentes modelos *in vitro* de sistemas biológicos alvos da ação do mesmo. No artigo 1 foi observado que o MeHg causou um aumento em marcadores de estresse oxidativo em um modelo utilizando fração mitocondrial de cérebro de camundongos. Esse resultado foi evidenciado através de um aumento da peroxidação lipídica, conteúdo de hidroperóxidos e uma significativa diminuição da atividade mitocondrial. Além disso, também foi demonstrada uma depleção significativa de tióis. Os compostos $(PhSe)_2$ e DFD foram capazes de proteger contra a redução na atividade mitocondrial e peroxidação lipídica causada pelo MeHg. O composto CLD também mostrou ser efetivo em reverter a oxidação de lipídeos causada pela exposição da fração mitocondrial de cérebro de camundongos ao MeHg. O $(PhSe)_2$ também mostrou ser capaz de degradar o peróxido de hidrogênio através de um estudo comparativo com a catalase. Esses compostos mostraram atividade protetora de acordo com a intensidade de sua atividade GPx-like ($(PhSe)_2$ > DFD > CLD> MD). Em uma segunda análise (manuscrito 1), o MeHg causou uma significativa inibição das enzimas antioxidantes TrxR e GPx em células de neuroblastomas (SH-SY5Y). Neste estudo o $(PhSe)_2$ apresentou melhor atividade protetora que o ebselen. O $(PhSe)_2$ causou um aumento substancial na atividade e expressão da TrxR e foi capaz de proteger da inibição desta enzima causada pelo MeHg. Sob as mesmas condições experimentais, o composto ebselen apresentou efeito similar, no entanto, de maneira mais modesta quando comparado ao $(PhSe)_2$. O $(PhSe)_2$ também causou um aumento da expressão da GPx, que foi maior quando comparado ao efeito do ebselen. Em modelo de leucócitos humanos *in vitro* (manuscrito 2) foi possível observar que o MeHg causou morte celular e danos ao DNA. Em paralelo, observou-se um aumento significativo na expressão de Nrf2, o principal regulador da resposta antioxidante celular. O co-tratamento com $(PhSe)_2$, foi capaz de reverter esses danos e normalizar a expressão do Nrf2. Além disso, o $(PhSe)_2$ causou um aumento substancial na expressão da enzima TrxR, resposta já demonstrada no estudo anterior em modelo de neuroblastoma humano. Em conjunto, os resultados apresentados nesta tese reforçam o papel central do estresse oxidativo e inibição de selenoezimas na toxicidade induzida pelo MeHg. Além disso, os resultados indicam que os compostos orgânicos de selênio, em especial o $(PhSe)_2$, exercem um importante papel protetor frente a toxicidade deste organometal. A reversão da inibição da enzima TrxR causada pelo $(PhSe)_2$ parece ser um importante mecanismo envolvido na proteção contra os efeitos deletérios causados pela exposição ao MeHg, um agente tóxico ambiental.

Palavras-chave: Metilmercúrio, estresse oxidativo, selenoenzimas, compostos orgânicos de selênio.

ABSTRACT

Thesis of Doctor' degree
Graduate course in biological Sciences: Toxicological Biochemistry
Federal University of Santa Maria

EFFECTS OF ORGANOSELENIUM COMPOUNDS ON METHYLMERCURY – INDUCED TOXICITY: *IN VITRO* STUDIES

AUTHOR: Daiane Francine Meinerz
ADVISOR: João Batista Teixeira da Rocha
CO-ADVISOR: Jeferson Luis Franco
PLACE AND DATE OF THE DEFENCE: Santa Maria, October 23th, 2014

Methylmercury (MeHg) is a known environmental pollutant that affects a variety of cellular functions, and the oxidative stress is one of the proposed mechanisms to explain its toxicity. Inhibition of selenoenzymes (Glutathione peroxidase (GPx) and Thioredoxin Reductase (TrxR)) has been demonstrated as an important factor related to oxidative stress induced by MeHg. On the other hand, synthetic selenium compounds have important antioxidant activity, mainly attributed to the GPx-like activity and the fact that they are substrates for TrxR, may even exert protection against MeHg toxicity. Whereas the exact mechanism by which MeHg exerts toxicity remains to be further investigated and that there are no effective therapies for this organometal toxicity, this study evaluated the different mechanisms involved in MeHg-induced toxicity as well a possible protective role of organic selenium compounds (ebselen, diphenyl diselenide $(PhSe)_2$ and analogues (3'3 ditrifluoromethyldiphenyl diselenide (DFD), *p*-chloro-diphenyl diselenide (CLD) and *p*-methoxy-diphenyl diselenide(MD)) against toxic effects of this organometal at different *in vitro* models of biological systems targets of MeHg action. In Article 1 it was observed that MeHg caused an increase in oxidative stress markers in a model of mouse brain mitochondrial-enriched fraction. This result was evidenced by an increase in lipid peroxidation, hydroperoxide formation and a significant decrease in mitochondrial activity. Furthermore, it was also demonstrated a significant depletion of thiols. $(PhSe)_2$ and DFD were able to protect against the decrease in mitochondrial activity, and lipid peroxidation caused by MeHg. The compound CLD was also effective in reversing the lipid peroxidation caused by exposure of mouse brain mitochondrial fraction to MeHg. The $(PhSe)_2$ was also able to degrade hydrogen peroxide through a comparative study with catalase. These compounds showed protective activity according their GPx-like activity intensity ((PhSe) 2> DFD> CLD> MD). In a second analysis (manuscript 1), MeHg caused a significant inhibition of antioxidant enzymes, TrxR and GPx, in neuroblastoma cells (SH-SY5Y). In this study $(PhSe)_2$ showed a better protective activity than ebselen. $(PhSe)_2$ caused a substantial increase in the activity and expression of TrxR, and it was able to protect against the inhibition of this enzyme caused by MeHg. Under the same experimental conditions, ebselen exhibited a similar effect, though more modestly compared to $(PhSe)_2$. Diphenyl diselenide also caused an increased in GPx expression, which was higher when compared to the effect of ebselen. In *in vitro* model of human leukocytes (manuscript 2), MeHg caused cell death and DNA damage. In parallel, there was a significant increase in Nrf2expression, the main cellular regulator of antioxidant response. Co-treatment with $(PhSe)_2$ was able to reverse these damages, and normalizing the expression of Nrf2. Furthermore, $(PhSe)_2$ caused a substantial increase in expression of TrxR enzyme, response that has been already demonstrated in the previous study in a model of human neuroblastoma. Together, the results presented in this thesis reinforce the central role of oxidative stress and inhibition of selenoezimas in MeHg-induced toxicity. Furthermore, the results indicate that the organic selenium compounds, especially $(PhSe)_2$, play an important protective role against MeHg toxicity. The reversal of TrxR inhibition caused by $(PhSe)_2$ appears to be an important mechanism involved in the protection against the deleterious effects caused by exposure to MeHg, a toxic environmental agent.

Key-words: Methylmecury, oxidative stress, selenoenzymes, organic selenium compounds.

LISTA DE FIGURAS

REVISÃO BIBLIOGRÁFICA

ARTIGO 1

MANUSCRITO 1

MANUSCRITO 2

LISTA DE TABELAS

REVISÃO BIBLIOGRÁFICA

MANUSCRITO 2

LISTA DE ABREVIATURAS

ANOVA: análise de variância

BSA: albumina sérica bovina

cDNA: DNA complementar

CLD: p-cloro-disseleneto de difenila

Cys: cisteína

DFD: 3’3-disselento de ditrifuorometildifenil

DIO: deiodinase

DMSO: Dimetilsulfóxido

DNA: ácido desoxirribonucleico

DNTP: desoxirribonucleotídeos fosfatados

DTNB: ácido 5,5-ditiobis-2-nitrobenzóico

EDTA: ácido etileno-dinitrilo-tetra-acético

EGTA: ácido etileno-glicol-tetra-acético

ERO: espécies reativas de oxigênio

GPx: Glutationa Peroxidase

GR: Glutationa Redutase

GSH: Glutationa reduzida

GSSG: Glutationa oxidada

H_2O_2: Peróxido de Hidrogênio

HCl: ácido clorídrico

HEPES: ácido N-2-Hidroxietilpiperazina-N'-2-etanosulfonico

Hg: mercúrio

$HgCl_2$: cloreto de mercúrio

MD: p-metoxi-disseleneto de difenila

MDA: malondialdeído

MeHg: metilmercúrio

MeSeCys: metilselenocisteína

mRNA: RNA mensageiro

MTT: brometo de 3-(4,5-Dimetiltiazol-2-il)-2,5-difenil-tetrazolium

Na_2SeO_3: selenito de sódio

NAPPH: nicotinamida adenina dinucleotídeo fosfato

PBS: tampão salina fosfato

$(PhSe)_2$ ou DD: Disseleneto de Difenila

RNA: ácido ribonucléico

SDS: dodecil sulfato de sódio

SDS-PAGE: eletroforese em gel de poliacrilamida contendo SDS

Se: selênio

Se^{2-}: seleneto

Sec: selenocisteína

SeMet: selenometionina

SeO_3^{2-}: selenito

SeO_4^{2-}: selenato

SFB: soro fetal bovino

SNC: sistema nervoso central

TBARS: substâncias reativas ao ácido tiobarbitúrico

Trx: Tiorredoxina

TrxR: Tiorredoxina Redutase

SUMÁRIO

APRESENTAÇÃO

No item INTRODUÇÃO é apresentado um referencial teórico sucinto sobre os temas trabalhados nesta tese, bem como a justificativa e os objetivos gerais e específicos da mesma.

Na REVISÃO BIBLIOGRÁFICA é apresentado um manuscrito de revisão da literatura sobre os temas trabalhados e relacionados a esta tese.

A metodologia realizada e os resultados que compõem esta tese estão apresentados sob a forma de artigos e manuscritos, os quais se encontram no item ARTIGOS CIENTÍFICOS. Neste constam as seções: introdução, materiais e métodos, resultados, discussão e referências bibliográficas.

Os itens DISCUSSÃO e CONCLUSÕES, encontrados no final desta tese, apresentam interpretações e comentários gerais sobre os artigos científicos contidos neste trabalho.

As REFERÊNCIAS BIBLIOGRÁFICAS referem-se somente às citações que aparecem nos itens INTRODUÇÃO, DISCUSSÃO e CONCLUSÕES desta tese.

INTRODUÇÃO

O metilmercúrio (MeHg), considerado a principal forma orgânica de mercúrio encontrada na natureza, é gerado através da biometilação do mercúrio inorgânico presente em sedimentos aquáticos (CLARKSON *et al*., 2003). Este organometal é um conhecido poluente ambiental, que nas últimas décadas causou contaminação em humanos em várias partes do mundo, sendo os incidentes mais severos observados na Baía de Minamata no Japão e no Iraque (TAKEUCHI *et al.*, 1962; BAKIR *et al*., 1973). No Brasil, estudos têm evidenciado que várias espécies de peixes da Amazônia apresentam altos níveis de MeHg (PINHEIRO *et al*., 2003). Consequentemente, comunidades ribeirinhas localizadas próximas às áreas de garimpo sofrem exposição crônica a níveis relativamente elevados de MeHg através da sua dieta, a qual é rica em peixes (PINHEIRO *et al*., 2003).

O MeHg afeta uma variedade de funções celulares podendo causar danos em muitos órgãos e sistemas, particularmente no sistema nervoso central (SNC) (FARINA *et al*., 2011). No entanto, o fígado, os rins, o sistema imune e cardiovascular parecem ser também alvos importantes para a toxicidade do MeHg (DIAZ *et al*., 2001; RUTOWSKI *et al*., 1998; MOSZCZYNSKI *et al.*, 2008; VIRTANEN *et al*., 2007). Além disso, danos ao material genético podem ser consequência da exposição a este composto (GROTTO *et al*., 2009).

Os mecanismos de ação envolvidos na toxicidade do MeHg ainda não estão completamente elucidados, porém, o estresse oxidativo, o qual é caracterizado por um desequilíbrio entre a produção de espécies reativas de oxigênio (ERO) e sua remoção por sistemas antioxidantes (ALI *et al*., 1992), exerce um papel central na toxicidade induzida por este composto orgânico de mercúrio (ASCHNER *et al.*,2007). Este efeito está principalmente relacionado à ação direta do MeHg sobre tióis endógenos, levando a uma depleção dos níveis de glutationa (GSH) e também ao seu efeito sobre uma variedade de sistemas enzimáticos, causando dano e morte celular (GERHARDSSON & SKERFVING, 1996). Desta forma, a depleção da GSH e inibição de sistemas antioxidantes enzimáticos induzidas por MeHg podem facilitar o aumento de espécies reativas de oxigênio (ERO) (FARINA *et al*, 2003) e, consequentemente, danificar macromoléculas biológicas tais como, ácidos nucléicos, proteínas, lipídios e carboidratos (XIONG *et al*., 2007). As mitocôndrias, que são as principais organelas envolvidas na produção de ERO, também representam um dos sítios mais suscetíveis à toxicidade induzida pelo MeHg (CECCATELLI *et al*., 2010).

Enzimas antioxidantes, como a Glutationa Peroxidase (GPx) e a Tiorredoxina Redutase (TrxR), podem ter sua atividade negativamente afetadas pela exposição ao MeHg

devido a presença do selenol nos sítios ativos destas enzimas (de acordo com Sugiura *et al.* o selenol demonstra maior afinidade pelo MeHg que grupos tiól) (FRANCO *et al*., 2009, GLASER *et al.,* 2010; SUGIURA *et al., 1976;* CARVALHO *et al.,* 2011*).* Em um estudo recente, de Usuki *et al.* também foi observado que o MeHg induz uma redução nos níveis de selênio (Se) intracelulares, o que acarreta uma redução da atividade e expressão destas selenoenzimas (GPx e TrxR), fato que também foi demonstrado em modelo *in vivo* por Zemolin *et al.* (ZEMOLIN *et al*.,2012), resultando em um distúrbio no sistema redox das células e estresse oxidativo (USUKI *et al*., 2011).

O Se é um elemento traço essencial (COMBS & COMBS, 1984) e exerce funções de grande importância no organismo desempenhando o seu principal papel como aminoácido selenocisteína (Sec), presente no sítio ativo de selenoproteínas como a GPx e a TrxR, enzimas com papel essencial no equilíbrio redox do organismo, agindo como importantes antioxidantes (FLOHÉ *et al.,* 1973; ARNER AND HOLMGREN, 2000). Além disso, o Se também desempenha proteção de membranas biológicas contra dano oxidativo e é capaz de interagir com metais pesados (incluindo o mercúrio) atenuando sua toxicidade (FALNOGA & TUSEK-ZNIDARIC, 2007). Vários estudos apontam para o efeito protetor de compostos inorgânicos de Se contra a toxicidade do MeHg (SUZUKI *et al*., 1998; BRANCO *et al*., 2012). No entanto, ainda são poucos os estudos sobre o possível papel protetor das formas orgânicas de Se.

Nos últimos anos, vários estudos têm demonstrado o potencial farmacológico de compostos sintéticos orgânicos contendo Se, já que algumas dessas moléculas podem formar espécies nucleofílicas potentes (NOGUEIRA & ROCHA, 2011). O ebselen (2-fenil-1,2-benzisoselenazol-3(2H)- one) foi um dos primeiros compostos utilizado na pesquisa, o qual demonstrou importantes propriedades antioxidantes. Posteriormente, também o composto disseleneto de difenila $(PhSe)_2$, o mais simples diaril disseleneto, também apresentou efeitos protetores com propriedades semelhantes ao ebselen, as quais têm sido atribuídas, principalmente, a atividade mimética à enzima GPx e também por sua ação como substrato da enzima TrxR, propriedades também observadas para os análogos do $(PhSe)_2$ (3'3-disseleneto de ditrifuorometildifenil (DFD), p-metoxi-disseleneto de difenila (MD) and p-cloro-disseleneto de difenila (CLD)) (WENDEL *et al*., 1984; NOGUEIRA *et al*., 2004; FREITAS *et al.*, 2010). O ebselen e $(PhSe)_2$, também tem demonstrado atividades neuroprotetora, anti-inflamatória, anti- aterosclerótica e anti-hipercolesterolêmica em vários modelos *in vitro* e *in vivo* (DE BEM *et al.*, 2009; FREITAS *et al.*, 2009; FREITAS *et al.*, 2010; NOGUEIRA & ROCHA, 2011; HORT *et al.*, 2011). Ainda, o ebselen e $(PhSe)_2$ foram

capazes de proteger contra efeitos tóxicos do MeHg em modelo *in vitro* e *in vivo* relacionados ao estresse oxidativo (FARINA et al., 2003; FREITAS *et al.*, 2009).

Considerando que (i) a toxicidade do MeHg está diretamente relacionada a um decréscimo na atividade de importantes selenoenzimas e consequentemente ao estresse oxidativo; (ii) déficits na função destas proteínas têm sido relacionados ao desenvolvimento de patologias cardíacas (DE LORGERIL & SALEN, 2006**)** desordens embriogênicas, doenças neurodegenerativas e câncer (STEINBRENNER & SIES, 2009); (iii) não existem formas de tratamento efetivas para a intoxicação com MeHg e (iv) compostos orgânicos de Se têm sido caracterizados por suas propriedades benéficas, principalmente a atividade antioxidante, torna-se importante o estudo do potencial protetor destes compostos contra a toxicidade induzida por MeHg, contribuindo, assim, para o desenvolvimento de novas abordagens terapêuticas para o tratamento de casos de intoxicação por este organometal.

Desta forma, este estudo focará na investigação de mecanismos celulares e moleculares envolvidos na toxicidade induzida por MeHg bem como na avaliação do potencial efeito protetor do ebselen, $(PhSe)_2$ e análogos, compostos com atividade mimética à GPx em modelos *in vitro*. A utilização de modelos *in vitro*, além de representar um passo importante para a avaliação do mecanismo de ação de drogas, possibilitando um ambiente controlado para observação de interações celulares e moleculares, reduz a variação experimental e é de fácil replicação (FRESHNEY, 2001). Sendo assim, para este estudo serão utilizados modelos *in vitro* que representam alvos da toxicidade do MeHg (como mitocôndrias cerebrais, células cerebrais e leucócitos isolados).

OBJETIVOS

Objetivo Geral

Compreender os diferentes mecanismos envolvidos na toxicidade do MeHg, bem como avaliar um possível papel protetor de compostos orgânicos de selênio frente aos efeitos tóxicos deste organometal em diferentes modelos *in vitro* de sistemas biológicos alvos da ação do mesmo.

Objetivos específicos

- Avaliar a toxicidade do MeHg frente a diferentes parâmetros bioquímicos e moleculares buscando compreender e elucidar mecanismos de ação tóxica deste composto;

- Estudar o possível efeito protetor do $(PhSe)_2$ e análogos na toxicidade induzida por MeHg em diferentes indicadores de estresse oxidativo e atividade mitocondrial em modelo de fração mitocondrial enriquecida de cérebro de camundongos;

- Investigar como o tratamento com $(PhSe)_2$ e ebselen pode alterar a atividade e expressão da TrxR e GPx e interferir na interação entre o mercúrio (MeHg) e estas selenoenzimas em modelo experimental de cultura celular de neuroblastoma humano (SH-SY5Y);

- Avaliar, em modelo de leucócitos *in vitro*, o possível papel protetor do $(PhSe)_2$ frente à toxicidade induzida por MeHg na viabilidade celular, danos ao DNA e expressão de enzimas antioxidantes (com foco para selenoenzimas).

1. REVISÃO BIBLIOGRÁFICA

1.1 **Manuscrito de revisão em preparação**:

SELENIUM, MERCURY AND THEIR INTERACTIONS

DAIANE F. MEINERZ, JEFERSON L. FRANCO, JOÃO BATISTA T. DA ROCHA

Review:

Selenium, mercury and their interactions

Meinerz D.F.[1], Franco, J.L.[2], Rocha J.B.T.[1]

[1] Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria RS, Brazil, 97105-900;

[2]Campus São Gabriel, Universidade Federal do Pampa, São Gabriel, RS, Brazil, 97300-000.

Abstract:

Selenium (Se) is an essential micronutrient occuring in a number of forms in the environment. In living organisms, the most common forms are selenomethionine and selenocysteine. As selenocysteine it plays major role in the cell defense against oxidative stress, being a component of endogenous selenoenzymes with antioxidant activity. In contrast, mercury, which is far from being an essential element, has been utilized by humans since ancient times. Methylmercury, an organic form of this metal, is demonstrated to negatively interact with Se-containing enzymes and to induce toxicity by means of oxidative stress. In this review, we emphasize the importance of Se in health and discuss on the biological actions of new syntethic organoselenium compounds and their role as protective compounds against the toxicity of mercury.

Key-words: selenium, seloenzymes, mercury.

1. Introduction

Selenium (Se) is a nonmetal element that occurs at different forms (organic and inorganic) in the environment (soil, water, air and incorporated to different organisms). Its essentiality, as trace element, for several species relies on the fact that it is constituent of selenocysteine on selenoproteins. This fact was first reported at 1970's by authors that described Glutathione Peroxidase (GPx), as a selenoenzyme. Thus, this micronutrient, with redox characteristics, plays major role in the cell defense against oxidative stress (Rotruck et al., 1973; Flohe et al., 1973; Frostom et al., 1978).

Moreover, Se is increasingly recognized for its biological importance and it is considered a nutraceutical component, having health benefits as part of prevention and treatment of diseases. Deficiency of Se, can lead to cardiovascular disorders (Allander et al., 1994), cognitive impairment, seizures, Parkinson's and Alzheimer's diseases (Zhang et al., 2010), increase of cancer incidence (Davis et al., 2012) and conditions associated with increased oxidative stress or inflammation (Whanger, 2002; Rayman, 2000). In this context, Se supplementation can be considered a valuable therapeutic alternative for such diseases as demonstrated on studies using inorganic and organic forms of this element (Velardos et al., 2004; Corcoran et al., 2010; Rayman et al., 2008). Furthermore, synthetic organic Se compounds, have also been investigated in this context, and despite some toxic effects, its GPx-like activity and action as Thioredoxin reductase (TrxR) substrate, lead to evidences of protection against some diseases in experimental models, such as inflammation, depression, cardiomyopathy, neuro disorders and other (Nogueira et al., 2003; Posser et al., 2009; de Bem et al., 2008, 2009; Saito et al., 1998).

In contrast, mercury (Hg), which is far from being an essential element, has been utilized by humans since ancient times. Methylmercury (MeHg), considered the main organic form of this metal found in nature, is a known environmental pollutant that caused human contamination around the world in the last decades (Clarkson et al., 2003; Bakir et al., 1973). The main source of contamination to this organometal can be through fish and seafood consumption (Pinheiro et al., 2003), where this toxic compound demonstrate affect a variety of cellular functions leading to damage of organs and tissues, particularly to central nervous system (Farina et al., 2011), but also to liver, kidney and immune and cardiovascular systems (Freitas et al., 2009; Shenker et al., 1997; Virtanen et al., 2007). This organometal negatively

interact with Se-containing enzymes, reducing their redox potential and induce toxicity by means of oxidative stress (Shanker et al., 2005; Franco et al., 2009; Branco et al., 2012).

As described before, Se is a potent antioxidant, and may, due to its chemical characteristics, be a promising class of therapeutic compounds against Hg toxicity particularly in its organic form, in order to reduce its toxic effects. This, compound also have a high ability to interact with heavy metals (Falnoga and Tusek-Znidaric, 2007), and it is a natural MeHg and inorganic Hg antagonist that potently counteracts or eliminates symptoms of toxicity that would otherwise accompany high MeHg/Hg exposures (Ralston and Raymond, 2010). Synthetic organic Se compounds have also been demonstrated potent agents to attenuate MeHg toxicity (Freitas et al., 2009, Farina et al., 2003; Meinerz et al., 2011), and their importance are emphasize here.

This review describes important aspects of Se in the environment and in living organism, with focus to selenoenzymes, and its importance to health. In addition, we describe some important characteristics of Hg at inorganic and mainly at organic forms, and their toxicological effects in the metabolism. Finally, this review will describe some Se and Hg interactions; their biochemical, physiological, and toxicological interactions, and the importance of this antioxidant compounds (especially as synthetic organic Se compounds) in the attenuation of MeHg toxicity.

2. Selenium

Selenium is a nonmetal element with atomic number 34. It was identified, in 1818 by Jöns Jacob Berzelius a Swedish chemist (Berzelius, 1818; Kabata-Pendias, 1998) that named it in reference to Selene, the Greek goddess of the moon (Kabata-Pendias, 1998), thus contrasting with tellurium, metalloid that has similar properties shared with Se, initially referred to the Earth.

Selenium is present in nature and in organisms as organic and/or inorganic forms. The main organic forms are selenomethionine (SeMet) and selenocysteine (Sec). The inorganic forms are selenite (SeO_3^{2-}), selenide (Se^{2-}), selenate (SeO_4^{2-}) and the Se element (Se). In addition, this element is often associated with sulphur containing compounds (Mehdi, 2013).

2.1 Selenium in the Enviornment

This trace element is present in the earth's crust at an average of 90 µg/Kg. Selenium concentration is high in carbonate rocks as well as volcanic and sedimentary soils (Mehdi et al., 2013). In soils, Se availability occurs mainly due to erosion of rocks containing selenites and selenides which are associated with sulphide minerals. It is also found in the form of elemental Se, selenate salts and ferric selenite or in its organic forms that derive mainly from the decomposition of plants that accumulate Se (Martens and Suarez, 1996; Barceloux, 1999). Basically, Se concentration in soil varies depending on the type, texture and organic matter content of the soil (Dumont et al., 2006).

In water, Se is originated from atmospheric precipitation and deposits or soil drainage and sub-soils which are naturally rich in this element. This element can be found mainly as selenide, selenate and selenite forms (Barceloux, 1999).

Regarding human health, Se in water has been considered less important than it in soil. However, the presence of this element in drinking water provided manly from agricultural fertilizers has been recently linked to an increased propensity to develop Amyotrophic lateral sclerosis (Vinceti et al., 2010)**.** In the atmosphere, the presence of Se is linked to natural activities, such as soil erosion, volcanism, forest fires, and anthropogenic activities like fossil fuel burning, incineration of garbage, tires and paper (Barceloux, 1999; Wen and Carignan, 2007). Three groups of Se compounds can be distinguished in the atmosphere: volatile organic compounds (dimethylselenide (DMSe), dimethyl diselenide (DMDSe) and methaneselenol), volatile inorganic compounds (Se dioxide), and elemental Se, linked to ashes or particles (Sannac, 2009) (**Figure 1**).

2.1.1 Selenium entry in the life cycle

Selenium assimilation by plants is influenced by the physico-chemical properties of the soil (redox status, pH and microbial activity). But the main critical factors for assimilation/absorption of Se are its concentration (Coughtrey et al., 1983) and its speciation or chemical form in the surrounding soil. Selenate, the most oxidized form of Se, can be found in alkaline soils, where it is soluble and easily available to plants. However, this Se (VI) competes with its sulfur analogue sulfate for uptake by root plasma membrane of plants, because these two elements are metabolized by the same methabolic pathways (Dumont et al.,

2006; Zhu et al., 2009). Selenite can be absorbed less effectively by plants, about ten times less than selenate (Coughtrey et al., 1983).

Se (VI), that is the most important form of Se that enters in the biological cycle, in a number of circumstances can dictate the natural or anthropogenic environmental toxicity of Se, depends on its concencentration in the surrounding soil (Madison, 1860; Dumont et al. 2006; Zhu et al. 2009). In plants, Se can also be found as organic compounds such as SeMet, Se- methylselenocysteine (MeSeCys) or Sec, that are important forms of this element that can be absorbed, bioaccumulated and metabolized by animals in the food chain (**Figure 1**).

2.1.2 Selenium Distribution in Living organisms

According to many studies, sea food, offal meats and Brazil nuts are the richest source of Se (Rayman, 2008; Rayman et al., 2008). In addition cereals, grains, egg, vegetables and dairy products are other important sources of Se (Yamashita and Yamashita, 2010). However, the quantities may vary widely according to the soil content of Se in different continents (Mahalingam et al., 1997).

2.2 Selenium in cell biology

2.2.1. The first evidences of selenium in cell biology

Selenium is an essential trace element in many, but not all, life forms. It was found as an essential micronutrient for some microorganisms around 1950, by Pinsent. In that study, the author found that traces of selenite were essential for the formation of an active formic dehydrogenase by strains of coli-aerogenes group of bacteria grown in a purified synthetic medium, although selenite had no influence on growth (Pinsent, 1954).

In 1957, Schwarz and Foltz demonstrated that Se could substitute vitamin E and prevent hepatic degeneration caused by the vitamin E deficient diet in rats. Indeed, as a pioneering discovery, they demonstrated that factor 3 (a fraction isolated from kidney) blunted the liver degeneration and, most importantly, they definitely demonstrated that pure Se (as selenite (Se(IV)) could substitute factor 3 as protective agent. Schwarz and Foltz did not demonstrate the essentiality of Se in mammals but stablished for the first time that selenite

could replace vitamin E in an aggressive model of liver disease, a finding that suggested Se to be a nutritional requirement for mammals (Schwarz and Foltz, 1957).

However, the molecular role of Se was only identified about 15 years after Schwarz and Foltz's publication**.** In the early 1970's, the first biological activity of Se was identified by independent groups. Two publications demonstrated that glutathione peroxidase (GPx) from blood, an enzyme that decompose hydrogen peroxide using reduced GSH as reducing equivalent donor, was a selenoenzyme. In addition, it was found that Se deficient rats exhibit decreased GPx activity, indicating that this element must be considered an essential component of this enzyme (Rotruck et al., 1973). However, the chemical nature of Se in GPx was unknown in 1973 (Flohe et al., 1973; Rotruck et al., 1973).

Forstrom et al. (1978) demonstrated that Se was present in the active site of rat GPx as selenocysteine, which corroborated previous findings with the selenocysteine-containing enzyme glycine reductase in a prokaryote - *Clostridium sticklandii* (Cone et al., 1976).

2.2.2 Incorporation of Selenium as selenocysteine in selenoproteins

Unlike other inorganic elements that interact with proteins as cofactors, Se is cotranslationally incorporated into the polypeptide chain as part of the amino acid Sec. Selenocysteine is known as the 21st amino acid in the genetic code as it has its own biosynthetic machinery, a tRNA and an elongation factor, and is inserted into newly synthesized polypeptides in response to the Sec codon, UGA, that in most circumstances signals translation termination. But, this codon duality is circumvented by the presence of evolutionary conserved *cis*- and *trans*-acting elements and protein factors dedicated to decoding of UGA as Sec (Copeland, 2003; Discroll and Copeland, 2003; Tujebajeva et al., 2000).

Sec insertion into protein requires the presence of a conserved stem–loop structure, known as the Sec insertion sequence (SECIS) element (Berry et al., 1991). In eukaryotes, this structure is located in the 3′-UTR (Walczak et al., 1996; Berry et al., 1993). Beyond the SECIS element, the selenoprotein synthesis machinery also include other factors such as, Sec-specific elongation factor, Sec-tRNASec, SBP2 (SECIS-binding protein 2), ribosomal protein L30, 43-kDa RNA binding protein, soluble liver antigen protein, and SPS1 thus work in concert to incorporate the Sec into a polypeptide at the site encoded by the UGA codon in mammalian cells (Hatfield et al., 2006; Squires and Berry, 2008).

Sec-containing proteins occur in all major lines of organism (for example, eukaryota, eubacteria and archaea), but not all of them have these proteins. Prokaryotic genomes have been extensively analyzed for the occurrence of selenoprotein genes (Kryukov and Gladyshev, 2004) and among eukaryotes, mainly the genomes of mammals (*Homo sapiens* and *Mus musculus*) (Kryukov et al., 2003), nematodes (*Caenorhabditis elegans* and *Caenorhabditis briggzae*) (Taskov et al., 2005), fruit fly (*Drosophila melanogaster*) (Castellano et al., 2001), green algae (*Chlamydomonas reinhardtii*) (Novoselov et al., 2002) and Plasmodia (Lobanov et al., 2006) have been analyzed with regard to the entire set of selenoproteins (a set of selenoproteins in an organism is known as the selenoproteome). **Table 1** shows the most used experimental organisms (eukaryotes) to the analysis of selenoproteins and the respective number of these proteins found on each of them.

However, no selenoproteins or Sec insertion machinery was detected in sequenced fungal genomes, which is consistent with the idea that selenoprotein genes were lost at this kingdom. Similarly, while large selenoproteomes occur in Chlamydomonas, Volvox and Ostreococcus – green algae genders, higher plants such as *Arabidopsis thaliana* and *Oryza sativa* completely lost both selenoproteins and Sec insertion machinery, instead, they express cysteine-containing homologues (Lobanov et al., 2007).

Recent studies also showed that aquatic organisms generally have larger selenoproteomes than terrestrial organisms, and that mammalian selenoproteomes show a trend toward reduced use of selenoproteins (Lobanov et al., 2007; 2008). Accordingly, the highest number of selenoproteins is found in aquatic organisms, whether they are animals (e.g., fish) or plants (e.g., algae). Some algae are especially rich in selenoproteins (Venturi and Venturi, 2007). One factor for the occurrence of large selenoproteomes in aquatic organisms is bioavailability of Se in oceans. In this environment, Se is highly available and can be easily uptake along the food chain (Cutter, 1989). In this case, an additional factor may be related to lower fluctuating levels of nutrients in the aquatic environments. In contrast, in terrestrial environments, changes on nutrients availability are more frequent. Other important factor to explain the differences between aquatic and terrestrial selenoproteomes may be oxygen content. Higher content of oxygen in air than in aquatic environments may pose highly reactive selenoproteins more susceptible to oxidation in terrestrial organisms and select against the use of these proteins (Lobanov et al., 2007). To reinforce this hypothesis, Zhang and coauthors demonstrated that decreases in oxygen concentration appeared to preserve or even stimulate the use of Sec in bacterias. Indeed, bacterias that have highest number of selenoproteins are anaerobics (Zhang et al 2006). Nevertheless, the hypothesis on the

preferential use of Se in aquatic environments is not clear and, more evidences and studies are nedded. Furthermore, these points of view may exclude large animals (vertebrates/ mammals) because their intra-organismal environment would be less affected by environmental conditions due to their complex morphology.

Finally, to identify the specific factors that might have favored introduction or exchange of selenocysteine in proteins, it may be imperative to look at the chemical differences between Se and sulfur. One of this differences is related to the polarizability of Se (3.8 $Å^3$) when compared to sulfur (2.9 $Å^3$) that can make Sec better nucleophile and better leaving group than cysteine (Cys) (Wada et al., 1999; Huber and Criddle, 1967), and can confers to a given selenol group a stronger redox potential than that of an analog thiol group (Nogueira and Rocha, 2010). In addition, Se has a lower pK_a than sulfur (5.2 for the selenolate versus 8.5 thiolate) and higher catalytic efficiency on Sec-dependent redox active selenoproteins as compared to Cys-dependent non-selenoprotein orthologous (Arnér, 2010).

Other important characteristic is that, even the Se being substantially more sensitive to oxidation than their sulfur counterparts in an environment with high levels of oxygen, the oxidation of selenocysteine does not result in an irreversible inactivation. That can occur due to the ability of Se-oxides to be recycled back to the parent selenol and due the ability of Se to resist inactivation by one-electron oxidation by one-electron oxidants. As a consequence, enzymes that need to be very resistant to oxidation would be expected to prefer selenocysteine to cysteine (Hondal and Ruggles, 2011).

2.3 Selenium in vertebrates

Selenium can occur in vertebrate cells incorporated as SeMet to proteins in place of methionine, but in this case this non- metal element does not exert any important function in cell, since methionine rarely has functional roles in proteins (Tuve and Williams, 1961; Hartmanis and Stadtman, 1982).

Selenium can also be found in cells as Se-binding proteins mainly related to toxification/ detoxification processes (Pumford et al., 1992) and cell growth regulation (Giometti et al., 2000). In addition, it can be found in some low-molecular weight Se compounds, such as methyl selenic acid, methyl-selenocysteine, and SeMet, as efficient antitumorigenic agents in animal (Ganther, 1999; Whanger, 2004). However, considering that Se is an essential trace element in vertebrates, the most important form of Se is found, as Sec, in proteins known as **selenoproteins**.

According to a study of Mariotti and colaborators, among all vertebrates, a set of 45 selenoproteins was identified, with at most 38 represented in a single organism (zebrafish). Fish selenoproteomes are among the largest known. For instance, fish have several selenoproteins (Fep15, SelJ, SelL) that are missing in mammals, as well as several Sec-containing copies of selenoproteins T, U and W, and two forms of SelP (Mariotti et al., 2012; Lobanov et al., 2009).

Twenty seven selenoproteins were found to be unique to vertebrates. Twenty of them were generated through duplication of an existing selenoprotein in some vertebrate lineage, while 6 of them were part of the predicted ancestral selenoproteome, a set of selenoproteins. This implies that these latter 6 proteins (GPx2, thyroid hormone deiodinases (DIO 2 and DIO 3), SelI, SelPb, Fep15) were generated at the root of vertebrates (Mariotti et al, 2012).

Mammalian selenoproteomes consist of 24/25 selenoproteins, from a set of 28, and they have remained relatively stable. When humans, rodents and other mammals were compared, selenoproteomes are very similar. The unique differences are GPx6 that in humans it is a selenoprotein, but the rodent GPx6 replaced Sec with Cys, decreasing the rodent selenoproteome to 24 selenoproteins, when compare to 25 in humans (Kryukov et al., 2003) and platypus retained a Sec-containing form of SelU, which was replaced in other mammals with a Cys homolog.

Mammals/ human selenoproteins have important functions as demonstrated in **Table 2**. However, most of them act as oxidoreductases that prevent damage to cellular components, repair this damage, regulate redox state of proteins or have other redox functions. In addition, they can participate in the biosynthesis of dNTPs for DNA in thyroid hormone metabolism, in immune responses, Se transport and storage and potentially protein folding (Papp et al., 2007). This class of proteins plays a major role in human health and disease (Surai, 2006; Hatfield et al., 2006).

Most selenoproteins belong to one of two main groups according to the location of the Sec residue. Selenoproteins of the first group contain Sec closely to the C-terminus of the protein. Mammalian selenoproteins K, S, O, I, R (methionine-R-sulfoxide reductase, MsrB) and thioredoxin reductases (TrxR) are examples of such proteins. Other proteins, such as mammalian selenoproteins H, M, T, V, W, Sep15, selenophosphate synthetase 2 (SPS2), thyroid hormone deiodinases (DIOs) and GPxs, have Sec in the N-terminal segments of proteins (almost all are thioredoxin fold proteins), often as part of the CxxU motif that corresponds to the CxxC motif (two cysteines separated by two residues) in the active site of thioredoxin (Lobanov et al., 2009).

2.4 Selenoenzymes

The best characterized selenoenzymes families are GPxs, TrxRs and DIOs, that are described below. These selenoenzymes have different enzymatic activities, but all require reductive cofactors which provide the electrons for their catalytic redox cycle.

2.4.1 Glutathione Peroxidase

Glutathione peroxidase was the first mammalian selenoprotein identified. This enzyme is associated with the antioxidant activity of Se (Forstrom et al., 1978), and its enzymatic activity is directly proportional to Se intake. Their catalytic redox cycle involves the oxidation of Sec to selenenic acid by hydrogen peroxide and organic hydroperoxides and reduction to the selenolate anion form by expenses of GSH (Epp et al., 1983). Thus, this selenoenzyme is able to protect cells from oxidative damage, ensuring the continued integrity of cell membranes (Brigelius-Flohe et al., 1994).

There are eight identified forms of GPx which are characterized by similar features. They have different modes and sites of action and different chemical forms. However, only five of them presents Sec in the catalytic active site, that include the following: the cytosolic GPx (cGPx/GPx1), a gastrointestinal-specific enzyme (GI-GPx/GPx2), a secreted protein found in plasma (pGPx/GPx3), an enzyme that acts on oxidized lipids, phospholipid hydroperoxide glutathione peroxidase (PHGPx/GPx4), and a glutathione peroxidase (GPx6) located in olfactory epithelium and embryonic tissues. GPx1–3 are homotetrameric proteins with a subunit molecular mass of 22–25 kDa, whereas GPx4 is a 20 –22- kDa monomeric enzyme.

Glutathione peroxidase- 1 is a ubiquitous **cytosolic enzyme** that is widespread throughout the whole body and is expressed at very high levels in erythrocytes, liver, kidneys and lungs (Flohé, 1989). Its main activity is antioxidant and it can metabolize only hydrogen peroxide and some organic hydroperoxides. It is the first enzyme to be affected in the case of Se deficiency (Fairweather-Tait et al., 2010). GPx1 can also be associated with protection against virus infection (Beck et al., 1998).

Glutathione peroxidase-2 (GPx-2) is an enzyme present in the cytosol localized in the human liver and predominantly in the **gastrointestinal** tissues. Therefore, GPx2 is often referred to as GI-GPx. Its distribution varies in the intestine, it is higher in the crypts and

lower in the luminal surface. It protects against oxidative damages and presents the structure and substrate specificity similar to that of GPx1 (Chu et al., 1993).

Glutathione peroxidase-3 (pGPx) is a glycosylated protein secreted to extracellular compartments. It uses a wide range of substrates including H_2O_2, fatty acid hydroperoxides, and phospholipid hydroperoxides and is an efficient antioxidant in the **plasma** (Brigelius-Flohe, 1999). GPx3 is the second most abundant selenoprotein in the plasma after selenoprotein P and represents 10 to 30% of Se found in plasma. In addition, it is also found in the liver, kidneys, heart, lungs, thyroid, gastrointestinal tract and breasts, and also in the placenta and the male reproductive system (Schwaab et al., 1998).

Glutathione peroxidase-4 (GPx-4) (**Phospholipid hydroperoxide GPx**) is widely spread in the human body. Unlike other GPxs, GPx4/PHGPx can reduce phospholipid- and cholesterol-hydroperoxides directly, by using electrons from protein thiols as well as from glutathione in mammalian cells (Imai and Nakagawa, 2003). GPx4 is present in cytosolic, mitochondrial, and nuclear isoforms with differential tissue distribution. Besides its antioxidant activity, it protects biomembranes from peroxidative degradation (an important role is suggested in the brain) (Papp et al., 2010).

Glutathione peroxidase-6 is a selenoprotein found only in humans and they expression is shown only in olfactory epithelium and embryonic tissues (Kryukov et al., 2003) it is a homologue of GPx-3 and its role remains unknown.

2.4.2 Thioredoxin Reductase

Thioredoxin Reductases (TrxR) are members of the pyridine nucleotide-disulfide oxidoreductase family. TrxR is part of the thioreoxin system, together with thioredoxin (Trx), and NADPH, and it is considered a major cellular redox system present in all living organisms (Arner and Holmgren, 2000). This selenoenzymes has ≥115 kDa and are highly homologous to glutathinone reductase, considering that the N-terminal active sites of them are identical, and use a CxxxxC motif in electron transfer to a C-terminal -Gly-Cys-Sec-Gly- active site without conformational changes (Zhong et al., 2000; Zhong and Holmgren, 2000).

Mammalian TrxRs are housekeeping yet inducible proteins with roles in many cellular processes. They act both by controlling the function of the central redox molecule thioredoxin, and by directly reducing numerous substrates. TrxRs contain an FAD domain, an NADPH-binding domain, an interphase domain, and a penultimate Sec residue in a 16-residue C-terminal extension, which is indispensable for their enzymatic activity (Luthman and

Holmgren, 1982, Zhong et al 2000). The proposed mechanism of mammalian TrxR-dependent reduction involves electron transfer from NADPH to FAD via the N-terminal active site of one subunit to the Cys–Sec selenenylsulfide bond within the C-terminal active site of the opposite subunit and finally to the substrate Trx (Zhong et al., 2000; Zhong and Holmgren, 2000).

Three mammalian TrxR selenoenzymes have been identified: the cytosolic enzyme (TrxR1), the mitochondrial enzyme (TrxR2), and a testis-specific enzyme thioredoxin-glutathione reductase (TGR/TrxR3) (the latter also possessing glutathione and glutaredoxin reductase activity).

Thioredoxin reductase 1 is a ubiquitous **cytosolic** homodimeric oxidoreductase containing 1 Sec and 1 FAD per 55 kDa subunit, with Sec as the penultimate amino acid (Gladyshev et al., 2004). This enzyme is involved in many aspects of cellular redox regulation (Soderberg et al., 2000**)** and DNA synthesis (Spyrou and Holmgren, 1996). In addition, it is capable to induce apoptosis if the Se of the enzyme was compromised or if this residue is blocked e. g. by a chemotherapeutic agent (Anestal and Arnér, 2003).

Thioredoxin reductase 2 located in **mitochondria**, is a ubiquitous homodimeric pyridine nucleotide disulfide oxidoreductase, with Sec as terminal amino acid. Highest levels are observed in prostate, testes, liver, uterus, and small intestine, with intermediate levels in brain, skeletal muscle, heart, and spleen (Miranda – Vizuete et al., 2000).

Thioredoxin glutathione reductase can reduce glutathione disulfide since it contains an N-terminal 1-Cys glutaredoxin-like domain, differentiating it from TrxR1 and TrxR2, but its specific physiological functions remain unknown at present. It is **testes-specific**, where it is localized in the ER (Sun et al., 2001).

TrxR is the only enzyme able to reduce oxidized Trx. Trx provides electrons to ribonucleotide reductase, which is essential for DNA synthesis by converting ribonucleotide to deoxyribonucleotides. Trx can also reduce methionine-sulfoxide reductase and thioredoxin peroxidase (peroxiredoxin) and thus is involved in the repair of methionine sulfoxide-oxidized proteins or redox signaling via hydrogen peroxide (Holmgren, 1989; Arnér and Holmgren, 2000). In addition, Trx system participates in many cellular signaling pathways by controlling the activity of transcription factors containing critical cysteines in their DNA-binding domains, such as NF-κB, AP-1, p53, and the glucocorticoid receptor (Lillig and Holmgren, 2007).

Since TrxR is essential for cell survival, it is an important target of many pharmaceutical drugs, such as anticancer and antirheumatic agents (Ralston and Raymond, 2010).

2.4.3 Iodothyronine Deiodinases

Iodothyronine hormones regulate many metabolic functions in vertebrates relating to growth, development, thermogenesis and regulation of basal metabolic rate. Three iodothyronine deiodinases (DIO) that differ in sequence, structure and also catalyze different reactions are responsible for activation and inactivation of these molecules. All three are selenoenzymes (DIO1, DIO2, DIO3), and they were the second type of selenoproteins to be characterized (Köhrle, 2000).

Deiodinase type I (DIO 1) was the first DIO identified, in 1990 (Behne et al., 1990). It is a homodimeric plasma membrane protein found primarily in the liver, kidneys, thyroid and brown fat that plays a role in thyroid hormone metabolism. It converts inactive L-thyroxine (T4) - the major form secreted by the thyroid - into active 3,3'-5'triiodothyronine (T3)- the major thyroid hormone in peripheral circulation - by catalyzing the removal of iodine from T4 (Behne et al., 1990).

Deiodinase type II is an ER- membrane protein abundant in the central nervous system, thyroid, in the brown adipose tissue and in the skeletal muscles. It provides an important regulatory function in the brain and central nervous system. The deiodinase type II also has a role in the activation of thyroid hormones.

DIO1 and DIO2 can also convert reverse T3 into 3,3’-diidothyronine (Kuiper et al., 2005).

Deiodinase III is localized in plasma membrane and has an activity in fetal and in the deactivation of thyroid hormones (converts thyroid hormone T3 to inactive rT3 by catalyzing the removal of iodine from T3). In addition this enzyme can also converts T4 into reverse T3 and also T3 into 3,3’-diidothyronine (Kiuper et al 2005). It is present in the placenta, uterus, fetus and central nervous system (Salvatore et al., 1995).

2.4.4 Other selenoenzymes

Selenophosphate synthetase 2 (SPS2) catalyzes the activation of selenide with adenosine 5'-triphosphate (ATP) to generate selenophosphate, the essential reactive Se donor for the formation of selenocysteine (Sec) and synthesizes the $SePO_4^{2-}$ precursor required for *de novo* Sec synthesis of all selenoproteins, including itself (Guimarães et al., 1996).

Methionine R-sulfoxide reductase (MsrB1, also known as SelR or SelX) employs one Sec and one Zn^{2+} ion bound to four Cys residues per 12-kDa molecule. MsrB1 is predominantly localized in the nucleus and the cytoplasm of cells (Kim and Gladyshev, 2004). It has the highest specific activity among three principal types of Met-R-sulfoxide reductases, all of which employ thioredoxin as the reductive cofactor. They contribute to antioxidant defenses by reducing oxidized methionine residues (methionine sulfoxide) in proteins back to methionine (Zhao et al., 2012). It also has role in counteracting aging and neurological disorders, and its expression is stimulated by oxidative stress (Ralston and Raymond, 2010).

3. Selenium compounds

3.1 The importance of selenium compounds to health

Selenium is essential for life, and undoubtedly, adequate amounts of this element are required for cell function. As a micronutrient, small quantities of dietary Se are necessary to maintain good health in several species (50-70 µg/day).

One major mechanism by which Se exerts its physiological roles can be attributed to its redox characteristics. The antioxidant mechanisms include radical scavenging, metal-binding activities and in the form of antioxidant selenoproteins, it constitutes the major Se containing antioxidant machinery in cells (Kieliszek et al., 2013). These mechanisms are generally associated with disease prevention by Se supplementation, due to the relationship between oxidative stress and disease (Weekley and Harris, 2013).

The essentiality of Se is further highlighted by the high incidence of Keshan disease, an endemic cardiomyopathy and well documented example of extreme Se deficiency in the diet, a condition which was first observed in Se-deficient areas in Keshan County, Heilongjiang Province, China in 1935. Clinical features of Keshan disease include acute or

chronic episodes of a heart disorder characterized by cardiogenic shock and congestive heart failure (Li et al., 2013; Allander, 1994).

Furthermore, some studies have suggested that supplementation of Se could reduce the risks associated with cardiovascular diseases, particularly on cardiac muscle integrity (Ricetti et al., 1999; Anderson, 1982). Low levels of plasma this element are associated with the increased risk of cardiovascular disease mortality (Neve, 1996). In addition, this essential trace element takes part in the biosynthesis of arachidonic acid derivatives involved in platelet and leucocyte functions, in the regulation of cholesterol, and also in the direct protection of endothelial and vascular smooth muscle cells and monocytes against the accumulation of undesired reactive oxygen species and consequently oxidative stress leading to cardiovascular disease and formation of atherosclerotic lesions (Neve, 1996; Salonen et al., 1982).

In Germany, authors found that patients with suspected coronary artery disease present low GPx1 activity in erythrocytes that were associated with an increased risk of cardiovascular events (Blankenberg et al., 2003). However, the studies about the protection of Se in cardiac pathologies are yet controversy, since some epidemiological studies suggest no correlation between the plasma Se levels and cardiovascular diseases (Strages et al., 2006) and some other clinical studies strongly support the beneficial effects of Se on cardiovascular functioning (Derneneve et al., 2012; Turan, 2010).

Considering that brain is particularly susceptible to oxidative stress due to its high oxygen consumption and presence of redox active metals (Valko et al., 2007), Se, as an antioxidant, can offer benefits to neurological system. In fact, studies have demonstrated evidences on the importance of Se to brain (Rayman, 2000). For instance, (i) Se depletion induces increased neuronal cells death in animal models of neurodegenerative diseases, (ii) genetic inactivation of cellular GPx increases the sensitivity to neurotoxins and cerebral ischaemia, while increased GPx activity as a result of increased Se supply or overexpression present beneficial outcomes; (iii) genetic inactivation of selenoprotein- P leads to a marked reduction in brain Se content with a parallel induction of movement disorder and spontaneous seizures in animal models (Schweizer et al., 2004).

Accordingly, evidence from clinical studies revealed that Se deficiency leads to cognitive impairment, seizures, Parkinson's and Alzheimer's disease, with important evidences for a role oxidative stress in these conditions (Zhang et al., 2010). Low Se levels also deactivate certain centers in the brain leading to changes in the mood (Benton, 2002; Sher, 2002) with incidence of depression and other negative mood states such as anxiety, confusion, and hostility (Rayman, 2000).

The immune system is also influenced by body levels of Se. Considering that immune cells regulating organs such as spleen and liver contain abundance of this nonmetal, it shows the importance of the Se in immune function/modulation (Hawkes et al, 2001). For example, as a constituent of selenoproteins, Se is needed for the proper functioning of neutrophils, macrophages, NK cells, T lymphocytes and some other immune mechanisms. In animal models, Se deficiency impairs the ability of phagocytic neutrophils and macrophages to destroy antigens. Selenium appears to be a key nutrient in counteracting the development of virulence and inhibiting HIV progression to AIDS (Ferencík et al., 2003). In HIV-infected patients, Se deficiency has been significantly correlated with total lymphocyte counts (Look et al., 1997). Thus, maintaining an optimal Se status in HIV-1 infected patients may help to increase the enzymatic defense and improve general health in those patients (Delmas-Beauvieux et al. 1996; Cirelli et al 1991).

Low Se levels can also lead to various thyroid and gastrointestinal problems. Adequate levels of this element is associated with a high activity of GPx3 and TrxR that protects thyroid cells from the hydrogen peroxide generated in the cells to be used by the thyroid peroxidase in the synthesis of thyroid hormone. In addition, this element is also required by selenoenzyme iodothyronine deiodinase for the production of active thyroid hormone (Schomburg and Köhrle, 2008). On the other hand, deficiency of Se in the diet leads to the lack of absorption of various other trace elements in the duodenum. Gastrointestinal GPx2 protects from the toxicity of ingested lipid hydroperoxides in mammals (Brigelius-Flohé and Maiorino, 2013).

Davis and coworkers showed that different selenoproteins are involved in cancer prevention, and that Se deficiency can be a cancer promoter factor. In addition, the authors demonstrated that Se had a protective effect against lung cancer in populations with low Se status and that the risk of cancer was 2–6 times lower in high Se serum levels compared to low levels (<100 ng/mL), or low Se intake (<55 μg/day) (Davis et al 2012). On the other hand, few studies reported that there is no significant effect on Se levels in plasma on the high risk cancer populations (Algotar et al., 2013). And also, there is no recommendation of Se supplementation for cancer prevention in healthy populations (Cortes-Jofre et al., 2012).

In fertility, Se can also represent an important helper. In men, this nonmetal is essential for sperm motility in the form of GPx4 and for Se supply to the testes from Selenoprotein P (Mesenguer et al., 2007; Shalini and Bansal, 2005). Se deficiency is likely to affect male fertility, particularly in the synthesis of testosterone and sperm (Rayman, 2000). Moreover, many studies have highlighted the involvement of Se in human and animal reproduction (reproductive parameters and performance). Indeed, many cases of infertility

were recorded in Se deficient areas. It also plays a specific role during embryo implantation. Selenium deficiencies have also been involved in metritis, retained placenta, miscarriage and preeclampsia. Some diseases during the gestation can be developed by low levels of Se ingestion, as obstetric cholestasis, and gestational diabetes mellitus (Mistry et al., 2012).

Despite the well-known deleterious effects of Se deficiency, high intake of organic/inorganic Se is also detrimental to health maintenance. High levels of this element can cause selenosis, resulting in hair loss, nail loss, brittleness, lesions in the skin and nervous system, fatigue and irritability (Nuttal, 2006; Navarro- Alarcon and Cabera-Vique, 2008). Acute Se toxicity causes severe neuronal lesions, gastrointestinal symptoms, respiratory symptoms, kidney failure, myocardial infarction and other cardiac disorders (Navarro-Alarcon and Cabera-Vique, 2008).

3.1.1 Inorganic forms of selenium

Sodium selenite (Na_2SeO_3) and selenate, as demonstrated below, are the most studied inorganic forms of Se and supplementation with these compounds are shown to improve health. Laboratory studies point to benefits of mainly Na_2SeO_3 supplementation in several disease models.

For instance, Schnabel and coauthors showed that Na_2SeO_3 supplementation increased the antioxidant capacity through GPx-1 activity in endothelial cells and in coronary artery disease patients (Schnabel et al, 2008). In another study, researchers showed that low doses of selenite (5–40 nM) increased both TrxR and GPx activities and protected human coronary artery and human umbilical vein endothelial cells from peroxide toxicity, but TrxR appeared to be most important in exerting the antioxidant effects (Miller et al., 2001). In rats supplemented with 1 ppm Se per day with the same compound for 5 weeks there was a modest increase in TrxR and GPx levels, with a corresponding modest improvement in cardiac function post ischaemia-reperfusion (Velardos et al., 2004).

Sodium selenite supplementation (200 µg/day) was also effective against auto immune thyroid disorders like Hashimoto's thyroiditis (HT) (Toulis et al., 2010). Then, according to this study, routine Se supplementation can be recommended in the treatment of HT, demonstrating an improvement in thyroid function and morphology.

Related to immune system, Roy and coauthors revealed that supplementation of Na_2SeO_3 (200 mg) enhanced T cell response (Roy et al., 1994, 1995). Sodium selenite

supplementation (200 µg/day) also lead to significant enhancement of cell mediated immune response in cancer patients who have undergone radiotherapy (Kiremidjian et al., 2000). Broome and collaborators also reported antiviral activities of Se using a supplementation of Na_2SeO_3 (100 µg/day) that cleared the poliovirus more rapidly than placebo (Broome et al., 2004).

In a diabetes model in rats, Se supplementation (1 µg Na_2SeO_3/kg body weight) reduced the expression of NF-kB, which is responsible for the expression of inflammatory proteins such as interleukins and tumor necrosis factor (Pillai et al., 2012). This fact can be related with the mechanism of Se in neutralizing the oxidative stress, one important cause of diabetes (Fatmi et al., 2013).

Kumar and coauthors reported the importance of Na_2SeO_3 against *Helicobacter pylori*, a gastric pathogen causing gastric ulcer. This *in vitro* and animal model studies revealed that inorganic Se compound showed the anti *H. pylori* activity. During *in vitro* condition, Na_2SeO_3 showed significant bactericidal effects however *in vivo* only low doses were not toxic to the animals (Kumar et al., 2010).

In Alzheimer's disease (AD) models, sodium selenate mitigates tau pathology, making it a promising lead compound for tau-targeted treatments of AD and related dementias (van Eersel et al., 2010). In addition sodium selenate was also able to specifically activates PP2A phosphatase, dephosphorylates tau and reverses memory deficits and neurodegeneration (Corcoran et al., 2010).

3.1.2 Organoselenium compounds

Vegetables are important sources of organoselenium compounds. Selenium in plants can be found mainly as SeMet, MeSeCys, Sec and selenoneine. Previous studies have shown that supplementation with organic forms of Se or use of Se enriched foods can improve health and disease (Rayman et al., 2008).

For instance, a study of Lovell and collaborators using a diet supplemented with selenised yeast (1 ppm Se in feed) decreaseD the amyloid burden and oxidative damage in a mouse model of Alzheimer's disease, supporting a role for SeMet as a potential therapeutic agent in neurologic disorders with increased oxidative stress (Lovell et al., 2009). Using a similar model of selenized yeast, Wood and coauthors found an increase in T cells count. Previous studies also reported the correlation of plasma SeMet levels with the respiratory distress syndrome (Wood et al., 2000).

Considering that, many studies are available on the Se deficiency and infertility in men, Scott and coauthors showed that supplementation with selenomethionine (100 µg/day) in men having low initial Se status and presenting low motility levels of sperm, caused improvement in the motility after 3 months (Scott et al., 1998).

Organoselenium compounds such as MeSeCys were demonstrated to be capable of binding copper and iron and preventing oxidative DNA damage induced by these metals (Battin et al., 2011).

3.1.3 Synthetic organoselenium compounds

Considering that physiological chemistry of Se in mammalian cells relies on the presence of selenocysteine, an organoselenium compound, in selenoproteins, and that GPx, an important selenoprotein, acts in the antioxidant process catalyzing the reduction of a wide variety of hydoperoxides, protecting against oxidative stress, efforts have been made to find organoselenium compounds capable of imitating the enzymatic properties of GPx.

In this way, several simple synthetic organoselenium compounds with glutathione peroxidase-like activity have been prepared and used in different models of diseases due its antioxidant activity that greatly rely in the formation of selenol group after interaction with reducing thiols**.** Basically these compounds interact with thiol-thiolates in the cell forming the selenol group that will than, catalyzed the peroxide degradation in a GPx- like manner (Figure 3) (Nogueira et al., 2004).

Ebselen was the first organoselenium compound presenting GPx- like activity described in the literature (Wendel et al., 1984). Later, other GPx mimetic compounds were also investigated, including diphenyl diselenide $(PhSe)_2$ and anologues, with a higher catalytic activity than ebselen (Nogueira et al., 2004).

In the last decades, the antioxidant activity of ebselen and other organoselenium compounds have been reported in experimental models, and a number of studies showed that these compounds can efficiently scavenge and eliminate reactive oxygen species. The foremost antioxidant abilities of such compounds relies on the protection against lipid peroxidation, radical-scavenging activity (Mishra et al., 2006; Prigol et al., 2009b), peroxynitrite scavenging activity (Sies and Masumoto, 1997; Woznichak et al., 2000) and also act as substrates of mammalian thioredoxine reductase enzyme (Freitas et al., 2010). In addition this seleno compounds were also able to induce the TrxR synthesis, by increasing its expression, as observerd by Zhang and coauthors (2013).

Moreover, synthetic organoselenium compound can act as detoxifying agents against xenobiotic compounds that exhibits toxicity through various mechanisms. For instance, xenobiotics that induce ROS production can be attenuated by ebselen antioxidative properties. This selenocompound is highly effective in reducing iron, cadmium and MeHg-induced cytotoxicity (Davis and Bartffay, 2004; Ardais et al., 2008; Yin et al., 2011). In the same context, $(PhSe)_2$ reduces the pro-oxidant effect of cadmium and methylmerucry (MeHg) (Santos et al., 2005; Roos et al. 2009).

Therefore, synthetic organoselenium compounds could represent novel therapeutic strategies against oxidative stress and inflammation related diseases (Arteel and Sies 2001, Wang et al., 2004; Gill et al., 2010). In fact, ebselen and other organoselenium compounds have been shown to be efficient anti-inflammatory agents (Parnham and Kindt 1984; Cotgreave et al. 1989; Nogueira et al., 2003).

Antinociceptive activity is also among the repertoire of biological actions delivered by organoselenium compounds. Diphenyl diselenide is shown as an effective antinociceptive compound on the tail-flick, formalin, acetic acid-induced abdominal writhing, and capsaicin animal models (Nogueira et al. 2003; Savegnago et al. 2008). Indeed, its activity in these models is more effective than ebselen. In addition, *p*-methoxyldiphenyl diselenide, an anolgue of $(PhSe)_2$ was also able to act as antinociptive drug (Nogueira and Rocha, 2010).

Depression and anxiety mood disorders are shown to be linked to low Se levels and increasing dietary Se intake is shown to improve these factors (Sher, 2007; Rayman, 2000; Benton, 2002). The antidepressant-like effect of ebselen was evidenced in the forced swimming test, a predictive model for antidepressant drugs (Posser et al., 2009). Such an effect was demonstrated to involve noradrenergic and dopaminergic neurotransmitter systems (Posser et al., 2009). By using a similar model, Savegnago et al (2007) pointed the serotonergic system as a main mediator for the antidepressant-like effect of $(PhSe)_2$. Diphenyl diselenide and its anologue *m*-trifluoromethyl- diphenyl diselenide have also been proposed to have anxiolytic action in different animal models (Ghisleni et al. 2008; Brünig et al., 2009).

Synthetic organoselenium compounds have also been investigated for its protective effects as antioxidants. In this regard, ebselen and $(PhSe)_2$ act as a hepatoprotetective agent against toxicants as Paracetamol (Li et al., 1994; Wilhelm et al., 2009a). The compounds *p*-methoxyldiphenyl diselenide and m-trifluoromethyl-diphenyl diselenide also showed hepatoprotective effects on acute liver injure by lipopolysaccharide/D glucosamine exposure and 2-nitropropane respectively (Wilhelm et al., 2009b;c). Ebselen and $(PhSe)_2$ have

demonstrated yet, renoprotective activity against acute renal failure injury (Noiri et al., 2001; Brandão et al., 2009).

In addition, considering that organoselenium compounds do not induce irritability of the gastric mucosa, in contrast to non-sterioidal antiinflamatory drugs (NSAIDS), organochalcogenides are suggested as gastroprotective drug candidates, as demonstrated in studies with ebselen that prevents ulceration induced by ethanol in mice (Tabuchi et al., 1995) and diclofenac in rats (Leyck and Parnham, 1990). Diphenyl diselenide showed similar effects in the ethanol and endomethacin models of ulceration in rats (Savegnago et al., 2006).

As depicted before, extreme deficiencies of Se in the diet can lead to Keshan and Kashin-Beck diseases (Navarro-Alarcon and López-Martínez, 2000). Then, some synthetic organoselenium compounds have been studied in different models of cardiovascular damage. Ebselen was effective against daunorubicin-induced cardiomyopathy in rats. In fact, ebselen can normalize serum cardiac enzymes creatine kinase, lactate dehydrogenase, and GPx (Saad et al., 2006). Diphenyl diselenide inhibited lipid peroxidation and prevented the oxidation of protein moieties in human isolated LDL *in vitro* (de Bem et al. 2008) and rabbits supplementation with 10 ppm of this compound reduced total cholesterol levels (de Bem et al., 2009). In addition, the hypolipidemic action of $(PhSe)_2$ was characterized besides the reduction in the total cholesterol also, non-high-density lipoprotein cholesterol, and triglycerides levels (da Rocha et al., 2009).

Synthetic organoselenium compounds, have also been investigated as potential neuroprotective drugs. Ebselen was used in clinical trials for the treatment of neuropathological conditions associated with oxidative stress (Saito et al. 1998). Ebselen has been proved to protect against brain damage from different models of permanent and transient focal ischemia (Johshita et al. 1990; Dawson et al. 1995), and hypoxia/ischemia-induced neuronal damage (Knollema et al. 1996). This compound demonstrated significant neuroprotection *in vitro*, in ischemic model where protected the brain against oxygen and glucose deprivation (Porciúncula et al., 2003) and attenuated, in concentration dependent manner, the degradation and depletion of glutathione in astrocytes (Gabryel and Malecki, 2006).

Diphenyl diselenide has been reported as a neuroprotector agent in a classical model of *in vitro* ischemia (Ghisleni et al. 2003), that can occur by the inhibition of excessive nitric oxide (NO) production by inducible nitric oxide synthase (iNOS) after brain ischemia in vitro. This compound was also able to attenuate the dyskinesia induced by haloperidol (Burger et al., 2005) and fluphenazine (Fachinetto et al., 2007). This compound has been demonstrated

yet, as an inductor of facilitation of long-term object recognition memory in rats (Rosa et al., 2003).

Diphenyl diselenide anologues have also been proposed as neuroprotective agents. Pinton and co-workers showed that *p*-methoxyldiphenyl diselenide was able to reverse the learning and memory impairments induced by intracerebroventricular injection of streptozotocin, a model of sporadic dementia of Alzheimer's type, in mice (Pinton et al., 2010). Another analogue known as *m*-trifluoromethyldiphenyl diselenide attenuated behavioral features associated with a mouse model of psychosis (Machado et al., 2006), it also present an anticonvulsivant effect pentylenetetrazole-induced seizures in mice (Prigol et al., 2009a).

3.1.4 Selenium compounds and cancer

Many studies in relation to anticarcinogenic effects of Se compounds have been carried out by several research groups.

For instance, Na_2SeO_3, due to its pro-oxidant characteristic, represents a promising alternative for cancer therapy (Brozmanová et al., 2010). Sodium selenite has been shown to be the most effective in *in vitro* and *in vivo* studies of carcinogenesis. As recently demonstrated, selenite possesses oxidizing properties in the presence of specific substrates. Selenite by virtue of oxidizing cell membrane thiols, can prevent the formation of the coat and consequently makes cancer cells vulnerable to the immune control and destruction. It is, therefore, postulated that Na_2SeO_3, in view of its relative low toxicity, and might become a drug of choice for many types of cancer including leukemia (Lipinski, 2005).

Accordingly, Combs demonstrated that in animal models, supplementation of inorganic Se in the diet protects against cancer induced by a variety of chemical carcinogens (Combs, 1997). In addition, in a study of Davis and coauthors, an enriched selenite salt supplementation in a community of 21,000 persons in China reduced liver cancer by 35% (Davis et al., 2012).

Selenium compounds like selenite and selenate have strong inhibitory effects particularly on mammalian tumor cell growth (Spyrou et al., 1996). In this context, the selenoenzyme, TrxR can represent an important trigger to anticancer activity of inorganic Se compounds, since TrxR1 represents a central enzyme for cell growth tumor, differentiation, and the protection against oxidative stress. TrxR1, which in several studies has been shown to be up-regulated in various tumor cells, is also a target for many anticancer drugs. In a study of

Selenius and colaborators, inhibition of TrxR resulted in enhanced selenite cytotoxicity, clearly connecting the thioredoxin system to the toxic effects mediated by selenite, inhibiting the cell tumor growth (Selenius et al., 2008).

Effects of organoselenium compounds (SeMet and MeSeCys) are also tested in several studies of prevention or treatment to cancer. Such as, a meta-analysis of various studies published on the effect of Se on gastrointestinal cancers showed that Se supplementation (SeMet) was associated with nearly 60% reduction in gastrointestinal cancers (Ma et al., 2012; Jayaprakash and Marshall, 2011; Ibiebele et al., 2013).

In accordance, the Nutritional Prevention of Cancer (NPC) trial carried out with selenised yeast supplements (200 µg per day) containing SeMet (18- 69%) but also other forms of Se (selenite, g-glutamyl-methylselenocysteine and Se-adenosylhomocysteine), showed significant reduction in total cancer mortality, total cancer incidence and the incidence of prostate, lung and colorectal cancers without any effect in the primary endpoint, nonmelanoma skin cancer (Clark et al., 1996). The chemopreventive mechanism of action of these compounds is not known, but as demonstrated in the literature it may be mediated through the Se dependent redox systems in the cells or through the T cell action (Cheng et al., 2012). T cells are the major components on cancer removal, and the synthesis and activation of which are Se dependent (Clarlson et al., 2010; Shrimali et al., 2008).

However, a Se and Vitamin E cancer prevention trial (SELECT) that was designed to study the effect of SeMet (the main form of Se found in food - 200µg) alone or in combination with vitamin E, did not presented enthusiastic results and in contrast, an increase in type 2 diabetes was observed together with a significant increase on the risk of prostate cancer (Lippman et al., 2005). These contrasting results evidenced the importance of considering the speciation of Se supplement and the Se status of population.

Futhermore, studies have demonstrated that MeSeCys can be more effective than other natural organic form of Se as an anticarcinogenic compound, which can be found some members of the *Brassica* and *Allium* families, as well as in Se-accumulating *Astragalus* species (Whanger., 2002; Ganther, 1992).

Accordingly, numerous studies have demonstrated the anticarcinogenic activities of some organic forms of Se against certain types of cancer (Whanger., 2002; Clark et al., 1996; Raid et al., 2002). In a long-term double-blind study, supplemental Se (MeSeCys) was associated with significant reductions in lung, colorectal and prostate cancers (Ganther, 1992). In addition, Se-enriched broccoli is shown to be protective against intestinal cancer

susceptibility in multiple intestinal neoplasia (MIN) mice and in chemically induced mammary and colon cancer in rats (Davis et al., 2002).

In parallel to a number of pharmacological properties reported by synthetic organoselenium compounds, ebselen has been also studied as a chemopreventive compound. In this context, ebselen inhibits the cell growth in human breast and colon cancer cells (Engman et al. 1997), and induces apoptosis in the human hepatoma cell line HepG2 (Yang et al. 2000a). The apoptotic effect of ebselen involves its ability to deplete thiols and to alter the mitochondrial permeability transition (Yang et al. 2000b). This compound has showed to be also an effective inhibitor of TrxR, one important mechanisms to decrease the growth of tumor cells. (Ganther and Ip, 2001).

Futhermore, diphenyl diselenide presents a protective effect against the tumor development, even when supplemented at a relatively low concentration (1 ppm), in a *N*-nitroso-*N*-methylurea (NMU)-induced mammary carcinogenesis model in rats (Barbosa et al. 2008), demonstrating potential effect as chemotherapeutic drug.

4. Mercury

The element Hg is a metal classified in the periodic table with the atomic number (80) and atomic mass 200.59 Da. Mercury can exist in three oxidation states: elemental mercury (Hg^0), mercurous ion (Hg_2^{2+}) and the mercuric ion (Hg^{2+}) – and can be found also in organic form as MeHg and dimethylmercury (Eisler, 2006). The elemental Hg is liquid at room temperature, but form amalgams in contact with metals such as gold and silver, and then it is used in the extraction process of these precious metals. At high temperatures it passes to gaseous phase, originating Hg vapor which is highly toxic. Both mercurous and mercuric ions can form chlorine salts, such as mercurous choride (Hg_2Cl_2) and corrosive sublimate mercuric chloride ($HgCl_2$) (Eisler, 2006).

4.1 Mercury in the environment

Mercury is considered a global pollutant. In the atmosphere, the major form of Hg is found as Hg vapor, which can be released from the earth's crust and volcanic eruptions (Mason, 2009). Atmospheric Hg^0 can be oxidized to Hg^{2+} under the action of ozone or halogens. Mercury (II) may be adsorbed to solid atmospheric particles and deposited on the

surface (dry deposition), or it can also be dissolved by atmospheric moisture and then deposited on the surface by rain (Ariya et al., 2009).

In water, Hg^{2+} can either be abiotically and biotically reduced again to Hg^0 and re-emitted to the atmosphere or adsorbed onto suspended particles and organic matter and then be deposited on the bottom sediments, or transformed by microbiological action, mainly by sulphate-reducing bacteria, into alkylated forms of Hg (Morel et al., 1998). The main alkylated forms of Hg resulting from microbial action are monomethylmercury (MeHg) and dimethylmercury (CH_3HgCH_3 or DMeHg) (Barkay and Wagner-Döbler, 2005).

Once methylated, MeHg becomes the main form of Hg in aquatic sediments, being available to be absorbed by phytoplankton and/or the phytobenthos, and subsequently, it becomes available to superior trophic levels, a phenomenon that results in Hg biomagnification along food chain (Morel et al., 1998). Moreover, the elimination of MeHg by organisms (e.g. fishes) is a rather slow process and the exposure level is more or less constant, leading to accumulation in tissues throughout the life-span of the fish (bioaccumulation), increasing this accumulation until the top of food chain (Jackson, 1998). Since humans stand at the very top of the food pyramid, populations relying on a diet containing high amounts of predatory marine or freshwater fishes are, consequently, exposed to high levels of MeHg (Carvalho et al., 2008).

Mercury and its compounds have been used in anthropogenic activities since ancient times by different civilizations (Goldwater, 1972). The Chineses (100 a.c.) utilized Hg in the form of cinnabar, as the pigment in red ink and by alchemists. The Europeans used therapeutic applications of Hg for the treatment of syphilis, which showed also Hg's toxic nature resulting in a large number of deaths (Clarkson and Magos, 2006). Moreover, although its use for the treatment of this disease declined during the 19th and early 20th centuries, Hg remained as a common treatment for a vast myriad of other pathologies (Clarke, 1912; Kollock, 1896). From 19th century, Hg begins to be utilizes in amalgams in dentistry, the main exposure route of the general population to Hg vapor (Clarkson, 2002).

Nowadays, the use of Hg in medicine is mainly related to the anti-septic properties of Hg compounds, such as phenylmercury and ethylmercury thiosalacylate (Thimerosal), that make them useful in preserving medical solutions and vaccines (Magos, 1997; Clarkson and Magos, 2006). Mercury is also used in the industry, as electronic components, as component of energy-efficient compact fluorescent lamps and in thermometers production (Clarkson and Magos, 2006). In agriculture this metal is used in insecticides and antifungals. Its use in mining represent an important problem of public health, and a good example is the a Amazon

region in Brazil, where the use of Hg compounds to gold extraction leads the workers to contamination (Malm et al, 1998; Myers et al., 2007).

At least two catastrophic episodes of environmental contamination with Hg occurred around the world. For instance, the incidents in Minamata, Japan in the 1950' decade and in 1970' in Iraq are the major environmental disasters reported involving Hg, leading to an unprecedented contamination of local populations causing severe neurological conditions (Harada, 1995; Johansson et al., 2007).

In the last decades, the human exposure still occurs, but in a less scale. Important avenues of human contamination are agriculture, industrial and mining activities, but the main source of Hg exposure to humans is suggested to be the consumption of MeHg-contaminated fish (Clarkson, 2002, Clarkson e Magos, 2006).

4.2 Human exposure to mercury

4.2.1 Elemental and Inorganic Mercury (Occupational exposition)

Elemental Hg is found as a liquid metallic that itself is not very toxic, since upon ingestion gastrointestinal absorption is extremely low (WHO, 2003; Rooney, 2007). Toxicity of Hg^0 results primarily from the inhalation of Hg vapor by workers in industries where Hg enters the manufacturing process or by general population exposure is the release of Hg^0 from dental amalgam surfaces (Clarkson, 2002; Rooney, 2007).

Inhaled air is the main route by which Hg^0 in the form of vapor enters the body (Clarkson et al., 2007). In the lungs, approximately 70% to 85% of Hg vapor diffuses from the alveoli into the blood stream (Magos, 1997). Considering that Hg vapor is an uncharged monoatomic gas, lipid soluble and highly diffusible, it can diffuse through cell membranes by passive diffusion and gain access to cell interior in all organs (WHO, 2003; Clarkson and Magos, 2006). Mercury vapor can easily cross the blood-brain barrier (BBB), reaching the central nervous system (CNS) and the placental barrier (Lettmeier et al., 2010). Once inside cells Hg vapor may be oxidized to Hg^{2+} by catalase (Clarkson et al, 2007). Thus, the mercuric ion (Hg^{2+}) is the direct responsible for Hg vapor toxicity (Clarkson and Magos, 2006) and since it cannot cross easily the BBB, it is retained in the CNS for long periods (Mottet et al, 1997; Rooney, 2007; WHO, 2003).

The liver is a major organ for metabolization of Hg vapor (Magos, 1997). The mercuric ion can then be conjugated with Se forming mercuric selenide (HgSe) precipitates which have been observed in the liver of several species and are thought to be a detoxification mechanism for Hg (Groth et al., 1976; Parizek et al., 1971).

The mercuric ion can also be conjugated with glutathione (GSH) by binding the thiol group (SH-) of the cysteine residue (Cys), forming a complex similar to oxidized glutathione (Clarkson and Magos, 2006). A fraction of this Hg-GSH adduct may be secreted into the bile followed by Hg^{2+} elimination in the feces (Ballatori and Clarkson, 1985). It can also enter systemic circulation and eventually reach the kidney, the major organ for Hg accumulation following exposure to Hg vapor similarly to what is observed for exposure to Hg^{2+} (Magos, 1997).

Inorganic Hg compounds (Hg salts) are also significant source of Hg intoxication. It has been used in numerous products, including medications, germinal soaps, teething powders and skin creams (Goldman and Shannon, 2001).

In addition, mercuric mercury in form of water soluble salt ($HgCl_2$) is considered a highly potent poison (Clarkson and Magos , 2006). Ingestion of as little as 1 g may be fatal. This dose of mercuric chloride leads to a complete breakdown in patient's renal function. It is also highly corrosive to the gastrointestinal tract. Lower doses cause selective damage to the kidneys, being the proximal tubules the main targets of this Hg compound.

Cases of occupational contamination have been reported to cause lung damage and the use of Hg^{2+} as an antiseptic in diapers for infant use was associated with Acrodynia (Warkany and Hubbard, 1953). In addition, there are also studies linking Hg^{2+} contamination and the development of autoimmune diseases (Pollard and Hultman, 1997).

4.2.2 Ethylmercury

Ethylmercury (EtHg) is an organic Hg compound mainly used in form of thimerosal as topical antiseptic and as a preservative in vaccines, routinely given to children. It represents the most widely used form of organic Hg (Ball et al., 2001). Ethylmercury was already used in the treatment of siphilis and commercial applied in the agriculture as fungicidal, which caused severe toxicity and some accidental death, for example the episode of Iraq in 1970' as related before (Jalili and Abbasi, 1961).

Thimerosal, still used nowadays in vaccines, contains about 50% Hg by weight and is metabolized to EtHg and thiosalicylate (Ball et al., 2001). Ethylmercury radical, in thimerosal, is attached to the sulfur atom of the thiol group of salicylic acid. Considering that, mercuric ions bind tightly but reversibly to thiol ligands (Carty and Malone., 1979), after thimerosal injection, the released EtHg would be expected to bind to small (cysteine, glutathione) and large (proteins) endogenous thiol-containing molecules. However, little is know about this process and less about how the bound EtHg is distributed throughout the blood and body (Dórea et al, 2013).

Studies have suggested that EtHg exposure may induce neuro-developmental disabilities such as attention deficit-hyperactivity disorder, language delay but especially autism spectrum disorder (Geier and Geier, 2005). The adverse effects of high dose EtHg are thought to be similar to those of high-dose MeHg (Ball et al., 2001; Halsey, 1999) but the effects of low dose EtHg are unclear.

The literature in general suggests that the role of multiple doses of thimerosal - containing vaccines in current immunization schedules can be public health issue. Even the exposure to Hg in the form of EtHg being lower than that from MeHg in fish-eating populations, is important considered that thimerosal is injected intramuscularly, and consequently, its absorption is approximately 100% at a very early age and in several doses (Dórea, 2007).

Furthermore, in the end of 20th century, a request to vaccine manufacturers about removing thimerosal from vaccines was performed and now, it has been removed from most vaccine in United States and in other European countries such as Italy but it is still used in some developing countries (Freed et al., 2002).

In terms of metabolization, the convertion of EtHg to inorganic Hg appears to be substantially faster than MeHg (Burbacher et al., 2005). As disclosed by a study of Matheson and coworkers (Matheson et al., 1980), after injection of thimerosal, 90% of the total Hg eliminated in urine was in the inorganic form, contrarily to MeHg, whose excretion in urine is considerably lower (Bakir et al., 1973).

The pattern of tissue disposition of EtHg was qualitatively similar to that of MeHg, with brain levels of the intact Hg being slightly higher for methyl than for ethyl forms. Moreover, passage of EtHg is hindered by its larger size and faster decomposition (Clarkson, 2002). Regarding the kinetic of EtHg elimination, little is known, however, feces are suggested the main pathway of elimination. Additionally, the residence time in the body is shown to be shorter for ethyl than to MeHg (Clarkson, 2002).

4.2.3. Methylmercury

The first evidences for contamination with MeHg came from the first half of the 20th century, attributed to its use in some industrial processes and in agriculture as a fungicide (Ahlmark, 1948). However, after the massive Hg contamination in Minamata (Japan) the scientific community and the general public realized that fish consumption is the major route by which humans are exposed to MeHg.

Today the main regions of contamination by MeHg are in close proximity to rivers where the gold mining is performed. For example in Brazil, the Tapajós region (Amazon), where fish consumption is the main source of daily food, levels of MeHg, measured in hair root of inhabitants, ranging from a few μg/g to over 150 μg de Hg/g hair (Malm, 1998; Pinheiro et al., 2003). That can represent 3 times higher than the level at which the first clinical signs and symptoms of Hg contamination occur. In addition, consuption of predatory fish (e.g. swordfish, shark and tuna) from oceans, are also important source of MeHg intoxication (Carvalho et al., 2008).

However, despite the toxicity in the consumption of fish contaminated with MeHg is important to consider that a diet rich in fish also provides many important high quality protein, vitamins, long-chain-3 polyunsaturated fatty acids (PUFA), and micronutrients as Se in fish muscle (Dorea, 2003; Ström et al., 2011). Then, the literature suggests that perhaps is better to advice consumers not to exclude fish and seafood from their diet but to consume preferably species with low MeHg levels (Mahaffey, 2004).

Methylmercury from fish, after ingested is hydrolyzed and the MeHg-Cys, complex found in fish muscle, is absorbed in the intestinal wall (Clarkson and Magos, 2006). This is a highly effective process, with more than 95% of MeHg being absorbed in the GI tract (Magos, 1997). The high motility in the body happens because the MeHg-Cys complex has a similar structure to the amino acid methionine (Met), and then, by molecular homology, it use the same neutral amino acid carrier (LAT) (Roos et al., 2010; Simmons-Willis et al., 2002).

The systemic distribution of MeHg is fast, in about 30 hours, considering that only 5 % of Hg is found in the blood-strem. From this compartment, MeHg is distributed virtually to all protein-rich tissues, such as the brain (found about 10% of Hg), kidney, liver, muscle, hair and nails (Clarkson et al., 2003, 2007).

In the liver MeHg is metabolized and it may be conjugated with reduced glutathione (GSH) followed by its secretion through the bile into the intestinal lumen (Mottet et al., 1997; Clarkson, 2002). Then, the MeHg pass by entero-hepatic reabsorption and recirculation

process and only 1% of the MeHg will be excreted in feces (Clarkson, 2002). Although inefficient, fecal excretion is still the major route of MeHg elimination, which occurs in the form of Hg^{2+} after cleavage of the Hg-C bond by intestinal microflora, known as demethylation (Clarkson et al., 2007). After demethylation, Hg^{2+} may conjugate with GSH and enter the systemic circulation to be delivered to the kidney to be excreted (Zalups, 2000). Mercury (II) can also conjugate with Se forming mercuric selenide (HgSe) (Groth et al., 1976; Parizek et al., 1971).

Methylmercury easily cross the Blood- brain barrier (BBB) by the same mimic mechanism described before and thus, gain access to endothelial cells of the BBB, and enter the CNS (Clarkson and Magos, 2006).

Methylmercury affects a variety of cellular functions and may cause damage to many organs and tissues, as well as: monocytes and lymphocytes (Insug et al., 1997; Shenker et al., 1997) kidney and liver tissues (Freitas et al., 2009), the cardiovascular system (Virtanen et al. 2007) and other. However, the CNS seems to be the main MeHg target (Choi, 1978; Atchison, 2005; Freitas et al., 2009).

In fact, the MeHg effects occur because of damage in the brain focal regions, usually involving areas related to sensory functions and motor coordination, particularly the cerebellum, and also surrounding areas of the visual cortex. The damage is irreversible due to the severe neuronal death (Clarkson and Magos, 2006).

One of the proposed mechanisms for MeHg neurotoxicity is related with the disruption of glutamate transport, which represents the main excitatory neurotransmitter in mammalian CNS (Fonfría et al., 2005). When released into the synaptic cleft it stimulates Ca^{2+} influx via NMDA receptors in the post-synaptic terminal. In a normal physiological scenario, the glutamate in the synaptic cleft is taken up by astrocytes through Na+/K+ transporters, allowing for the signal transmission to terminate (Fonfría et al., 2005). However, MeHg (Aschner et al., 1993, 1994; Fonfría et al., 2005) affect glutamate mediated excitatory signaling by simultaneously enhancing its release from the pre-synaptic terminal and by impeding their uptake by astrocytes. Increased levels of glutamate in the synaptic cleft promote an increased calcium influx to the post synaptic terminal.

This excess of calcium can affect the mitochondria causing mitochondrial swelling and depolarization. These factors may contribute to increase ROS levels, alteration of mitochondrial homeostasis (Seegal et al., 2007) and consequently cause apoptosis / cell death. All of these events may culminate in neuronal loss and contribute to the onset of various diseases related to contamination with MeHg (Clarkson, 2002; Mutter et al., 2004).

Furthermore other mechanisms can be involved in the MeHg toxicity such as:

Depletion of Intracellular Glutathione (GSH): GSH is the most abundant cellular thiol, especially in hepatocytes and, it is involved in many cellular processes (e.g. in the regeneration of oxidized proteins and as a radical scavenger) and constitutes one of the most important antioxidant defense lines (Meister, 1988). Conjugation with GSH is an important step in the metabolization and systemic transport of mercurials. However, an excessive MeHg burden may lead to the depletion of reduced glutathione, with serious consequences in the cell antioxidant response (Farina et al., 2011).

Oxidative stress: Oxidative stress is defined as an imbalance between the levels of pro-oxidant and antioxidant molecules (Halliwell e Gutteridge, 1999). Oxidative stress induced by MeHg is apparently due to its direct action on endogenous thiols (Shanker et al., 2005) and also its effect on antioxidant enzymes (Farina et al., 2004). Once inside the cell, MeHg covalently binds to the -SH groups of biomolecules, including GSH as depicted before, thus causing oxidative stress (Franco et al., 2009). The H_2O_2 production, as well as other ROS, can be form at different ways. However, we can mention the mitochondria as a primary source of ROS, that can occur via eletrons escape from respiratory chain to oxygen, forming superoxide anion (O^{2-}) (Halliwell e Guitteridge, 1999). Moreover, free radicals, such as some ROS, may cause direct oxidative damage in mitochondria, resulting in reduce enzymatic activity and compromising mitochondrial functionality (Radi et al., 2002; Galindo et al., 2003). Incrases in lipid peroxidation can also be an indicator of MeHg induces oxidative stress (Huang et al 2011; Mahboob et al., 2001). Antioxidant enzymes, involved in the degradation of ROS, can have their activity changed by MeHg. Changes in the activities of other enzymes involved in ROS scavenging enzymatic systems such as catalase (CAT) and superoxide dismutase (SOD) are also frequently observed as Hg induced oxidative stress. However, the results in the literature are not consistent regarding the effect of mercurials over the activity of these enzymes and some studies have showed the opposite effect (Hussain et al., 1997).

Changes in the activity of selenoenzymes: Considering that the selenol present in the selenocysteine molecule is more nucleophilic than thiol present in cysteine, as depicted in the first chapter of this review, selenoenzymes are more susceptible to MeHg toxicity, than

thiol enzymes. In this context, there are some studies about the interactions/inhibition of selenoenzymes, particularly GPx and TrxR, by MeHg, that will be better explained in the next section.

5. Interaction between Mercury and Selenium

The interaction between Hg and Se has been reported since 1967, where the first study about the protective role of Se against Hg toxicity was carried out by Parizek and Ostadalova. In this work, injections of Na_2SeO_3 reduced the renal toxicity induced by $HgCl_2$ in rats (Parizek and Ostadalova, 1967).

The mechanism on how and why these two compounds interact was proposed in the 1990's decade by Imura and Naganuma (1991). Sodium selenite forms a high molecular weight complex with Hg (Se-Hg at equimolar ratio) and a plasma protein. The formation of the complex causes a marked decrease of Hg accumulation in the kidney, the Hg target organ, that reduce Hg renal toxicity (Magos and Webb, 1976). The protein which Hg-Se attaches was identified as selenoprotein P, the major plasma selenoprotein (Yoneda and Suzuki, 1997). Basically, intravenously administered selenite is reduced by GSH in red-blood cells to selenide (Se^{2-}) and in the plasma binds Hg^{2+} forming a complex (HgSe)n, that binds to SelP excreted from the liver (Suzuki and Ogra, 2001). Additionally, observations of insoluble granules of HgSe have been reported in the organs of several species (Eley, 1990; Arai et al., 2004). Moreover, Na_2SeO_3 and Hg can form an inert high molecular weight complex and accumulate in the red blood cells causing no harm to other tissues (Imura and Naganuma, 1991).

There is also evidence that various forms of Se, including selenate and selenide, when administered to rats together with mercuric chloride, reduce the toxicity of both Se and Hg, probably by forming a Hg-Se complex that can be detected in nuclei of renal tubular cells by electron microscopy (Goyer, 1997).

However, the benefit of Se supplementation is also dependent on the chronology of exposure treatments, and is frequently reported that is only observable when both compounds ($HgCl_2$ and Na_2SeO_3) are provided simultaneously. When Na_2SeO_3 is administered delayed, it has been reported as having no protective effect (Nagamuna et al., 1984). Agarwal and Behari (2007) noted that treatment of rats previously exposed to $HgCl_2$ with Na_2SeO_3 resulted in

greater accumulation of Hg in the brain and kidney with increased lipid peroxidation and decreased GPx activity and did not decrease toxicity of Hg^{2+}.

Considering organic forms of Hg, Ganther and coauthors (1972) were the first to show that Se was able to alleviate the lethal effects of MeHg in rats co-exposed. Based on the study of Ganther et al (1972), several authors have shown similar results in various mammal, birds and fish species (Cuvin-Aralar and Furness, 1988; Koeman et al., 1975; Beijer and Jernelov, 1978; Ohi et al., 1980; Ralston et al., 2007) and also in humans (Kosta et al., 1975).

Initially this phenomenon had been thought to be a result of supplemental dietary Se binding to Hg, thereby preventing Hg from exerting its toxic effects (Suzuki, 1997; Whanger, 1992). However, considering the role of Se in physiology, authors suggested then that Hg's propensity for Se sequestration in several tissues could inhibit formation of essential Se-dependent proteins (selenoproteins).

Selenium and Hg have a high affinity. Then, these elements selectively bind together and form insoluble Hg selenides that are retained in the brain (Moller-Madsen and Danscher, 1991; Moller- Madsen, 1990). The affinity constant between Hg and the Se of Sec is estimated to be$\sim 10^{22}$, much higher than Hg's affinity for the sulfur of Cys; 10^{14}. Futhermore, the selenides that form during each cycle of Sec synthesis have an exceptionally high affinity constant for Hg (10^{45}) which is a million times higher than that of sulfide (10^{39}), Hg's second-best binding partner. This fact leads to extreme difficulties in the release of complex components (Hg-Se), thereby reducing the concentration of free Se in the organism.

In this context, the supplementation with Se, suggested by several authors, could have a protective effect by ensuring adequate levels of Se to replace the amount of Se lost to Hg sequestration, thereby maintaining normal selenoprotein synthesis.

Furthermore, Se may also decrease Hg toxicity by counteracting the effects of free radicals generated by inorganic Hg (Ganther, 1978). In general, Se has a protective effect in that it delays the onset of Hg toxicity or reduces the severity of the effects of both inorganic forms of Hg and MeHg (Goyer, 1997).

A recent study by Li et al. in China also reported that in humans exposed to Hg, daily supplementation with Se-enriched yeast for three months decreased the urinary levels of biomarkers of oxidative stress, such as malondialdehyde and 8-hydroxy- 2-deoxyguanosine. In addition, in the same study the authors demonstrated another important property of Se in counteracting Hg toxicity, showing an increase in the excretion of Hg after supplementation of volunteers with selenomethionine (Li et al. 2012).

Nevertheless, other works (Magos and Webb, 1977; Ralston et al., 2008) showed that Hg-Se interaction is not always beneficial, as it may lead to Se depletion making it unavailable to participate in metabolism. As observed by Khan and Wang (2009) the overall net effect of Se over Hg toxicity will depend on the relative concentrations of Hg and their bioavailability. Other variables to be taken into account are the organism species and/or the target organ.

5.1 Interaction between MeHg and selenium

In this review, we emphasized the interaction between MeHg and Se instead of other forms of Hg. Considering that, perhaps it is the most significant Hg form for non-occupational human population and with important toxic effects to human health.

As depicted before, MeHg has a high affinity to Se. In this way, the Hg and Se interactions also occur in aquatic environment, low levels of Se in rivers or oceans may lead to higher accumulation of Hg in fish (MeHg) and consequently, increase the accumulation of MeHg in the the food chain. Ocean fish tend to be generally rich in Se relative to Hg. However, in freshwater fish, the health risks of MeHg exposure may vary in response to individual and regional differences in Se intake. Therefore, fish from low-Se lakes would not only have low-Se contents, they would also tend to have higher MeHg contents, a dangerous combination for consumption by pregnant and other vulnerable subpopulations (Ralston and Raymond, 2010). Thus, Hg – Se ratio interaction can consequently, decrease or increase the levels of MeHg in fish consumed by population.

Considering that Se has an important physiological role, MeHg exposures that approach or exceed 1:1 molar ratios with Se appear to induce toxicity as a result of selenoenzyme inhibition.

Under normal conditions, homeostatic regulation mechanisms prevent brain Se content declines higher than 60% of normal levels, approximately. However, consumption of diets with high MeHg contents (50µmol MeHg/kg; ~10ppm Hg) cause significant decreases in brain Se levels up to 43% of normal in weanling rats raised on low-Se diets (Ralston et al., 2008). These remarkably low brain Se contents apparently occurred because MeHg impairs redistribution of somatic Se reserves in other tissues. Moreover, in the study of Ralston (2008), it was shown that dietary Se has a potent influence on toxic effects of chronic MeHg exposure. Low-Se diets showed the greatest toxic effects from high-MeHg exposures. Blood Hg:Se molar ratios were proportional to observed toxicity. Since increasing blood Hg:Se

ratios are indicative of increasing risk of harm, assessments of MeHg exposure should evaluate blood Hg:Se ratios rather than just Hg levels (Ralston, 2008).

The protective effect of supplemental Se appears to occur because additional dietary Se is able to offset the Se sequestered by Hg (Watanabe et al., 1999a, b; Ralston et al., 2007) and maintain uninterrupted selenoenzyme activities. Contrary to earlier expectations, it has been shown that Hg does not cause oxidative damage directly (Seppanen et al., 2004). Instead, high MeHg exposures cause increased oxidative damage secondary to their inhibition of selenoenzyme activities, that will be better explain below.

Ganther and coauthors (1972) were the first to report the interaction between MeHg and supplemented Se. They showed alleviation of Na_2SeO_3 treatment on MeHg induced mortality in a mice model treated with low Se diet. Moreover, the results showed enhance in neurotoxicity when the animals were exposed to MeHg with low Se when compared to Se-sufficient mice (Ganther et al. 1972).

Moreover, related to mechanisms of Hg-Se interaction, a study by Iwata et al (1981) demonstrated *in vitro* that conversion of albumin-bound MeHg to insoluble complex (HgSe) occur by reaction of MeHg and Se compound (possibly H_2Se) reduced by GSH and /or protein sulphydril groups in the soluble fraction of rat liver, kidney and brain. The Se compounds used in this study, that demonstrated significant results, are selenite, selenate, GSSeSG and selenocystine, and all of them form this insoluble and inert complex only in the presence of a reducing thiols (GSH and sulphydril groups). Selenomethionine, was ineffective in this experiment. In addition, this study proposed that this conversion activity was specific for Se compounds. Together, these studies suggest that selenite induce redistribution of MeHg, which has been reported to be the mechanism of protection by Se against Hg toxicity.

Studies of several authors (Beyrouty and Chan, 2006; Ganther et al., 1972; Hoffman and Heinz, 1998; Ralston et al., 2008; Satoh et al., 1985) suggested that co-exposure to Se compounds has been indicated to alleviate MeHg toxicity. Dietary Se was recently shown to diminish the neurotoxic effects caused by MeHg in rats (Ralston et al., 2008).

It has been proposed that MeHg (mostly bound to thiols), due to its higher affinity to selenols (-SeH), could exchange ligands forming MeHg-Se-R compounds that are less reactive, more stable and so less bioavailable (Canty et al., 1983). Such compounds include MeHg–Sec and bis(methylmercuric)selenide [$(MeHg)_2Se$]. The latter compound is thought to be rapidly eliminated from tissues (Watanabe, 2002).

Selenium has been also reported to have a role in MeHg demethylation, which is known to occur *in vivo*, especially in the liver, but also in the brain (Mottet et al., 1997). The

mechanism by which the C-Hg bond is cleaved remains unknown but, several hypotheses concerning the involvement of Se in this process have been suggested (Yang et al., 2008), such as a direct role in the demethylation; the generation of superoxide radicals by R-SeH compounds, that will promote demethylation and the diffusion of $(MeHg)_2Se$ into the mitochondria where demethylation occurs by radical attack.

However, the beneficial effects of Se co-exposure over MeHg toxicity are still controversial. In some studies (e.g. Magos and Webb, 1977; Masukawa et al., 1982), $(MeHg)_2Se$ was shown to form in the blood, modifying MeHg distribution and increasing concentrations in the brain, which could increase MeHg toxicity. A study by Newland and co-workers (2006) also showed that a Se rich diet increased levels of MeHg in the brain of female rats and their pups. This increase of MeHg levels in the brain, can potential enhance its interference with the activity of selenoenzymes.

5.1.1 Interaction between MeHg and selenoenzymes

Chemically, MeHg is classified as soft electrophile and, consequently, it reacts preferentially with soft nucleophiles (Pearson and Songstad, 1967). So, in the living organisms MeHg can react with ―SH (sulfhydryl or thiol groups) and with ―SeH (selenohydryl or selenol groups). It is important to emphasize that selenol groups are softer nucleophiles than thiol groups; consequently, the affinity of MeHg for selenol is expected to be higher than that for an analog thiol (Sugiura et al., 1976; Khan et al., 2009). Unfortunately, these features that make it so valuable physiologically also make it very vulnerable to MeHg toxicity.

Biochemically, an irreversible inhibitor is one that forms covalent bonds with components of the active site of an enzyme. Since selenocysteine is the principal active site catalytic component of selenoenzymes, MeHg is by definition a highly specific irreversible selenoenzyme inhibitor since it forms covalent bonds between its Hg moiety and the Se of the enzyme's selenocysteine (Seppanen et al., 2004).

Therefore, selenoproteins are prone to be targeted by mercurials leading to loss of activity (Ralston and Raymond, 2010). Since, Se exists primarily in selenoproteins many of which are enzymes with important roles in cellular and tissue homeostasis (Papp et al., 2007), impairment of their functions by mercurials is probably a determinant factor in the development of toxicity. Formation of MeHg–SeCys (proposed term; pseudomethionine) will

not only directly inhibit selenoenzyme activities, but the Se trapped in this form will become unavailable for reuse in future cycles of selenocysteine synthesis.

There are several reports in the literature concerning interaction of MeHg with selenoproteins. In this context, glutathione peroxidase (GPx), which is one of the main scavengers of H_2O_2 and organic hydroperoxides, has been shown to be decreased/inhibited following exposure to MeHg (Franco et al. 2009; Freitas et al. 2009). This can be the result of several factors, such as GSH depletion, Se depletion or direct interaction between mercurials and its active site. In fact, Franco et al. (2009) showed, in neuroblastoma cells exposed to MeHg, a concentration dependent decrease (up to 40%) in GPx activity. Zemolin (2013) also demonstrated an inhibition on GPx activity and a decrease in GPx 4 expression by MeHg in cortex and cerebellum of treated mice. Several animal studies (Chang and Suber, 1982; Franco et al., 2009; Fredriksson et al., 1993;Nishikido et al., 1987; Watanabe et al., 1999) also reported decreased GPx activity in rodents after exposure to MeHg (up to 50%) followed by recovery when Se was provided.

Furthermore, TxrR is particularly sensitive to MeHg both upon *in vitro* and *in vivo* exposure. The active site of TrxR enzyme is more susceptible to MeHg inhibition than GPx, due to the presence of a selenocysteine (Sec) residue in close proximity to three Cys residues in the open C-terminus of mammalian TrxR when compared to the GPx active site, where the Sec residue is close to Gln and Trp residues. This fact associated with the ability of the TrxR to bind electrophilic agents such as Hg compounds, significantly increases the reactivity of MeHg with the active site of TrxR (Branco et al., 2012).

Recently several studies have demonstrated the susceptibility of TrxR to MeHg toxicity. For instance, Carvalho et al., (2008) showed the inhibitory effect of MeHg towards thioredoxin reductase (TrxR) and thioredoxin (Trx) *in vitro* (Carvalho et al. 2008). In this study, Carvalho and coauthors showed that mammalian TrxR activity was affected by MeHg in HeLa and HEK cells, indicating that mercurials targeted the active site SeH (Carvalho et al., 2008b). In addition, Wagner et al. (2010) showed that exposure of rats to MeHg reduced the activity of TrxR in the liver and kidney, however, no inhibition of TrxR was observed in the brain (Wagner et al., 2010) after 24 hours of exposure to this organometal. Moreover, Branco et al. showed *in vivo* exposure to MeHg profoundly affected TrxR activity both in brain and liver of fish (*Diplodus cervinus),* corroborating the vulnerability of TrxR to this organomercury compound (Branco et al., 2011). Dalla Corte and collaborators also showed a significant inhibition in liver, brain and kidney TrxR acitivity in rats treated with MeHg for 21 days (Dalla Corte et al., 2013), showing a time dependent

effect of MeHg on TrxR enzyme activity.

Since the thioredoxin system is upstream of several biochemical pathways, it can be anticipated that its inhibition by mercurials is a key step in the development of Hg toxicity (Carvalho et al., 2008), and this inhibitory effect can lead to deleterious effects, leading to cytotoxicity and cell death.

5.1.2 Interaction between MeHg and synthetic organoselenium compounds

In addition to inorganic and naturally occurring organic Se compounds, synthetic organoselenium compounds can also exhibit protective effects against MeHg. Especially considering that some synthetic organic Se compounds (as $(PhSe)_2$ and ebselen) form a "selenol intermediate" which MeHg has a high affinity, or can indirectly modulate the oxidative stress.

Freitas and coauthors demonstrated that $(PhSe)_2$ (0.4 and 1 mg / kg, sc, for 35 days) was able to minimize the oxidative stress caused to adult mice by consumption of MeHg (2 mg/ kg/ day for 35 days). The researchers also showed that the animals treated with $(PhSe)_2$ together with MeHg consumption presented lower tissue levels of this toxicant. The authors argued that the benefits conferred by $(PhSe)_2$ against MeHg intoxication are beyond the antioxidant effect, suggesting that formation of a complex between a selenol intermediate, which possesses high nucleophilicity and high affinity for MeHg, accounts for the ability of $(PhSe)_2$ to ameliorate MeHg-induced toxicity, and that the body can more easily excrete the toxicant (Freitas et al. 2009). In addition, $(PhSe)_2$ decreased the deposition of Hg in cerebrum, cerebellum, kidney and liver (Freitas et al., 2009).

To justify the protective effect of this selenocompound to counteract the toxicity of MeHg, and the best activity when compared to other organic Se compounds, a recent study demonstrated that selenomethionine, which is not expected to generate a selenol/selenolate intermediate, did not reduce the levels of Hg after intoxication with MeHg (Santos et al., 2007). The same study also suggested that selenomethionine protected against MeHg-induced toxicity by increasing the antioxidant defense, but not by a reduction in Hg body burden. Accordingly, is possible suggest that $(PhSe)_2$ could possess additional beneficial properties against MeHg-induced toxicity when compared to selenomethionine.

Glaser et al (2013) also demonstrated important protective effect to $(PhSe)_2$, showing that it is a potent neuroprotective agent for preventing MeHg-induced brain poisoning, and

probably other neurotoxic and neurodegenerative conditions, mainly based on its capacity to quench MeHg protecting cells from mitochondrial dysfunction.

$(PhSe)_2$ and analogues were also able to protect against against MeHg-induced mitochondrial dysfunction and oxidative stress in mitochondrial-enriched fractions from adult Swiss mouse brain. In this study the protective effects of compounds was shown to be directly proportional to their GPx-like activity (Meinerz et al., 2011).

In addition, Ebselen, another synthetic organoselenium compound, protected developing rats from MeHg neurotoxicity, by disruption in glutamate homeostasis (release and uptake), during lactation period (Farina et al., 2003a). In adult mice, Ebselen blunted the MeHg inhibited glutamate uptake by cortical brain slices and decreased cortical glutathione peroxidase activity (Farina et al., 2003b).

Since ebselen can form both “selenol intermediates” and an ebselen diselenide (Zhao et al., 2002 a, b; Sausen de Freitas et al., 2010), part of the observed neuroprotective effect of ebselen against MeHg could also be a consequence of the reduction in Hg deposition in the brain.

In contrast, Dalla Corte et al (2013) did not observed any protective effect of $(PhSe)_2$ to MeHg, except when total and no-proteic thiol levels were tested. In this study, Se compound increased the accumulation of Hg in the tissues, leading to an increase in the toxicity.

6. Concluding remarks

Selenium is an essential trace element and being a constituent of selenoenzymes (e.j. GPx and TrxR), it plays its essentiality on the maintenance of cellular redox balance (Brigelius-Flohe et al., 1994; Arner and Holmgren, 2000). Moreover, this element (organic and inorganic forms) has been used due to its important health benefits, acting on prevention and mitigation of many diseases (Velardos et al., 2004; Rayman et al., 2008; Nogueira et al., 2004). On the other hand, some studies have demonstrated a toxic potential for Se compounds, suggesting the need for additional studies aiming to investigate the actual limits between toxicity and protective effects exerted by these compounds.

In contrast to this essential element, Hg is an extremely toxic element, especially at its organic form, MeHg, which is consumed by the population through diet (Clarkson et al.,

2003). This organometal has harmful effects especially to the CNS and can lead to significant cognitive impairment (Farina et al, 2003). The interaction of Hg compounds with intracellular Se pools has been shown as a major mechanism of toxicity, being selenoezymes important targets during Hg compounds poisoning events.

In this context, considering the protective effects of Se compounds, they may be valuable allies in the treatment of MeHg poisoning, and also they can reduce the toxic effects caused by this compound mainly by reducing the interaction with important intracellular macromolecules containing Se. However, studies regarding the protective effect of these compounds are still controversial. While some Se compounds are shown to protect against MeHg toxicity (Freitas et al., 2009; Glaser et al., 2013; Meinerz et al., 2011), others can exacerbate MeHg deleterious effects (Dalla Corte et al., 2013). Thus, a question remains unsolved: what is the actual mechanism involved in this equation?

Furthermore, despite the high levels of MeHg presented in seafood, it offers abundant natural protection against MeHg. This has important implications in human health, since fish contains many essential nutrients that are often needlessly avoided because of misguided fears associated with its consumption. Then, another important question to be addressed is the Hg:Se ration in the ecosystems, considering that small changes can lead to a greater accumulation of MeHg in fish, and consequently higher intake by humans. Such analysis is also important considering fish as a great source of micronutrients, important to healthy development.

REFERENCES

Agarwal R, Behari, JR. Role of Selenium in Mercury Intoxication in Mice. Ind Health 2007: 45,388-95.

Ahlmark, A. Poisoning by Methyl Mercury Compounds. Brit J Ind Med 1948; 5:117-119.

Algotar AM, Hsu C-H, Singh P, Stratton SP. Selenium supplementation has no effect on serum glucose levels in men at high risk for prostate cancer. J Diabetes 2013; 5(4):465–70.

Allander E. Kashin-Beck disease. An analysis of research and public health activities based on a bibliography 1849-1992. Scand J Rheumatol Suppl 1994; 99:1–36.

Anderson RA. Trace elements and cardiovascular diseases. Acta Pharmacol Toxicol (Copenh) 1986; 59(7):317–24.

Anestal K, Arnér ESJ. Rapid induction of cell death by selenium compromised thioredoxin reductase 1 but not by the fully active enzyme containing selenocysteine. J Biol Chem 2003; 6:6.

Arai T, Ikemoto T, Hokura A, Terada Y, Kunito T, Tanabe S, et al. Chemical Forms of Mercury and Cadmium in Marine Mammals and Seabirds as Determined by XFAS Analysis. Environ Sci Technol 2004; 38:6468-74.

Ardais AP, Santos FW, Nogueira CW. Ebselen attenuates cadmium-induced testicular damage in mice. J Appl Toxicol 2008; 28(3):322–328.

Ariya PA, Peterson K, Snider G, Amyot M. Mercury Chemical Transformation in the Gas, Aqueous and Heterogeneous Phases: State-of-the-art Science and Uncertainties. In : Mercury Fate and Transport in the Global Atmosphere; Pirrone N, Mason R (Eds.), Springer, New York, 2009; 459-501.

Arner ES, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem 2000; 267: 6102–6109.

Arnér ES. Selenoproteins-What unique properties can arise with selenocysteine in place of cysteine? Exp Cell Res 2010; 316(8):1296-303.

Arteel GE, Sies H. The biochemistry of selenium and the glutathione system. Environ Toxicol Phar 2001; 10:153–158.

Aschner M, Du YL, Gannon M, Kimelberg HK. Methylmercury-induced Alterations in Excitatory Amino Acid Transport in Rat Primary Astrocyte Cultures. Brain Res 1993; 602:181–186.

Aschner M, Mullaney KJ, Fehm MN, Wagonerc DE Jr, Vitarella D. Astrocytes as Potential Modulators of Mercuric Chloride Neurotoxicity. Cell Mol Neurobiol 1994; 14:637–652.

Atchison WD. Is chemical neurotransmission altered specifically during methylmercury-induced cerebellar dysfunction? Trends Pharmacol Sci 2005; 26(11):549-57.

Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, al-Rawi NY, Tikriti S, Dhahir HI, Clarkson TW, Smith JC, et al. Methylmercury poisoning in Iraq. Science 1973; 181:230–241.

Ball LK, Ball R, Pratt RD. An assessment of thimerosal use in childhood vaccines. Pediatrics 2001; 107:1147–1154.

Ballatori N, Clarkson TW. Biliary Secretion of Glutathione and of Glutathione-metal Complexes. Fund Appl Toxicol 1985; 5:816–831.

Barbosa NBD, Nogueira CW, Guecheva TN, Bellinaso MD, Rocha JBT. Diphenyl diselenide supplementation delays the development of *N*-nitroso-*N*-methylurea-induced mammary tumors. Arch Toxicol 2008; 82:655–663.

Barceloux DG. Selenium. J Toxicol ClinToxicol 1999; 37:145–172.

Barkay T, Wagner-Döbler I. Microbial Transformations of Mercury: Potentials, Challenges, and Achievements in Controlling Mercury toxicity in the Environment. Adv Appl Microbiol 2005; 57:1-52.

Battin EE, Zimmerman MT, Ramoutar RR, Quarles CE, Brumaghim JL. Preventing metal-mediated oxidative DNA damage with selenium compounds. Metallomics 2011; 3(5):503-12.

Beck MA, Esworthy RS, Ho YS, Chu FF. Glutathione peroxidase protects mice from viral-induced myocarditis. FASEB J 1998; 12:1143–1149.

Behne D, Kyriakopoulos A, Meinhold H, Köhrle J. Identifi cation of type I iodothyronine 5'-deiodinase as a selenoenzyme. Biochem Biophys Res Commun 1990; 173:1143–1149.

Beijer K, Jernelov A. Ecological aspects of mercury–selenium interaction in the marine environment. Environ Health Perspect 1978;25:43–45.

Benton D. Selenium intake, mood and other aspects of psychological func-tioning. Nutr Neurosci 2002; 5(6):363–74.

Berry MJ, Banu L, Harney JW, Larsen PR. Functional characterization of the eukaryotic SECIS elements which direct selenocysteine insertion at UGA codons. EMBO J 1993; 12:3315–3322.

Berry MJ, Banu L, Chen YY, Mandel SJ, Kieffer JD, Harney JW, Larsen PR. Recognition of UGA as a selenocysteine codon in type I deiodinase requires sequences in the 3′untranslated region. Nature 1991; 353:273–276.

Berzelius JJ. Additional observations on lithion and selenium. Ann Philos 1818; 11:373–5.

Beyrouty P, Chan HM. Co-consumption of Selenium and Vitamin E altered the Reproductive and Developmental Toxicity of Methylmercury in Rats. Neurotoxicol Teratol 2006;28:49-58.

Blankenberg S, Rupprecht H, Bickel C, Torzewski M, Hafner G, Tiret L, Smieja M, Cambien F, Meyer J, Lackner K. N Engl J Med 2003; 349:1605–1613.

Branco V, Canário J, Holmgren A, Carvalho C. Inhibition of the Thioredoxin System in the Brain and Liver of Zebra-Seabreams Exposed to Waterborne Methylmercury. Toxicol Appl Pharmacol 2011;251:95-103.

Branco V, Canário J, Lu J, Holmgren A, Carvalho C. Mercury and selenium interaction in vivo: effects on thioredoxin reductase and glutathione peroxidase. Free Radical Biology and Medicine 2012; 52(4):781-93.

Brandao R, Acker CI, Leite MR, Barbosa NBV, Nogueira CW. Diphenyl diselenide protects against glycerol induced renal damage in rats. J Appl Toxicol 2009; 29:612–618.

Brigelius-Flohe R, Aumann KD, Blocker H, Gross G, Kiess M, Kloppel KD, Maiorino M, Roveri A, Schuckelt R, Usani F. *et al.* Phospholipid-hydroperoxide glutathione peroxidase. Genomic DNA, cDNA, and deduced amino acid sequence. J Biol Chem 1994; *269*: 7342–7348.

Brigelius-Flohé R, Maiorino M. Glutathione peroxidases. Biochim Biophys Acta 2013; 1830(5):3289–303.

Brigelius-Flohe R. Tissue-specific functions of individual glutathione peroxidases. Free Radic Biol Med 1999; 27:951–965.

Broome CS, McArdle F, Kyle JAM, Andrews F, Lowe NM, Hart CA, et al. An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status. Am J Clin Nutr 2004; 80(1):154–62.

Broussard LA, Hammett-Stabler CA, Winecker RE, Ropero-Miller JD. The Toxicology of Mercury. Laboratory medicine 2002; 33:614-25.

Brozmanová J, Mániková D, Vlčková V, Chovanec M. Selenium: a double-edged sword for defense and offence in cancer. Arch Toxicol 2010; 84(12):919-38.

Brummer M, Hayes S, Dawson KA, Lawrence LM. Measures of antioxidantstatus of the horse in response to selenium depletion and repletion. J Anim Sci 2013; 91(5):2158–68.

Brüning CA, Prigol M, Roehrs JA, Nogueira CW, Zeni G. Involvement of the serotonergic system in the anxiolytic-like effect caused by m-trifluoromethyl-diphenyl diselenide in mice. Behav Brain Res 2009; 205:511–517.

Burbacher TM, Shen DD, Liberato N, Grant KS, Cernichiari E, Clarkson T. Comparison of blood and brain mercury levels in infant monkeys exposed to methylmercury or vaccines containing thimerosal. Environ Health Perspect 2005; 113:1015–1021.

Burger ME, Fachinetto R, Zeni G, Rocha JBT. Ebselen attenuates haloperidol-induced orofacial dyskinesia and oxidative stress in rat brain. Pharmacol Biochem Behav 2005; 81:608–615.

Burke KE, Combs GF, Gross EG, et al. The effects of topical and oral L-selenomethionine on pigmentation and skin cancer induced by ultraviolet irradiation. Nutr Cancer 1992; 17:123–37.

Canty AJ, Carty AJ, Malone SF. Methylmercury(II) Selenolates: Synthesis and Characterization of MeHgSeMe and MeHgSePh and 1H and 199Hg NMR Studies of Ligand Exchange in Methylmercury(II) Thiolates and Selenolates, Including Amino Acid Complexes. J Inorg Biochem 1983;19:133–42.

Carlson BA, Yoo M-H, Shrimali RK, Irons R, Gladyshev VN, Hatfield DL, et al. Role of selenium-containing proteins in T-cell and macrophage function. Proc Nutr Soc 2010; 69(3):300–10.

Carty AJ, Malone SF. The chemistry of mercury in biological systems. In: The Biogeochemistry of Mercury in the Environment (Nriagu JO, ed). New York: Elsevier/North Holland, 1979; 433–459.

Carvalho CML, Chew E, Hashemy SI, Lu J, Holmgren A. Inhibition of Human Thioredoxin System: a Molecular Mechanism of Mercury Toxicity. J Biol Chem 2008; 283:11913-11923.

Castellano S, Morozova N, Morey M, Berry MJ, Serras F, Corominas M, Guigo R: In silico identification of novel selenoproteins in the Drosophila melanogaster genome. EMBO Rep 2001; 2:697-702.

Chang LW, Suber R. Protective Effect of Selenium on Methylmercury Toxicity: a Possible Mechanism. B Environ Contam Tox 1982;29:285-89.

Cheng W-H, Holmstrom A, Li X, Wu RTY, Zeng H, Xiao Z. Effect of dietaryselenium and cancer cell xenograft on peripheral T and B lymphocytes inadult nude mice. Biol Trace Elem Res 2012; 146(2):230–5.

Choi BH. Abnormal neuronal migration, deranged cerebral cortical organization, and diffuse white matter astrocytosis of human fetal brain: a major effect of methylmercury poisoning in utero. Journal Neuropathology Expression Neurology 1978; 37:719-733.

Chu FF, Doroshow JH, Esworthy RS. Expression, characterization, and tissue distribution of a new cellular selenium-dependent glutathione peroxidase, GSHPx-GI. J Biol Chem 1993; *268*:2571–2576.

Cirelli A, Ciardi M, de Simone C, Sorice F, Giordano R, Ciaralli L, et al. Serum selenium concentration and disease progress in patients with HIV infection. Clin Biochem 1991; 24:211–4.

Clark LC, Combs Jr GF, Turnbull BW, Slate EH, Chalker DK, Chow J, et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Preventionof Cancer Study Group. JAMA 1996; 276(24):1957–63.

Clarke C. Sterilization of the Skin by Spiritous Solution of Mercury Perchloride. Brit Med J 1912; 2:764-765.

Clarkson TW, Vyas JB, Ballatori N. Mechanisms of Mercury Disposition in the Body. Am J Ind Med 2007; 50:757–764.

Clarkson TW, Magos L, Myers GJ. Human Exposure to mercury: The Three Modern Dilemmas. J Trace Elem Exp Med 2003; 16:321-343.

Clarkson TW, Magos L. The Toxicology of Mercury and Its Chemical Compounds. Crit Rev Toxicol 2006; 36:609-662.

Clarkson TW. The Three Modern Faces of Mercury. Environ Health Perspect 2002; 1:11-23.

Combs Jr GF. Selenium and cancer. In: Garewal HS, editor. Antioxidants and disease prevention. New York, NY: CRC Press 1997. p. 97–113.

Cone JE, Del Río RM, Davis JN, Stadtman TC. Chemical characterization of the selenoprotein component of clostridial glycine reductase: identification of selenocysteine as the organoselenium moiety. Proc Natl Acad Sci USA. 1976; 73(8):2659-63.

Copeland PR. Regulation of gene expression by stop codon recoding:selenocysteine. Gene 2003; 312:17-25.

Corcoran NM, Martin D, Hutter-Paier B, Windisch M, Nguyen T, Nheu L, Sundstrom LE, Costello AJ, Hovens CM. Sodium selenate specifically activates PP2A phosphatase, dephosphorylates tau and reverses memory deficits in an Alzheimer's disease model. J Clin Neurosci 2010; 17(8):1025-33.

Cortes-Jofre M, Rueda JR, Corsini-Munoz G, Fonseca-Cortes C, Caraballoso M, Bonfill Cosp X. Drugs for preventing lung cancer in healthy people. Cochrane Database Syst Rev 2012; 10:CD002141.

Cotgreave IA, Duddy SK, Kass GEN, Thompson D, Moldeus P. Studies on the anti-inflammatory activity of ebselen: Ebselen interferes with granulocyte oxidative burst by dual inhibition of nadph oxidase and protein kinase C. Biochem Pharmacol 1989; 38:649– 656.

Coughtrey PJ, Jackson D, Thorne MC. Selenium. In Radionuclide Distribution and Transport in Terrestrial and Aquatic Ecosystems; A. Balkema: Rotterdam. The Netherlands 1983; 3:372.

Culvin-Aralar ML, Furness RW. Mercury and selenium interaction: a review. Ecotoxicol. Environ Saf 1991;21:348–364.

Cutter GA. The estuarine behaviour of selenium in San Francisco Bay. Estuarine Coastal Shelf Sci 1989; 28:13-34.

da Rocha JT, Sperança A, Nogueira CW, Zeni G. Hypolipidaemic activity of orally administered diphenyl diselenide in Triton WR-1339-induced hyperlipidaemia in mice. J Pharm Pharmacol 2009; 61:1673–1679.

Dalla Corte CL, Wagner C, Sudati JH, Comparsi B, Leite GO, Busanello A, et al. Effects of diphenyl diselenide on methylmercury toxicity in rats. Biomed Res Int 2013, 983821.

Davis CD, Tsuji PA, Milner JA. Selenoproteins and cancer prevention. Annu Rev Nutr 2012; 32:73–95.

Davis CD, Zeng H, Finley JW. Selenium enriched broccoli decreases intestinal tumorigenesis in multiple intestinal neoplasia mice. J Nutr 2002; 132:307–9.

Davis MT, Bartfay WJ. Ebselen decreases oxygen free radical production and iron concentrations in the hearts of chronically iron-overloaded mice. Biol Res Nurs 2004; 6(1):37–45.

Dawson DA, Masayasu H, Graham DI, Macrae IM. The neuroprotective eficacy of ebselen (a glutathione-peroxidase mimic) on brain-damage induced by transient focal cerebral-ischemia in the rat. Neurosci Lett 1995; 185:65–69.

de Bem AF, Farina M, Portella RL, Nogueira CW, Dinis TC, Laranjinha JA, Almeida LM, Rocha JBT. Diphenyl diselenide, a simple glutathione peroxidase mimetic, inhibits human LDL oxidation in vitro. Atherosclerosis 2008; 201:92–100.

de Bem AF, Portella RD, Colpo E, Duarte MMMF, Frediane A, Taube PS, Nogueira CW, Farina M, da Silva EL, Rocha JBT. Diphenyl diselenide decreases serum levels of total cholesterol and tissue oxidative stress in cholesterol-fed rabbits. Basic Clin Pharmacol Toxicol 2009; 105:17–23.

Delmas-Beauvieux MC, Peuchant E, Couchouron A, Constans J, Sergeant C, Simonoff M, et al. The enzymatic antioxidant system in blood and glutathione status in human immunodeficiency virus (HIV)-infected patients: effects of supplementation with selenium or beta-carotene. Am J Clin Nutr 1996; 64:101–7.

Derbeneva SA, Bogdanov AR, Pogozheva AV, Gladyshev OA, Vasilevskaia LS, Zorin SN, et al. Effect of diet enriched with selenium on the psycho-emotional and adaptive capacity of patients with cardiovascular diseases and obesity. Vopr Pitan 2012; 81(4):35–41.

Dórea JG, Farina M, Rocha JB. Toxicity of ethylmercury (and Thimerosal): a comparison with methylmercury. J Appl Toxicol 2013; 33(8):700-11.

Dórea JG. Exposure to mercury during the first six months via human milk and vaccines: modifying risk factors. Am. J. Perinatol 2007; 24:387–400.

Dorea JG. Fish are Central in the Diet of Amazonian Riparians: Should We Worry About Their Mercury Concentrations? Environ Res 2003; 92:232-244.

Driscoll DM, Copeland PR: Mechanism and regulation of selenoprotein synthesis. Annu Rev Nutr 2003; 23:17-40.

Dumont E, Vanhaecke F, Cornelis R. Selenium speciation from food source to metabolites: a critical review. Anal Bioanal Chem 2006; 385:1304-1323.

Eersel van J, Ke YD, Liu X, Delerue F, Kril JJ, Götz J, Ittner LM. Sodium selenate mitigates tau pathology, neurodegeneration, and functional deficits in Alzheimer's disease models. Proc Natl Acad Sci USA 2010; 107(31):13888-93.

Eisler R. Mercury Hazards to Living Organisms. CRC Press, Taylor & Francis Group, Boca Raton FL, 2006; 312.

Eley BM. A Study of Mercury Redistribution, Excretion and Renal Pathology in Guinea-pigs Implanted with Powdered Dental Amalgam for between 2 and 4 years. J Exp Pathol 1990;71:375-93.

Engman L, Cotgreave I, Angulo M, Taylor CW, Paine-Murrieta GD, Powis G. Diaryl chalcogenides as selective inhibitors of thioredoxin reductase and potential antitumor agents. Anticancer Res 1997;17:4599–4605.

Epp O, Ladenstein R, Wendel A. The refined structure of the selenoenzyme glutathione peroxidase at 0.2-nm resolution. Eur J Biochem 1983; 133: 51– 69.

Fachinetto R, Villarinho JG, Wagner C, Pereira RP, Puntel RL, Paixão MW, Braga AL, Calixto JB, Rocha JBT, Ferreira J. Diphenyl diselenide decreases the prevalence of vacuous chewing movements induced by fluphenazine in rats. Psychopharmacology 2007; 194:423–432.

Fairweather-Tait SJ, Collings R, Hurst R. Selenium bioavailability: Current knowledge and future research requirements. Am. J. Clin. Nutr. 2010; *91*: 1484S-1491S.

Farina M, Aschner M, Rocha JT. Oxidative Stress in MeHg-Induced Neurotoxicity. Toxicol Appl Pharm 2011; 256:405–417.

Farina M, Dahm KC, Schwalm FD, Brusque AM, Frizzo ME, Zeni G, Souza DO, Rocha JB. Methylmercury increases glutamate release from brain synaptosomes and glutamate uptake by cortical slices from suckling rat pups: modulatory effect of ebselen. Toxicol Sci 2003b;73(1):135–40.

Farina M, Frizzo ME, Soares FA, Schwalm FD, Dietrich MO, Zeni G, Rocha JB, Souza DO. Ebselen protects against methylmercury-induced inhibition of glutamate uptake by cortical slices from adult mice. Toxicol Lett 2003a;144(3):351–57.

Farina M, Soares FA, Zeni G, Souza DO, Rocha JB. Additive pro-oxidative effects of methylmercury and ebselen in liver from suckling rat pups. Toxicol Lett 2004; 146:227-235.

Fatmi W, Kechrid Z, Nazıroˇglu M, Flores-Arce M. Selenium supplementationmodulates zinc levels and antioxidant values in blood and tissues of diabeticrats fed zinc-deficient diet. Biol Trace Elem Res 2013; 152(2):243–50.

Ferencík M, Ebringer L. Modulatory effects of selenium and zinc on the immune system. Folia Microbiol (Praha) 2003; 48(3):417-26.

Ferguson AD, Labunskyy VM, Fomenko DE, Araç D, Chelliah Y, Amezcua CA, et al. NMR structures of the selenoproteins Sep15 and SelM reveal redox activity of a new thioredoxin-like family. J Biol Chem 2006; 281(6):3536-43.

Flohe L, Gunzler WA, Schock HH. Glutathione Peroxidase: Selenoenzyme. FEBS Letters 1973; 32:132-134.

Flohé L. The Selenoprotein Glutathione Peroxidase. In Glutathione: Chemical, Biochemical and Medical Aspects, Part A; Dolphin D, Poulson R, Avramovic O, Eds; John Wiley & Sons Inc: New York, NY, USA 1989; 643–731.

Fomenko DE, Novoselov SV, Natarajan SK, Lee BC, Koc A, Carlson BA, et al. MsrB1 (methionine-R-sulfoxide reductase 1) knock-out mice: roles of MsrB1 in redox regulation and identification of a novel selenoprotein form. J Biol Chem 2009;284(9):5986-93.

Fonfría E, Vilaró MT, Babot Z, Rodríguez-Farré E, Suñol C. Mercury Compounds disrupt Neuronal Glutamate in cultured Mouse Cerebellar Granule Cells. J Neurosci Res 2005; 79:545-553.

Forstrom JW, Zakowski JJ, Tappel AL. Identification of the catalytic site of rat liver glutathione peroxidase as selenocysteine. Biochemistry 1978; 17:2639–2644.

Franco JL, Posser T, Dunkley PR, Dickson PW, Mattos JJ, Martins R, Bainy AC, Marques MR, Dafre AL, Farina M. Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. Free Radic Biol Med 2009; 47:449-57.

Franco R, Sanchez-Olea R, Reyes-Reyes EM, Panayiotidis MI. Environmental toxicity, oxidative stress and apoptosis: Ménage à Trois. Mutat Res 2009; 674:3-22.

Fredriksson A, Gardlund AT, Bergman K, Oskarsson A, Ohlin B, Danielsson B, Archer T. Effects of Maternal Dietary Supplementation with Selenite on the Post-natal Development of Rat Offspring Exposed to Methyl Mercury in Utero. Pharmacol Toxicol 1993;72:377–82.

Freed GL, Andreae MC, Cowan AE, Katz SL. The process of public policy formulation: the case of thimerosal in vaccine. Pediatrics 2002; 109:1153-9.

Freitas AS, Funck VR, Rotta Mdos S, Bohrer D, Mörschbächer V, Puntel RL, et al. Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice. Brain Res Bull 2009;79(1):77-84.

Freitas AS, Prestes AS, Wagner C, Sudati JH, Alves D, Porciúncula LO, Kade IJ, Rocha JBT. Reduction of diphenyl diselenide and analogs by mammalian thioredoxin reductase is independent of their gluthathione peroxidase-like activity: a possible novel pathway for their antioxidant activity. Molecules 2010; 15:7699–7714.

Gabryel B, Malecki A. Ebselen attenuates oxidative stress in ischemic astrocytes depleted of glutathione. Comparison with glutathione precursors. Pharmacol Rep 2006; 58:381–392.

Galindo MF, Jordan J, Gonzalez-Garcia C, Cena VReactive oxygen species induce swelling and cytochrome c release but not transmembrane depolarization in isolated rat brain mitochondria. Brain Journal Pharmacology 2003; 139:797-804.

Ganther HE, Goudie C, Sunde ML, Kopecky MJ, Wagner P. Selenium: Relation to Decreased Toxicity of Methylmercury Added to Diets Containing Tuna. Science 1972;175:1122–24.

Ganther HE, Ip C. Thioredoxin reductase activity in rat liver is not affected by supranutritional levels of monomethylated selenium in vivo and is inhibited only by high levels of selenium in vitro. J Nutr 2001; 131:301–304.

Ganther HE. Modification of methyl mercury toxicity and metabolism by vitamin E and selenium. Environ Health Perspect 1978;25:71–76

Ganther HE. Relationship between the chemical form of selenium and anticarcinogenic activity. In Cancer Chemoprevention. Edited by Wattenberg I, Lipkin M, Boon CW, Kellott GJ. Boca Raton, FL. CRC Press 1992; 479-488

Ganther HE. Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. Carcinogenesis 1999; 20:1657–1666.

Geier MR, Geier DA. The potential importance of steroids in the treatment of autistic spectrum disorders and other disorders involving mercury toxicity. Med Hypotheses 2005; 64:946–954.

Ghisleni G, Kazauckas V, Both FL, Pagnussat N, Mioranzza S, Rocha JBT, Souza DO, Porciúncula LO. Diphenyl diselenide exerts anxiolytic-like eVect in Wistar rats: putative roles of GABAA and 5HT receptors. Prog Neuro-Psychopharmacol Biol Psychiatry 2008; 32:1508–1515.

Ghisleni G, Porciúncula LO, Cimarosti H, Rocha JBT, Salbego CG, Souza DO. Diphenyl diselenide protects rat hippocampal slices submitted to oxygen-glucose deprivation and diminishes inducible nitric oxide synthase immunocontent. Brain Res 2003; 986:196–199.

Gill R, Tsung A, Billiar T. Linking oxidative stress to inflammation: toll-like receptors. Free Rad Biol Med 2010; 48:1121–1132.

Giometti CS, Liang X, Tollaksen SL, Wall DB, Lubman DM, Subbarao V, Rao MS. Mouse liver selenium-binding protein decreased in abundance by peroxisome proliferators. Electrophoresis 2000; 21:2162–2169.

Gladyshev VN, Kryukov G, Fomenko D E, Hatfield D L. Identification of trace element-containing proteins in genomic databases. Ann Rev Nutr 2004; 24:579–596.

Glaser V, Moritz B, Schmitz A, Dafré AL, Nazari EM, Rauh Müller YM, et al. Protective effects of diphenyl diselenide in a mouse model of brain toxicity. Chem Biol Interact 2013;206(1):18-26.

Goldman LR, Shannon MW. American Academy of Pediatrics: committee on Environmental Health Technical report: mercury in the environment: implications for pediatricians. Pediatrics 2001; 108: 197–205.

Goldwater L. Mercury: A history of quicksilver. Baltimore, MD: York Press. 1972.

González S, Huerta JM, Fernández S, Patterson AM, Lasheras C. Life-quality indicators in elderly people are influenced by selenium status. Aging Clin Exp Res 2007; 19(1):10-5.

Goyer RA. Toxic and essential metal interactions. Annu Rev Nutr 1997;17:37-50.

Gromer S, Eubel JK, Lee B L, Jacob J. Human selenoproteins at a glance. Cell Mol Life Sci 2005;62:2414–2437.

Groth DH, Stettler L, Mackay G. Interactions of Mercury, Cadmium, Selenium, Tellurium, Arsenic and Beryllium. In: Effects and Dose Response Relationships of Toxic Metals; Nordberg, G. (ed.). Elsevier Science Publishers, Amsterdam 1976; 527-543.

Guimaraes MJ, Peterson D, Vicari A, Cocks BG, Copeland NG, Gilbert DJ, et al. Identification of a novel selD homolog from eukaryotes, bacteria, and archaea: is there an autoregulatory mechanism in selenocysteine metabolism? Proc Natl Acad Sci USA 1996; 93:15086–15091.

Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Oxford University press 1999.

Halsey NA. Limiting infant exposure to thimerosal in vaccines and other sources of mercury. JAMA 1999; 282:1763–1766.

Han SJ, Lee BC, Yim SH, Gladyshev VN, Lee SR. Characterization of Mammalian Selenoprotein O: A Redox-Active Mitochondrial Protein. PLOS ONE 2014; 9(4):e95518.

Harada M. Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit Rev Toxicol 1995; 25:1-24.

Hartmanis MG, Stadtman TC. Isolation of a selenium-containing thiolase from Clostridium kluyveri: identification of the selenium moiety as selenomethionine. Proc Natl Acad Sci USA 1982; 79(16):4912-6.

Hatfield DL, Berry M J, Gladyshev V N. Selenium: Its Molecular Biology and Role in Human Health, 2nd Ed., Springer-Verlag New York Inc., New York. 2006.

Hawkes WC, Kelley DS, Taylor PC. The effects of dietary selenium on theimmune system in healthy men. Biol Trace Elem Res 2001; 81(3):189-213.

Heitaroh I, Tohru M, Hirokazu K, Masaaki H. Involvement of tissue sulfhydryls in the formation of a complex of methylmercury with selenium 1981; 30(23):3159-63.

Hoffman DJ, Heinz, G.H. Effects of Mercury and Selenium on Glutathione Metabolism and Oxidative Stress in Mallard Ducks. Environ Toxicol Chem 1998;17:161-66.

Holmgren A. Thioredoxin and glutaredoxin systems. J Biol Chem 1989; 264:13963–13966.

Hondal RJ, Ruggles EL. Differing views of the role of selenium in thioredoxin reductase. Amino Acids 2011; 41:73–89.

Huang CF, Liu SH, Hsu CJ, Lin-Shiau SY. Neurotoxicological Effects of Low-Dose Methylmercury and Mercuric Chloride in Developing Offspring Mice. Toxicol Lett 2011; 201:196-204.

Huber RE, Criddle RS. Comparison of the chemical properties of selenocysteine and selenocystine with their sulfur analogs. Arch Biochem Biophys 1967; 122:164–173.

Hussain S, Rodgers DA, Duhart HM, Ali SF. Mercuric Chloride-Induced Reactive Oxygen Species and Its Effect on Antioxidant Enzymes in Different Regions of Rat Brain. J Environ Sci Heal B1997; 32:395-409.

Ibiebele TI, Hughes MC, Nagle CM, Bain CJ, Whiteman DC, Webb PM, et al. Dietary antioxidants and risk of Barrett's esophagus and adenocarcinoma of the esophagus in an Australian population. Int J Cancer 2013; 133(1):214–24.

Imai H, Nakagawa Y. Biological significance of phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPx4) in mammalian cells. Free Radic Biol Med 2003; 34: 145–169.

Imura, N. Naganuma A. Possible Mechanism of Detoxifying Effect of Selenium on the Toxicity of Mercury Compounds. Advances in mercury toxicology, Rochester Series on Environmental Toxicity 1991, pp 275-288

Insug O, Datar S, Koch C J, Shapiro IM, Shenker BJ. Mercuric compounds inhibit human monocyte function by inducing apoptosis: Evidence for formation of reactive oxygen

species, development of mitochondrial membrane permeability transition and loss of reductive reserve. Toxicology 1997; 124:211-224.

Jackson TA. Mercury in Aquatic ecosystems. In: Metal Metabolism in Aquatic Environments; W.J. Langston and M. J. Bebianno (Eds.); Chapman & Hall Publishers, London 1998; 77-318.

Jalili MA, Abbasi AH. Poisoning by ethyl mercury toluene sulfonanilide. Br J Ind Med 1961; 18:303–308.

Jayaprakash V, Marshall JR. Selenium and other antioxidants for chemoprevention of gastrointestinal cancers. Best Pract Res Clin Gastroenterol 2011; 25(4–5):507–18.

Johansson C, Castoldi AF, Onishchenko N, Manzo L, Vahter M, Ceccatelli S. Neurobehavioural and molecular changes induced by methylmercury exposure during development. Neurotox Res 2007; 11:241-260.

Johshita H, Sasaki T, Matsui T, Hanamura T, Masayasu H, Asano T, Takakura K. Effects of ebselen (PZ-51) on ischaemic brain oedema after focal ischaemia in cats. Acta Neurochir Suppl (Wien) 1990; 51:239–241.

Kabata-Pendias A. Geochemistry of selenium. J Environ Pathol Toxicol Oncol 1998;17(3–4):173–7.

Khan MAK, Wang F. Mercury-Selenium Compounds and their Toxicological Significance: Toward a Molecular Understanding of the Mercury-Selenium Antagonism. Environ Toxicol Chem 2009; 28:1567–77.

Kieliszek M, Bła˙zejak S. Selenium: significance, and outlook for supplemen-tation. Nutrition 2013; 29(5):713–8.

Kim HY, Gladyshev VN. Methionine sulfoxide reduction in mammals: characterization of methionine-R-sulfoxide reductases. Mol Biol Cell 2004; 15:1055–1064.

Kiremidjian-Schumacher, Roy M, Glickman R. Selenium and immunocom-petence in patients with head and neck cancer. Biol Trace Elem Res 2000; 73:97–111.

Knollema S, Elting JW, Dijkhuizen RM, Nicolay K, Korf J, Ter Horst GJ. Ebselen (PZ-51) protects the caudate putamen against hypoxia/ischemia induced neuronal damage. Neurosci Res Commun 1996; 19:47–56.

Koeman JH, Van de Ven WSM, de Goeij JJM, Tijoe PS, Van Haaften JL. Mercury and Selenium in Marine Mammals and Birds. Sci Total Environ 1975;3:279–87.

Köhrle J.The deiodinase family: selenoenzymes regulating thyroid hormone availability and action. Cell Mol Life Sci 2000; 57(13-14):1853-63.

Kollock CW. Use of Mercury in Traumatic Irido-Choroiditis. Trans Am Ophthalmol Soc 1896; 7:602-606.

Kosta L, Byrne AR, Zelenko V. Correlation between Selenium and Mercury in Man Following Exposure to Inorganic Mercury. Nature 1975;254:238–39.

Kryukov GV, Castellano S, Novoselov SV, Lobanov AV, Zehtab O, Guigo R, Gladyshev VN. Characterization of mammalian selenoproteomes. Science 2003; 300:1439-1443.

Kryukov GV, Gladyshev VN. The prokaryotic selenoproteome. EMBO Rep 2004; 5:538-543.

Kuiper GG, Kester MH, Peeters RP, Visser TJ. Biochemical mechanisms of thyroid hormone deiodination .Thyroid 2005; 15:787–798.

Kumar BS, Kunwar A, Singh BG, Ahmad A, Priyadarsini KI. Anti-hemolytic andperoxyl radical scavenging activity of organoselenium compounds: an in vitrostudy. Biol Trace Elem Res 2011; 140(2):127–38.

Kumar BS, Tiwari SK, Manoj G, Kunwar A, Amrita N, Sivaram G, et al. Anti-ulcer and antimicrobial activities of sodium selenite against Helicobacterpylori: in vitro and in vivo evaluation. Scand J Infect Dis 2010; 42(4):266–74.

Kunwar A, Jain VK, Priyadarsini KI, Haston CK. A selenocysteine derivativetherapy affects radiation-induced pneumonitis in the mouse. Am J Respir Cell Mol Biol 2013; 49(4):654–61.

Lettmeier B, Boese-O'Reilly S, Drasch G. Proposal for a Revised Reference Concentration (RfC) for Mercury Vapour in Adults. Sci Total Environ 2010; 408:3530–3535.

Leyck S, Parnham MJ. Acute antiinflammatory and gastric effects of the seleno-organic compound ebselen. Agents Actions 1990; 30:426–431.

Li Q, Liu M, Hou J, Jiang C, Li S, Wang T. The prevalence of Keshan disease in China. Int J Cardiol 2013; 168(2):1121–6.

Li QJ, Bessems JG, Commandeur JN, Adams B, Vermeulen NP. Mechanism of protection of ebselen against paracetamol- induced toxicity in rat hepatocytes. Biochem Pharmacol 1994; 48:1631–1640.

Li YF, Dong Z, Chen C, Li B, Gao Y, Qu L, et al. Organic Selenium Supplementation Increases Mercury Excretion and Decreases Oxidative Damage in Long-Term Mercury Exposed Residents from Wanshan. Environ Sci Technol 2012;46:11313−18.

Lillig CH, Holmgren A. Thioredoxin and related molecules-from biology to health and disease Antioxid. Redox Signal 2007; 9:25–47.

Lipinski B. Rationale for the treatment of cancer with sodium selenite. Medical Hypotheses 2005;64:806–810.

Lippman SM, Goodman PJ, Klein EA, Parnes HL, Thompson IM Jr, Kristal AR, Santella RM, Probstfield JL, Moinpour CM, Albanes D, Taylor PR, Minasian LM,Hoque A, Thomas SM, Crowley JJ, Gaziano JM, Stanford JL, Cook ED, Fleshner NE, Lieber MM, Walther PJ, Khuri FR, Karp DD, Schwartz GG, Ford LG, Coltman CA Jr. Designing the Selenium and Vitamin E Cancer Prevention Trial. J Natl Cancer Inst 2005; 97(2):94-102.

Liu J, Rozovsky S. Contribution of selenocysteine to the peroxidase activity of selenoprotein S. Biochemistry 2013;52(33):5514-6.

Lobanov AV, Delgado C, Rahlfs S, Novoselov SV, Kryukov GV, Gromer S, Hatfield DL, Becker K, Gladyshev VN. The Plasmodium selenoproteome. Nucleic Acids Res 2006; 34:496-505.

Lobanov AV, Fomenko DE, Zhang Y, Sengupta A, Hatfield DL, Gladyshev VN. Evolutionary dynamics of eukaryotic selenoproteomes: large selenoproteomes may associate with aquatic life and small with terrestrial life. Genome Biol 2007; 8:R198.

Lobanov AV, Hatfield DL, Gladyshev VN. Eukaryotic selenoproteins and selenoproteomes. Biochim Biophys Acta 2009; 1790:1424–1428.

Lobanov AV, Hatfield DL, Gladyshev VN. Reduced reliance on the trace element selenium during evolution of mammals. Genome Biol 2008; 9:R62.

Look MP, Rockstroh JK, Rao GS, Kreuzer KA, Spengler U, Sauerbruch T. Serum selenium versus lymphocyte subsets and markers of disease progression and inflammatory response in human immunodeficiency virus-1 infection. Biol Trace Elem Res 1997; 56:31–41.

Lovell MA, Xiong S, Lyubartseva G, Markesbery WR. Organoselenium (Sel-Plex diet) decreases amyloid burden and RNA and DNA oxidative damage in APP/PS1 mice. Free Radical Biology and Medicine 2009; 46:1527–1533.

Luthman M, Holmgren A. Rat liver thioredoxin and thioredoxin reductase: purification and characterization. Biochemistry 1982; 21:6628–6633.

Ma J-L, Zhang L, Brown LM, Li J-Y, Shen L, Pan K-F, et al. Fifteen-year effects of Helicobacter pylori, garlic, and vitamin treatments on gastric cancer incidenceand mortality. J Natl Cancer Inst 2012; 104(6):488–92.

Machado MS, Rosa RM, Dantas AS, Reolon GK, Appelt HR, Braga AL, Henriques JAN, Roesler R. An organic selenium compound attenuates apomorphine-induced stereotypy in mice. Neurosci Lett 2006; 410:198–202.

Magos L, Webb M. Difference in distribution and excretion of selenium and cadmium or mercury after their simultaneous administration subcutaneously in equimolar doses. Arch Toxicol 1976;36:63–69.

Magos L, Webb M. The Effect of Selenium on the Brain Uptake of Methylmercury. Arch Toxicol 1977;38:201-07.

Magos L. Physiology and Toxicology of Mercury. In: Metal Ions in Biological Systems, Volume 34: Mercury and its Effects on Environment and Biology; A. Sigel and H. Sigel (Eds.), Marcel Dekker Inc., New York, 1997; 321-370.

Mahaffey KR. Fish and Shellfish as Dietary Sources of Methylmercury and the omega-3 Fatty Acids, Eicosahexaenoic acid and Docosahexaenoic acid: Risks and Benefits. Environ Res 2004; 95:414–428.

Mahalingam TR, Vijayalakshmi S, Prabhu RK, Thiruvengadasami A, Wilber A, Mathews CK, et al. Studies on some trace and minor elements in blood. Asurvey of the Kalpakkam (India) population. Part III: studies on dietary intakeand its correlation to blood levels. Biol Trace Elem Res 1997;57(3):223–38.

Mahboob M, Shireen KF, Atkinson A, Khan AT. Lipid Peroxidation and Antioxidant Enzyme Activity in Different Organs of Mice Exposed to Low Level of Mercury. J Environ Sci Heal B 2001; 36:687-697.

Malm O. Gold mining as a source of mercury exposure in the Brazilian Amazon. Environ Res 1998; 77:73-78.

Mariotti M, Ridge P G, Zhang Y, Lobanov A V, Pringle T H, Guigo R. Hatfield D L, Gladyshev V N. Composition and Evolution of the Vertebrate and Mammalian Selenoproteomes. PLoS ONE 2012; 7:1-18.

Martens DA, Suarez DL. Selenium speciation of soil/sediment determined with sequential extractions and hydride generation atomic absorption spectrophotometry. Environ Sci Technol 1996; 31:133–139.

Mason R. Mercury Emissions from Natural Processes and their Importance to the global Mercury Cycle. In : Mercury Fate and Transport in the Global Atmosphere; N. Pirrone and R.Mason (Eds.), Springer, New York 2009; 173-192.

Masukawa T, Kito H, Hayashi M, Iwata H. Formation and Possible Role of Bis(Methylmercuric) Selenide in Rats Treated with Methylmercury and Selenite. Biochem Pharmacol 1982;31:75-78.

Matheson DS, Clarkson TW, Gelfand EW. Mercury toxicity (acrodynia) induced by long-term injection of gamma globulin. J Pediatr 1980; 97:153 155.

Mehdi Y, Hornick J-L, Istasse L, Dufrasne I. Selenium in the environment, metabolism and involvement in body functions. Molecules 2013; 18(3):3292–311.

Meinerz DF, de Paula MT, Comparsi B, Silva MU, Schmitz AE, Braga HC, et al. Protective effects of organoselenium compounds against methylmercury-induced oxidative stress in mouse brain mitochondrial-enriched fractions. Braz J Med Biol Res 2011;44(11):1156-63.

Meister A. Glutathione Metabolism and its Selective Modification. J Biol Chem 1988; 263:17205-17208.

Mertz W. Trace minerals and atherosclerosis. Fed Proc 1982; 41(11):2807-12.

Meseguer M, Martínez-Conejero JA, Muriel L, Pellicer A, Remohí J, Garrido N. The human sperm glutathione system: a key role in male fertility andsuccessful cryopreservation. Drug Metab Lett 2007; 1(2):121–6.

Miller S, Walker SW, Arthur JR, Nicol F, Pickard K, Lewin MH, Howie AF, Beckett GJ. Selenite protects human endothelial cells from oxidative damage and induces thioredoxin reductase. Clin Sci 2001; 100:543–550.

Miranda-Vizuete A, Damdimopoulos E, Spyrou G. The mitochondrial thioredoxin system. Antioxid. Redox Signal 2000; 2: 801–810.

Mishra B, Priyadarsini KI, Mohana H, Mugesh G. Horseradish peroxidase inhibition and antioxidant activity of ebselen and related organoselenium compounds. Bioorg Med Chem Lett 2006; 16:5334–5338.

Mistry HD, Broughton Pipkin F, Redman CW, Poston L. Selenium in reproductive health. Am J Obstet Gynecol 2012; 206(1):21-30.

Moghadaszadeh B, Beggs AH. Selenoproteins and their impact on human health through diverse physiological pathways. Physiology (Bethesda). 2006; 21:307-15.

Moller-Madsen B, Danscher G. Localization of mercury in CNS of the rat. IV. The effect of selenium on orally administered organic and inorganic mercury. Toxicol Appl Pharmacol 1991;108:457–73.

Moller-Madsen B. Localization of mercury in CNS of the rat. II. Intraperitoneal injection of methylmercuric chloride (CH3HgCl) and mercuric chloride (HgCl2). Toxicol Appl Pharmacol 1990;103:303–23.

Morel FMM, Kraepiel AML, Amyot M. The Chemical Cycle and Bioaccumulation of Mercury. Annu Rev Ecol Syst 1998; 29:543-566.

Mottet NK, Vahter ME, Charleston JS, Friberg LT. Metabolism of Methylmercury in the Brain and its Toxicological Significance. In: Metal Ions in Biological Systems, Mercury and its Effects on Environment and Biology; A. Sigel and H. Sigel (Eds.), Marcel Dekker Inc., New York 1997; 34: 371-403.

Mottet NK, Vahter ME, Charleston JS, Friberg LT. Metabolism of Methylmercury in the Brain and its Toxicological Significance. In: Metal Ions in Biological Systems, Volume 34: Mercury and its Effects on Environment and Biology; A. Sigel and H. Sigel (Eds.), Marcel Dekker Inc, New York 1997; 371-403.

Myers GJ, Davidson PW, Strain J J. Nutrient and methyl mercury exposure from consuming fish. J Nutr 2007; 137:2805-2808.

Nagamuna A, Ishii Y, Imura N. Effect of Administration Sequence of Mercury Chloride and Sodium Selenite on their Fates and Toxicities in Mice. Eco Env Safe 1984; 8: 572- 80.

Navarro-Alarcon M, Cabrera-Vique C. Selenium in food and the human body: a review. Sci Total Environ 2008; 400(1–3):115–41.

Navarro-Alarcón M, López-Garcia de la Serrana H, Pérez-Valero V, López-Martínez C. Ann Nutr Metab 1999; 43:30–36.

Navarro-Alarcon M, López-Martínez MC. Essentiality of selenium in the human body: relationship with different diseases. Sci Total Environ 2000; 249:347–371.

Neve J. Selenium and cardiovascular pathology. Pathol Biol (Paris). 1989; 37(10):1102-6.

Newland MC, Reed MN, LeBlanc A, Donlin WD. Brain and Blood Mercury and Selenium after Chronic and Developmental Exposure to Methylmercury. Neurotoxicology 2006;27:710-20.

Nogueira CW, Quinhones EB, Jung EAC, Zeni G, Rocha JBT. Anti-inflammatory and antinociceptive activity of diphenyl diselenide. Inflamm Res 2003; 52:56.

Nogueira CW, Rocha J BT. Diphenyl Diselenide a Janus-Faced Molecule. J Braz Chem Soc 2010; 21(11):2055-2071.

Nogueira CW, Zeni G, Rocha JBT. Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem Rev 2004; 104:6255-6285.

Noiri E, Nakao A, Uchida K, Tsukahara H, Ohno M, Fujita T, Brodsky S, Goligorsky MS. Oxidative and nitrosative stress in acute renal ischemia. Am J Physiol Renal Physiol 2001; 281:F948–F957.

Novoselov SV, Rao M, Onoshko NV, Zhi H, Kryukov GV, Xiang Y, Weeks DP, Hatfield DL, Gladyshev VN. Selenoproteins and selenocysteine insertion system in the model plant cell system. Chlamydomonas reinhardtii. EMBO J 2002; 21:3681-3693.

Nuttall KL. Evaluating selenium poisoning. Ann Clin Lab Sci 2006; 36(4):409–20.

Ohi G, Nishigaki S, Seki H, Tamura Y, Maki T. Efficacy of selenium in tuna and selenite in modifying methylmercury intoxication. Environ Res 1976;12:49–58.

Papp LV, Holmgren A, Khanna KK. Selenium and selenoproteins in health and disease. Antioxid. Redox Signal 2010; *12*:793–795.

Papp LV, Lu J, Holmgren A, Khanna KK. From Selenium to Selenoproteins: Synthesis, Identity, and Their Role in Human Health. Antioxidants & Redox Signaling 2007; 9:775-806.

Parizek J, Ostadalova I, Kalouskva J, Babichy A, Benes J. The detoxifying effects of selenium. Interrelation between compounds of selenium and certain metals. In: Newer Trace Elements in Nutrition; W. Mertz and W.E. Cornatzer (Eds.) Marcel Dekker Inc, New York, USA, 1971; 85-122.

Parizek J, Ostadalova I. Protective Effect of Small Amounts of Selenite in Sublimate Intoxication. Experientia 1967;23:142-43.

Parnham MJ, Kindt S. A novel biologically-active organoselenium compound 3. EVects of PZ-51 (ebselen) on glutathione-peroxidase and secretory activities of mouse macrophages. Biochem Pharmacol 1984; 33:3247–3250.

Pillai SS, Sugathan JK, Indira M. Selenium downregulates RAGE and NF-Bexpression in diabetic rats. Biol Trace Elem Res 2012; 149(1):71–7.

Pinheiro MCN, Harada M, Yasoda E, Nakanishi J, Oikawa T, Vieira JLF. Toxicological and epidemiological data on human exposure to mercury in the Tapajós River basin: 1994-1998. Environmental Science 2003; 10:99- 105.

Pinsent J. The need for selenite and molybdate in the formation of formic dehydrogenase by members of the coli-aerogenes group of bacteria. Biochem J 1954; 57:10-16.

Pinton S, da Rocha JT, Zeni G, Nogueira CW. Organoselenium improves memory decline in mice: involvement of acetylcholinesterase activity. Neurosci Lett 2010; 472:56–60.

Pollard KM, Hultman P. Effects of mercury on the immune system. Met Ions Biol Syst 1997; 34:421-440.

Porciúncula LO, Rocha JBT, Cimarosti H, Vinade L, Ghisleni G, Salbego CG, Souza DO. Neuroprotective effect of ebselen on rat hippocampal slices submitted to oxygen-glucose deprivation: correlation with immunocontent of inducible nitric oxide synthase. Neurosci Lett 2003; 346:101–104.

Posser T, Kaster MP, Baraúna SC, Rocha JBT, Rodrigues ALS, Leal RB. Antidepressant-like eVect of the organoselenium compound ebselen in mice: evidence for the involvement of the monoaminergic system. Eur J Pharmacol 2009; 602:85–91.

Prevost G, Arabo A, Jian L, Quelennec E, Cartier D, Hassan S, et al. The PACAP regulated gene selenoprotein T is abundantly expressed in mouse and human β-cells and its targeted inactivation impairs glucose tolerance. Endocrinology 2013; 154(10):3796-806.

Prigol M, Brüning CA, Godoi B, Nogueira CW, Zeni G. m-Tri-fluoromethyl-diphenyl diselenide attenuates pentylenetetrazoleinduced seizures in mice by inhibiting GABA uptake in cerebral cortex slices. Pharmacol Rep 2009a; 61:1127–1133.

Prigol M, Bruning CA, Zeni G, Nogueira CW. Protective effect of disubstituted diaryl diselenides on cerebral oxidative damage caused by sodium nitroprusside. Biochem Eng J 2009b; 45:94–99.

Pumford NR, Martin BM, Hinson JA. A metabolite of acetaminophen covalently binds to the 56 k Da selenium binding protein. Biochem Biophys Res Commun 1992; 182:1348–1355.

Radi R, Cassina A, Hodara RNitric oxide and peroxynitrite interactions with mitochondria. Biological Chemistry 2002; 383:401- 9.

Ralston NV, Raymond LJ. Dietary selenium's protective effects against methylmercury toxicity. Toxicology. 2010;278(1):112-23.

Ralston NVC, Blackwell JL, Raymond LJ. Importance of molar ratios in selenium dependent protection against methylmercury toxicity. Biol Trace Elem Res 2007;119:255–68.

Ralston NVC, Ralston CR, Blackwell III JL, Raymond LJ. Dietary and tissue selenium in relation to methylmercury toxicity. Neurotoxicology 2008;29:802–11.

Rayman MP, Infante HG, Sargent M. Food-chain selenium and human health: spotlight on speciation. Br J Nutr 2008; 100(2):238–53.

Rayman MP. Food-chain selenium and human health: emphasis on intake. BrJ Nutr 2008;100(2):254–68.

Rayman MP. Selenium and human health. The Lancet 2012; 379(9822):1256 – 1268.

Rayman MP. The importance of selenium to human health. Lancet 2000; 356:233–241.

Reeves MA, Bellinger FP, Berry MJ. The neuroprotective functions of selenoprotein M and its role in cytosolic calcium regulation. Antioxid Redox Signal. 2010; 12(7):809-18.

Reichl FX, Walther UI, Durner J, Kehe K, Hickel R, Kunzelmann KH, Spahl W, Hume WR, Benschop H, Forth W. Cytotoxicity of dental composite components and mercury compounds in lung cells. Dental Material 2001; 17:95-101.

Reid M, Duffield-Lillico AJ, Garland L, Turnbull BW, Clark LC, Marshall JR. Selenium supplementation and lung cancer incidences: an update of the nutritional prevention cancer trial. Cancer Epidemiol Biomarkers Prev 2002; 11:1285-1291.

Ricetti MM, Guidi GC, Tecchio C, Bellisola G, Rigo A, Perona G. Effects of sodium selenite on in vitro interactions between platelets and endothelial cells. Int J Clin Lab Res 1999; 29(2):80–4.

Rooney JPK. The role of thiols, dithiols, nutritional factors and interacting ligands in the toxicology of mercury. Toxicology 2007; 234:145-156.

Roos DH, Robson Luiz Puntel RL, Lugokenski TH, Ineu RP, Bohrer D, Burger ME, Franco JL, Farina M, Aschner M, Rocha JBT, Barbosa NBV. Complex Methylmercury–Cysteine Alters Mercury Accumulationin Different Tissues of Mice. Basic Clin Pharmacol 2010; 107:789-792.

Roos DH, Puntel RL, Santos MM, Souza DO, Farina M, Nogueira CW, Aschner M, Burger ME, Barbosa NB, Rocha JB. Guanosine and synthetic organoselenium compounds modulate methylmercury-induced oxidative stress in rat brain cortical slices: involvement of oxidative stress and glutamatergic system. Toxicol In Vitro 2009; 23:302–307.

Rosa RM, Flores DG, Appelt HR, Braga AL, Henriques JA, Roesler R. Facilitation of long-term object recognition memory by pretraining administration of diphenyl diselenide in mice. Neurosci Lett 2003; 341:217–220.

Rotruck J T, Pope A L, Ganther H E, Swanson A B, Hafeman D G, Hoekstra W G. Selenium: Biochemical Role As a Component of Glutathione Peroxidase . Science 1973; 179:588-590.

Roy M, Kiremidjian-Schumacher L, Wishe HI, Cohen MW, Stotzky G. Supplementation with selenium restores age-related decline in immune cell function. Proc Soc Exp Biol Med 1995; 209(4):369–75.

Roy M, Kiremidjian-Schumacher L, Wishe HI, Cohen MW, Stotzky G. Supplementation with selenium and human immune cell functions. I. Effect onlymphocyte proliferation and interleukin 2 receptor expression. Biol Trace Elem Res 1994; 41(1–2):103–14.

Saad SY, Najjar TA, Arafah MM. Cardioprotective effects of subcutaneous ebselen against daunorubicin-induced cardiomyopathy in rats. Basic Clin Pharmacol Toxicol 2006; 99:412–417

Saito I, Asano T, Sano K, Takakura K, Abe H, Yoshimoto T, Kikuchi H, Ohta T, Ishibashi S. Neuroprotective effect of an antioxidant, ebselen, in patients with delayed neurological deficits after aneurysmal subarachnoid hemorrhage. Neurosurgery 1998; 42:269–277.

Salonen JT, Alfthan G, Huttunen JK, Pikkarainen J, Puska P. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. Lancet 1982; 2(8291):175–9.

Salvatore D, Low S C, Berry M, Maia A , Harney J W, Croteau W. et al. Type 3 lodothyronine deiodinase: cloning, in vitro expression, and functional analysis of the placental selenoenzyme. J Clin Invest 1995; 96: 2421–2430.

Santos APM, Mateus ML, Carvalho CML, Batoréu MCC. Biomarkers of exposure and effect as indicators of the interference of selenomethionine on methylmercury toxicity. Toxicol Lett 2007;169:121–128.

Santos FW, Zeni G, Rocha JBT, Weis SN, Fachinetto JM, Favero AM, Nogueira CW. Diphenyl diselenide reverses cadmium-induced oxidative damage on mice tissues. Chem Biol Interact 2005; 15:159.

Satoh M, Nishimura N, Kanayama Y, Naganuma A, Suzuki T, Tohyama C. Enhanced renal toxicity by inorganic mercury in metallothionein-null mice. J Pharmacol Exp Ther 1997; 283:1529–1533.

Savegnago L, Jesse CR, Pinto LG, Rocha JBT, Nogueira CW, Zeni G. Monoaminergic agents modulate antidepressant-like effect caused by diphenyl diselenide in rats. Prog Neuro Psychopharmacol Biol Psychiatry 2007; 31:1261–1269.

Savegnago L, Jesse CR, Santos ARS, Rocha JBT, Nogueira CW. Mechanisms involved in the antinociceptive eVect caused by diphenyl diselenide in the formalin test. J Pharm Pharmacol 2008; 60:1679–1686.

Savegnago L, Trevisan M, Alves D, Rocha JBT, Nogueira CW, Zeni G. Antisecretory and antiulcer effects of diphenyl diselenide. Environ Toxicol Pharmacol 2006; 21:86–92.

Schnabel R, Lubos E, Messow CM, Sinning CR, Zeller T, Wild PS, et al. Selenium supplementation improves antioxidant capacity in vitro and in vivoin patients with coronary artery disease. The Selenium Therapy in Coronary Artery disease Patients (SETCAP) Study. Am Heart J 2008; 156(6):1201[e1–11].

Schomburg L, Köhrle J. On the importance of selenium and iodine metabolismfor thyroid hormone biosynthesis and human health. Mol Nutr Food Res 2008; 52(11):1235–46.

Schwaab V, Faure J, Dufaure JP, Drevet JR. Gpx3: The plasma-type glutathione peroxidase is expressed under androgenic control in the mouse epididymis and vas deferens. Mol Reprod Dev 1998; *51*:362–372.

Schwarz K, Foltz CM. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. 1951. Jornal of the American chemical society 1957; 29:3292-3293.

Schweizer U, Bräuer AU, Köhrle J, Nitsch R, Savaskan NE Selenium and brain function: a poorly recognized liaison. Brain Res Brain Res Rev 2004; 45:164–178.

Scott R, MacPherson A, Yates RW, Hussain B, Dixon J. The effect of oral selenium supplementation on human sperm motility. Br J Urol 1998; 2(1):76–80.

Seegal RF, Dreiem A. Methylmercury-induced changes in mitochondrial function in striatal synaptosomes are calcium-dependent and ROS- independent. NeuroToxicology 2007; 28:720-726.

Selenius M, Fernandes AP, Brodin O, Björnstedt M, Rundlöf AK. Treatment of lung cancer cells with cytotoxic levels of sodium selenite: Effects on the thioredoxin system. Biochem Pharmacol 2008; 75:2092-2099.

Seppanen K, Soininen P, Salonen JT, Lotjonen S, Laatikainen R. Does mercury promote lipid peroxidation? An in vitro study concerning mercury, copper, and iron in peroxidation of low-density lipoprotein. Biol Trace Elem Res 2004;101:117–32.

Shalini S, Bansal MP. Role of selenium in regulation of spermatogenesis: involvement of activator protein 1. Biofactors 2005; 23(3):151–62.

Shanker G, Syversen T, Aschner JL. Aschner MModulatory effect of glutathione status and antioxidants on methylmercury-induced free radical formation in primary cultures of cerebral astrocytes. Brain Res Mol Brain Res 2005; 137:11-22.

Shenker BJ, Datar S, Mansfield K, Shapiro IM. Induction of apoptosis in human T-cells by organomercuric compounds: A flow cytometric analysis. Toxicological Applie Pharmacology 1997; 143:397-406.

Sher L. Selenium, thyroid hormones, mood, and behaviour. Can J Psychiatry 2002; 47(3):284–5.

Shrimali RK, Irons RD, Carlson BA, Sano Y, Gladyshev VN, Park JM, et al. Seleno-proteins mediate T cell immunity through an antioxidant mechanism. J BiolChem 2008; 283(29):20181–5.

Sies H, Masumoto H. Ebselen as a glutathione peroxidase mimic and as a scavenger of peroxynitrite. Adv Pharmacol 1997; 38:229–246.

Simmons-Willis TA, Koh AS, Clarkson TW, Ballatori N. Transport of a Neurotoxicant by Molecular Mimicry: The Methylmercury–L-cysteine Complex is a Substrate for Human L-type large Neutral Amino acid Transporter (LAT) 1 and L AT2. Biochem J 2002; 367:239–246.

Soderberg A, Sahaf B, Rosen A. Thioredoxin reductase, a redox-active selenoprotein, is secreted by normal and neoplastic cells: presence in human plasma. Cancer Res 2000; 60:2281–2289.

Spyrou G, Holmgren A. Deoxyribonucleoside triphosphate pools and growth of glutathione-depleted 3T6 mouse fibroblasts. Biochem Biophys Res Commun 1996; 220:42–46.

Spyrou G, Björnstedt M, Skog S, Holmgren A. Selenite and selenite inhibit human lymphocyte growth via different mechanisms. Cancer Res 1996; 56:4407–12.

Squires J E, Berry M J. Eukaryotic Selenoprotein Synthesis: Mechanistic Insight Incorporating New Factors and New Functions for Old Factors. IUBMB Life 2008; 60:232–235.

Stranges S, Marshall JR, Trevisan M, Natarajan R, Donahue RP, Combs GF, et al. Effects of selenium supplementation on cardiovascular disease incidence and mortality: secondary analyses in a randomized clinical trial. Am J Epidemiol 2006; 163(8):694–9.

Ström S, Helmfrid I, Glynn A, Berglund M. Nutritional and toxicological aspects of seafood consumption - An integrated exposure and risk assessment of Methylmercury and polyunsaturated fatty acids. Environ Res 2011; 111:274-280.

Sugiura Y, Hojo Y, Tamai Y, Tanaka H. Letter: Selenium protection against mercury toxicity. Binding of methylmercury by the selenohydryl-containing ligand. J Am Chem Soc 1976;98(8):2339–41.

Sun QA, Kirnarsky L, Sherman S, Gladyshev VN. Selenoprotein oxidoreductase with specifi city for thioredoxin and glutathione systems. Proc Natl Acad Sci USA 2001; 98**:** 3673–3678.

Surai PF. Selenium in Nutrition and Health. Nottingham University Press; Nottingham. 2006.

Suzuki KT, Ogra Y. Metabolism of Selenium and its Interaction with Mercury: Mechanisms by a Speciation Study. Phosphorus Sulfur 2001;171:135-69.

Suzuki KT. Equimolar Hg–Se complex binds to selenoprotein P. Biochem. Biophys. Res. Commun. 1997; 231:7–11.

Szefer P, Domagal-Wieloszewska M, Warzocha A, Garbacik-Wesolowska A, Ciesielski T. Distribution and relationships of mercury, lead, cadmium, copper and zinc in perch (Perca fluviatilis) from the Pomeranian Bay and Szczecin Lagoon, southern Baltic. Food Chem 2003; 81:73-83.

Tabuchi Y, Sugiyama N, Horiuchi T, Furusawa M, Furuhama K. Ebselen, a seleno-organic compound, protects against ethanolinduced murine gastric mucosal injury in both in vivo and in vitro systems. Eur J Pharmacol 1995; 272:195–201.

Taskov K, Chapple C, Kryukov GV, Castellano S, Lobanov AV, Korotkov KV, Guigo R, Gladyshev VN: Nematode selenoproteome: the use of the selenocysteine insertion system to decode one codon in an animal genome? Nucleic Acids Res 2005; 33:2227-2238.

Toulis KA, Anastasilakis AD, Tzellos TG, Goulis DG, Kouvelas D. Selenium supplementation in the treatment of Hashimoto's thyroiditis: a systematic review and a meta-analysis. Thyroid 2010; 20(10):1163–73.

Tujebajeva RM, Copeland PR, Xu XM, Carlson BA, Harney JW, Driscoll DM, et al. Decoding apparatus for eukaryotic selenocysteine insertion. EMBO Rep 2000; 1:158-163.

Turan B. Role of antioxidants in redox regulation of diabetic cardiovascular complications. Curr Pharm Biotechnol 2010; 11(8):819–36.

Tuve T, Williams HH. Metabolism of selenium by Escherichia coli: biosynthesis of selenomethionine. J Biol Chem 1961; 236:597-601.

Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39(1):44-84.

Venardos K, Harrison G, Headrick J, Perkins A. Effects of dietary selenium on glutathione peroxidase and thioreduxin reductase activity and recovery from cardiac ischemia-reperfusion. Journal of Trace Elements in Medicine and Biology 2004; 18:81-88.

Venturi S, Venturi M. Evolution of dietary antioxidant defences. European EPI-Marker 2007; 11:1–12.

Vinceti M, Bonvicini F, Bergomi M, Malagoli C. Possible involvement of overexposure to environmental selenium in the etiology of amyotrophic lateral sclerosis: a short review. Ann Ist Super Sanita. 2010; 46(3):279-83.

Virtanen KJ, Rissanen TH, Voutilainen S, Tuomainen TP. Mercury as a risk factor for cardiovascular diseases. J Nutr Biochem 2007*;* 18:75–85.

Wada M, Nobuki S-I, Tenkyuu Y, Natsume S, Asahara M, Erabi T. Bis(2,6-dimethoxyphenyl) sulfide, selenide and telluride, and their derivatives. J Organomet Chem 1999; 580:282–289.

Wagner C, Sudati JH, Nogueira CW, Rocha JBT. In vivo and in vitro inhibition of mice thioredoxin reductase by methylmercury. Biometals 2010; 23:1171-1177.

Walczak R, Westhof E, Carbon P, Krol A. A novel RNA structural motif in the selenocysteine insertion element of eukaryotic selenoprotein mRNAs. RNA 1996; 2:367–379.

Wang T, Liu B, Qin L, Wilson B, Hong JS. Protective effect of the SOD/catalase mimetic MnTMPyP on inflammation-mediated dopaminergic neurodegeneration in mesencephalic neuronal-glial cultures. J Neuroimmunol 2004; 147:68–72.

Warkany J, Hubbard DM. Acrodynia and mercury. J Pediatr 1953; 42:365-386.

Watanabe C, Yin K, Kasanuma Y, Satoh H. In utero exposure to methylmercury and Se deficiency converge on the neurobehavioral outcome in mice. Neurotoxicol Teratol 1999a;21:83–88.

Watanabe C, Yoshida K, Kasanuma Y, Kun Y, Satoh H. In utero methylmercury exposure differentially affects the activities of selenoenzymes in the fetal mouse brain. Environ. Res. 1999b;80: 208–14.

Watanabe C. Modification of Mercury Toxicity by Selenium: Practical Importance? Tohoku J Exp Med 2002;196:71-77.

Weekley CM, Harris HH. Which form is that? The importance of selenium speciation and metabolism in the prevention and treatment of disease Chem Soc Rev 2013; 42:8870-8894.

Weiss JF, Landauer MR. Protection against ionizing radiation by antioxidantnutrients and phytochemicals. Toxicology 2003; 189(1–2):1–20.

Weiss JF, Landauer MR. Radioprotection by antioxidants. Ann N Y Acad Sci2000; 899:44–60.

Weiss JF, Srinivasan V, Kumar KS, Landauer MR. Radioprotection by metals: selenium. Adv Space Res 1992; 12(2–3):223–31.

Wen H, Carignan J. Reviews on atmospheric selenium: Emissions, speciation and fate. Atmospheric Environ 2007; *41*: 7151–7165.

Wendel A, Fausel M, Safayhi H, Tiegs G, Otter R. A novel biologically active seleno-organic compound-II. Activity of PZ 51 in relation to glutathione peroxidase. Biochem Pharmacol 1984; 33:3241–3246.

Whanger PD. Selenium and its relationship to cancer: an update dagger. Br J Nutr 2004; 91:11–28.

Whanger PD. Selenium in the treatment of heavy metal poisoning and chemical carcinogenesis. J. Trace Elem. Electrolytes Health Dis. 1992; 6 (4): 209–21.

Whanger PD. Selenocompounds in plants and animals and their biological significance. J Am Coll Nutr 2002; 21:223-232.

Wilhelm EA, Jesse CR, Leite MR, Nogueira CW. Studies on preventive eVects of diphenyl diselenide on acetaminopheninduced hepatotoxicity in rats. Pathophysiology 2009a; 16:31–37.

Wilhelm EA, Jesse CR, Nogueira CW, Savegnago L. Introduction of trifluoromethyl group into diphenyl diselenide molecule alters its toxicity and protective eVect against damage induced by 2-nitropropane in rats. Exp Toxicol Pathol 2009c; 61:197–203.

Wilhelm EA, Jesse CR, Nogueira CW. Protective eVect of p-methoxyl-diphenyl diselenide in lethal acute liver failure induced by lipopolysaccharide and d-galactosamine in mice. Fundam Clin Pharmacol 2009b; 23:727–734.

Wood, Beckham, Yosioka, Darban. Watson. beta-Carotene and selenium supplementation enhances immune response in aged humans. Integr Med 2000; 2(2):85–92.

World Health Organization (WHO). Elemental Mercury and Inorganic Mercury Compounds: Human Health Aspects. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series 2003; 922, Geneva, 176 .

Woznichak MM, Overcast JD, Robertson K, Neumann HM, May SW. Reaction of phenylaminoethyl selenides with peroxynitrite and hydrogen peroxide. Arch Biochem Biophys 2000; 379:314–320.

Yamashita Y, Yamashita M. Identification of a novel selenium containing compound, selenoneine, as the predominant chemical form of organic selenium in the blood of bluefin tuna. J Biol Chem 2010; 285(24):18134–8.

Yang CF, Shen HM, Ong CN. Ebselen induces apoptosis in HepG(2) cells through rapid depletion of intracellular thiols. Arch Biochem Biophys 2000a; 374:142–152.

Yang CF, Shen HM, Ong CN. Intracellular thiol depletion causes mitochondrial permeability transition in ebselen-induced apoptosis. Arch Biochem Biophys 2000b; 380:319–330.

Yang D, Yu-Wei C, Gunn JM, Belzille N. Selenium and Mercury in Organisms: Interactions and Mechanisms. Environ Rev 2008;16:71-92.

Yin Z, Lee E, Ni M, Jiang H, Milatovic D, Rongzhu L, Farina M, Rocha JBT, Aschner M. Methylmercury-induced alterations in astrocyte functions are attenuated by ebselen. Neurotoxicology 2011; 32(3):291–299.

Yoneda S, Suzuki KT. Equimolar Hg-Se complex binds to selenoprotein P. Biochem Biophys Res Commun. 1997;231(1):7-11.

Zalups RK. Molecular Interactions with Mercury in the Kidney. Pharmacol Rev 2000; 52:113-143.

Zhang S, Rocourt C, Cheng W-H. Selenoproteins and the aging brain. Mech Ageing Dev 2010; 131(4):253–60.

Zhang G, Nitteranon V, Guo S, Qiu P, Wu X, Li F, Xiao H, Hu Q, and Parkin K L. Organoselenium Compounds Modulate Extracellular Redox by Induction of Extracellular Cysteine and Cell Surface Thioredoxin Reductase. Chem Res Toxicol 2013; 26, 456−464.

Zhao H, Kim G, Levine RL. Methionine sulfoxide reductase contributes to meeting dietary methionine requirements. Archives of Biochemistry and Biophysics 2012; 522:37-43.

Zhao R, Holmgren A. A novel antioxidant mechanism of ebselen involving ebselen diselenide, a substrate of mammalian thioredoxin and thioredoxin reductase. J Biol Chem 2002; 277(42):39456–62.

Zhong L , Holmgren A. Essential role of selenium in the catalytic activities of mammalian thioredoxin reductase revealed by characterization of recombinant enzymes with selenocysteine mutations. J Biol Chem 2000; 275:18121–18128.

Zhong L, Arner ES, Holmgren A. Structure and mechanism of mammalian thioredoxin reductase: the active site is a redoxactive selenolthiol/selenenylsulfide formed from the conserved cysteine-selenocysteine sequence. Proc Natl Acad Sci USA 2000; 97:5854–5859.

Zhu YG, Pilon-Smits EAH, Zhao FJ, Williams PN, Meharg AA. Selenium in higher plants: understanding mechanisms for biofortification and phytoremediation. Trends Plant Sci 2009; 14:436-442.

Table 1: The most known organisms (eukaryotes) used experimentally to analysis of selenoproteins.

Division	Species	Number of selenoproteins
Chordata	Mus musculus	24
	Homo Sapiens	25
	Danio rerio	38
Arthropoda	*Drosophila melanogaster*	3
	Apis mellifera	1
Chlorophyta	*Chlamydomonas reinhardtii*	10
	Ostreococcus tauri	26
	Ostreococcus lucimarinus	29
Amoeba	*Dictyostelium discoideum*	5
Nematoda	*Caenorhabditis elegans and C. briggsae*	1
Apicomplexan parasites	*Plasmodium sp.*	4
	Toxoplasma	5
Kinetoplastidan parasites	*Leishmania and Trypanosoma*	3
Crustacea	*Daphnia magna*	7
Heterokontophyta	*Thalassiosira pseudonana*	16

Information presented in this table was compiled from: Lobanov et al., 2007; Lobanov et al., 2009; Novoselov et al., 2007; Gladyshev et al., 1999; Castellano et al., 2001; Clark et al., 2007; Kryukov et al., 2003; Gregory et al., 2004.

Table 2: Human selenoproteins and their functions.

Selenoprotein/Selenoenzyme	Fuctions
Cytosolic GPx (GPx 1)	Catalyzes the reduction of H_2O_2 and some organic hydroperoxides in aqueous compartment of cytosol.
Gastrointestinal GPx (GPx 2)	Protects against oxidative damage (similar to GPx1)
Plasma GPx (GPx 3)	Antioxidant in extracellular fluids, efficient in the plasma; active in plasma Se transport to other tissues.
Phospholipid hydroperoxide GPx (GPx4)	Can directly reduce phospholipid and cholesterol hydroperoxides; it protects biomembranes from peroxidative degradation
GPx 6	Protects against oxidative damage. (n.d.)
Thioredoxin reductase 1 (TrxR1)	Catalyzes NADPH-dependent reduction of oxidized thioredoxin which is involved in cellular redox regulation.
Thioredoxin reductase 2 (TrxR2)	Similar to TrxR1 (localized in mitochondria).
Thioredoxin glutathione reductase (TrxR3)	Catalyzes the reduction of thioredoxin and glutathione; it has a disulfide bond isomerization activity probably involved in spermatogenesis.
Type 1 deiodinase (DIO 1)	Activates thyroid hormone - converts thyroid prohormone T4 to active hormone T3.
Type 2 deiodinase (DIO 2)	Similar to DIO1; regulatory function in the brain and central nervous system.
Type 3 deiodinase (DIO 3)	Activity in fetal and in the deactivation of thyroid hormones.
Methionine sulfoxide reductase B1 (MsrB1 or X or R)	Redox control; it contributes to antioxidant defenses by reducing methionine sulfoxide in proteins back to methionine
Selenophosphate synthetase (SPS2)	Catalyzes the conversion of selenide to selenophosphate, which provides selenium for the biosynthesis of selenocysteine.
Selenoprotein 15 kDa (Sep 15)	Thiol-disulfide isomerase activity, possibly involved in disulfide bond formation and in protein folding and transport from ER.
Selenoprotein S	Influences inflammatory response; might protect cells from ER stress-induced apoptosis; linked to glucose metabolism and insulin sensitivity.
Selenoprotein P	Act as antioxidant, heavy metal chelator and selenium transporter and delivery in plasma (10Sec/molecule)
Selenoprotein N	Regulate calcium in ER mobilization required for early muscle development (mutations cause myopathies)
Selenoprotein W	Needed for muscle function; potencial antioxidant function.; may regulate the redox state of 14-3-3 proteins
Selenoprotein T	Involvement in the control of glucose homeostasis
Selenoprotein H	Nucleolar oxidoreductase function
Selenoprotein K	ER-membrane protein important for promoting effective $Ca^{(2+)}$ flux during immune cell activation; involved in the protection from ER stress agents-induced apoptosis

Selenoprotein M	Thiol disulfide oxidoreductase that participate in the formation of disulfide bonds in ER; neuroprotective properties
Selenoprotein O	Redox-active mitochondrial selenoprotein
Selenoprotein I	**n.d**
Selenoprotein V	**n.d**

Information presented in this table was compiled from: Moghadaszadeh and Beggs, 2006; Papp et al., 2007; Rayman et al., 2012, 2000; Gromer et al., 2005; Fomenko et al., 2009; Fergunson et al., 2005; Liu and Rozovsky, 2013; Yao et al., 2013; Prevost et al., 2013; Verma et al., 2011; Du et al., 2010; Reeves et al., 2010; Zhao et al., 2012; Han et al., 2014.

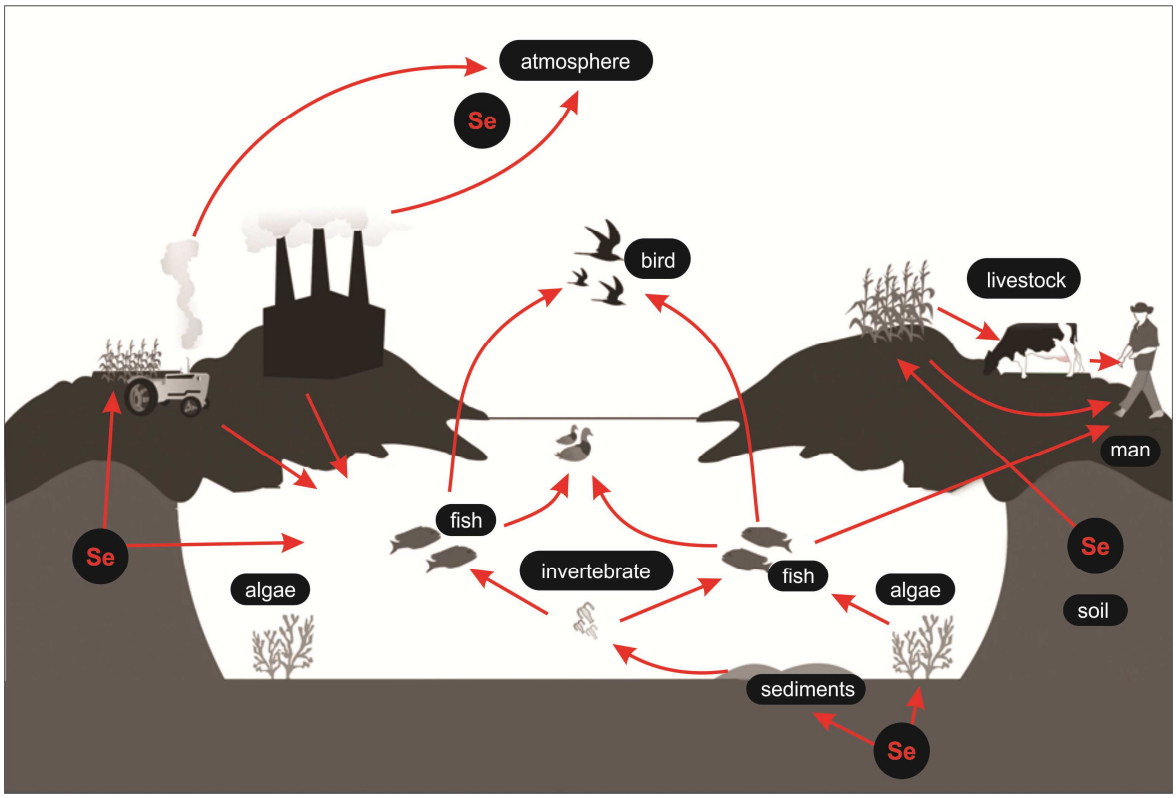
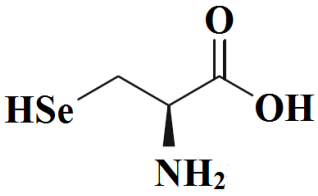
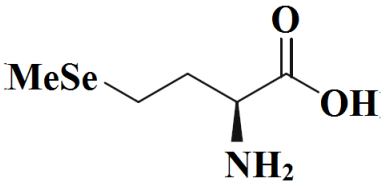


Figure 1. Biogeochemistry cycle of selenium

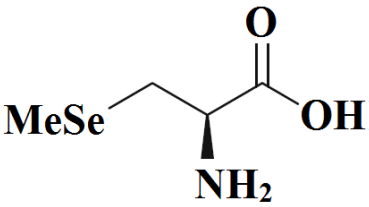
Naturally organoselenium compounds



Selenocysteine

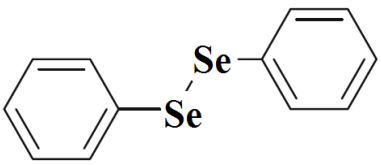


Selenomethionine

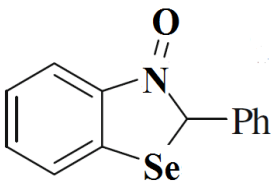


Se-Methylselenocysteine

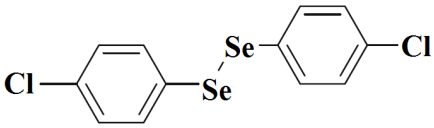
Syntethic organoselenium compounds



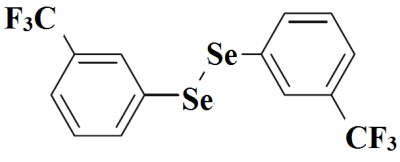
Diphenyl diselenide



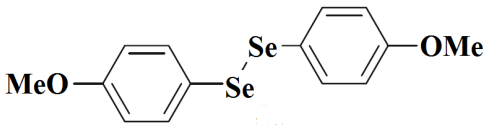
Ebselen



p-chloro diphenyl diselenide

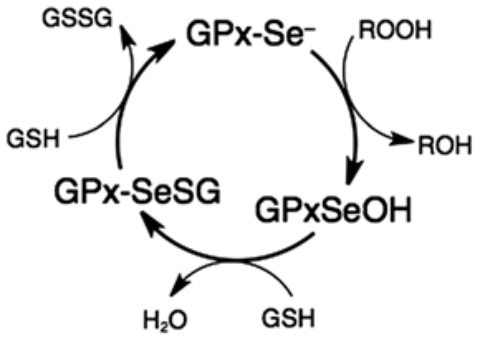


m-trifluoromethyl-diphenil diselenide

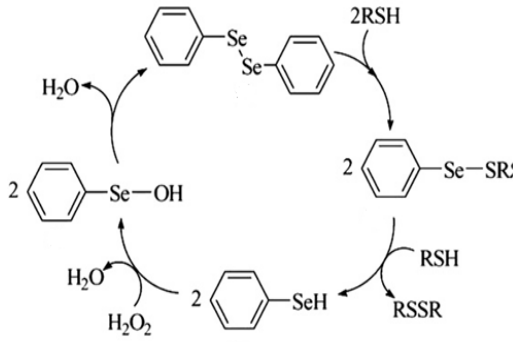


p-methoxyldiphenyl diselenide

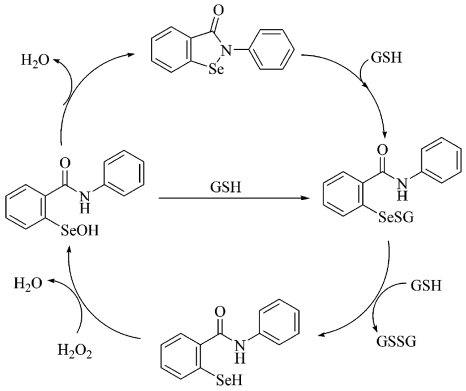
Figure 2. Structure of organoselenium compounds referred in this review



(A)



(B)



(C)

Figure 3. Comparison between the catalytic cycle of the selenoenzyme GPx (A) and the GPx-like cycle of $(PhSe)_2$ (B) and ebselen (C).

2. ARTIGOS CIENTÍFICOS

Os resultados que fazem parte desta tese estão apresentados sob a forma de artigo científico (1) e manuscritos (2). Os itens materiais e métodos, resultados, discussão e referências bibliográficas, encontram-se nos próprios artigos e manuscritos. O **artigo** está disposto na forma em que foi publicado na revista Brazilian Jornal of Medical and Biological Research. Os **manuscritos** estão dispostos na forma em que normalmente submete-se à publicação.

2.1 Artigo 1:

PROTECTIVE EFFECTS OF ORGANOSELENIUM COMPOUNDS AGAINST METHYLMERCURY-INDUCED OXIDATIVE STRESS IN MOUSE BRAIN MITOCHONDRIAL-ENRICHED FRACTIONS

D.F. MEINERZ, M.T. DE PAULA, B. COMPARSI, M.U. SILVA, A.E. SCHMITZ, H.C. BRAGA, P.S. TAUBE, A.L. BRAGA, J.B.T. ROCHA, A.L. DAFRE, M. FARINA, J.L. FRANCO AND T. POSSER

(Artigo publicado na revista “Brazilian Journal of Medical and Biological Research”)

Brazilian Journal of Medical and Biological Research (2011) 44: 1156-1163
ISSN 0100-879X

Protective effects of organoselenium compounds against methylmercury-induced oxidative stress in mouse brain mitochondrial-enriched fractions

D.F. Meinerz[2], M.T. de Paula[2], B. Comparsi[2], M.U. Silva[3], A.E. Schmitz[3], H.C. Braga[3], P.S. Taube[2], A.L. Braga[2], J.B.T. Rocha[2], A.L. Dafre[3], M. Farina[3], J.L. Franco[1,2] and T. Posser[1,2]

[1]Campus São Gabriel, Universidade Federal do Pampa, São Gabriel, RS, Brasil
[2]Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil
[3]Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, SC, Brasil

Abstract

We evaluated the potential neuroprotective effect of 1-100 µM of four organoselenium compounds: diphenyl diselenide, 3'3-ditrifluoromethyldiphenyl diselenide, *p*-methoxy-diphenyl diselenide, and *p*-chloro-diphenyl diselenide, against methylmercury-induced mitochondrial dysfunction and oxidative stress in mitochondrial-enriched fractions from adult Swiss mouse brain. Methylmercury (10-100 µM) significantly decreased mitochondrial activity, assessed by MTT reduction assay, in a dose-dependent manner, which occurred in parallel with increased glutathione oxidation, hydroperoxide formation (xylenol orange assay) and lipid peroxidation end-products (thiobarbituric acid reactive substances, TBARS). The co-incubation with diphenyl diselenide (100 µM) completely prevented the disruption of mitochondrial activity as well as the increase in TBARS levels caused by methylmercury. The compound 3'3-ditrifluoromethyldiphenyl diselenide provided a partial but significant protection against methylmercury-induced mitochondrial dysfunction (45.4 ± 5.8% inhibition of the methylmercury effect). Diphenyl diselenide showed a higher thiol peroxidase activity compared to the other three compounds. Catalase blocked methylmercury-induced TBARS, pointing to hydrogen peroxide as a vector during methylmercury toxicity in this model. This result also suggests that thiol peroxidase activity of organoselenium compounds accounts for their protective actions against methylmercury-induced oxidative stress. Our results show that diphenyl diselenide and potentially other organoselenium compounds may represent important molecules in the search for an improved therapy against the deleterious effects of methylmercury as well as other mercury compounds.

Key words: Methylmercury; Mitochondria; Oxidative stress; Organoselenium compounds; Diphenyl diselenide

Introduction

Reactive oxygen/nitrogen species (ROS/RNS) such as superoxide anion, hydrogen peroxide and nitric oxide induce damage to key biological components and cell membranes. In order to counteract the deleterious effects of reactive species, cells developed a specialized machinery of antioxidant defense. Cellular defense against ROS involves enzymes such as catalase, superoxide dismutase and glutathione peroxidase, which play a central role in the detoxification of reactive species (1,2).

Seleno-organic compounds such as ebselen and diphenyl diselenide (DD) (3,4) have a catalytic activity similar to that of the enzyme glutathione peroxidase involving the reduction of peroxides at the expense of thiol compounds (2,5) and represent important molecules whose protective and antioxidant properties against experimental oxidative stress conditions have been reported (6-8). These studies stimulated the search for new organoselenium compounds with catalytic properties similar to those of ebselen and DD, which could provide antioxidant and protective effects in biological systems.

Methylmercury (MeHg) has been recognized as a ubiquitous environmental toxicant whose toxicity is associated with neurological and developmental deficits in animals and humans (9). Nowadays, especially in the Amazon Region,

Correspondence: T. Posser and/or J.L. Franco, Campus São Gabriel, Universidade Federal do Pampa, 97300-000 São Gabriel, RS, Brasil and/or Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brasil.
E-mail: thaisposser@unipampa.edu.br and/or jefersonfranco@unipampa.edu.br

Received January 13, 2011. Accepted September 21, 2011. Available online October 14, 2011. Published November 14, 2011.

gold mining activity has been associated with intense environmental and human contamination with mercury compounds (10,11). Among the mechanisms involved in MeHg neurotoxicity, oxidative stress (12,13) appears to play a central role. MeHg-induced oxidative stress seems to be related to the direct oxidative properties of MeHg toward endogenous thiols (14) and to its inhibitory effects toward antioxidant enzymes like glutathione peroxidase (6,15,16). Mitochondria appear to be important cellular organelles targeted by MeHg (17), which is known to accumulate in mitochondria, where it can change mitochondrial membrane permeability and cause disruption of mitochondrial membrane potential (18,19). Considerable efforts have been made in the search for new drugs that counteract mercury toxicity. However, until now, no effective treatments are available to completely abolish the toxic effects of MeHg (20).

In previous studies, the organoselenium compounds DD and ebselen demonstrated potential protective effects against MeHg toxicity (6,21). Moreover, in *in vivo* studies, DD demonstrated lower toxicity than ebselen (5) and DD reversed MeHg-induced oxidative stress *in vivo* and in cortical slices (22,23). These data support the potential use of organoselenium compounds against MeHg poisoning.

The aim of the present study was to investigate the potential protective effects of DD and three novel selenium compounds: 3'3-ditrifluoromethyldiphenyl diselenide (DFD), *p*-methoxy-diphenyl diselenide (MD) and *p*-chloro-diphenyl diselenide (CLD) against MeHg-induced mitochondrial dysfunction.

Material and Methods

Chemicals

Glutathione reductase (G3664), reduced glutathione (GSH), oxidized glutathione (GSSG), *t*-butyl-hydroperoxide (*t*-bOOH), 5,5'-dithiobis(2-nitrobenzoic acid (DTNB), methylmercury (II) chloride, xylenol orange salt, and methylthiazolyldiphenyl-tetrazolium bromide (MTT) were purchased from Sigma-Aldrich (USA). All other chemicals used in this study were of the highest analytical grade available.

Synthesis and preparation of organoselenium compounds

All organoselenium compounds (Figure 1) tested in the present study were prepared as previously described (4). Analysis of the hydrogen-1 nuclear magnetic resonance (^{1}H NMR) and carbon-13 NMR (^{13}C NMR) spectra showed that all compounds presented analytical and spectroscopic data in full agreement with their assigned structures (data not shown). The diselenides were purified by flash chromatography on silica gel (hexane) and identified by (^{1}H NMR), ^{13}C NMR and gas chromatography-mass spectrometry (GCMS), which revealed homogeneous product (data not shown). Compounds were dissolved in ethanol immediately prior to use in each assay. The final ethanol in each experiment was 0.1%, and did not affect any of the parameters analyzed when compared to a control sample without ethanol (data not shown). We also investigated the basal activity of the compounds tested for the parameters analyzed in this study. We did not find significant changes when comparing the effects of compounds alone in all experiments performed (data not shown).

Preparation of mouse brain mitochondrial-enriched fractions

All procedures involving animals were performed according to the Animal Care Guidelines from the National Institutes of Health of the United States of America, and all procedures were approved by the Universidade Federal de Santa Catarina Ethics Committee for animal use (313/CEUA; 23080.026023/2004-39/UFSC). Mouse brain mito-

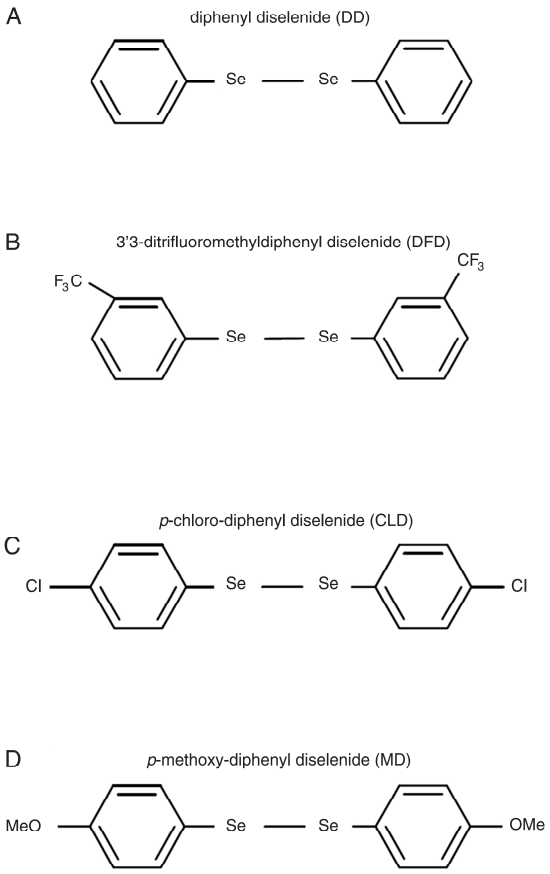


Figure 1. Structures of the organoselenium compounds tested in the present study.

chondrial-enriched fractions were prepared as described previously (24). Briefly, adult (8-10 weeks) male Swiss mice were sacrificed by decapitation. The whole brain (minus the cerebellum) was removed and homogenized on ice in 10 volumes of isolation medium (10 mM HEPES buffer, pH 7.0, containing 220 mM mannitol, 68 mM sucrose, 10 mM KCl, and 0.1% serum albumin) and the homogenate was centrifuged at 4°C for 10 min at 1000 *g*. The supernatant was then centrifuged at 17,500 *g* for 10 min at 4°C, providing a myelin-rich supernatant and a pellet (P2) consisting of synaptosomes and free (extra-synaptosomal) mitochondria. The supernatant was discarded, and the pellet was suspended in the isolation medium without albumin. The samples were kept on ice until the experiments were performed, usually within 10-15 min.

Incubations

P2 (2 mg protein) was incubated with different concentrations of MeHg (0, 10, 30, and 100 µM) diluted in incubation buffer, and/or selenium compounds (1, 10, 30, and 100 µM) in a incubation medium containing 10 mM HEPES buffer, pH 7.0, 220 mM mannitol, 68 mM sucrose, and 10 mM KCl (total incubation volume = 300 µL). In previous studies, we found a concentration of about 10 µM Hg in the brain of mice treated orally with 40 mg/L MeHg in drinking water (24). Considering the high amount of protein (2 mg), in the present study we used concentrations of MeHg up to 100 µM in order to obtain clear detection of all parameters tested during the *in vitro* assays. Incubations were carried out at 25°C. After incubation, mitochondrial dehydrogenase activity, GSH content, total hydroperoxides, and lipid peroxidation (TBARS) were determined. Parallel experiments with the presence of catalase (200 U) were also carried out in order to test the role of hydrogen peroxide (H_2O_2) in the mechanisms of toxicity and protection of MeHg and organoselenium compounds, respectively.

Assessment of mitochondrial activity

Mitochondrial activity was assessed by the conversion of the MTT dye to formazan (17). This assay is based on the ability of mitochondrial enzymes to metabolize MTT into formazan, a reaction that takes place only in functionally intact mitochondria. Briefly, samples (300 µL) were incubated for 30 min at 25°C. The purple formazan crystals were pelleted by centrifugation, and the supernatant was discarded. The pellets were dissolved in DMSO and the formazan was quantified spectrophotometrically by absorbance measurements at 550 nm. Data are reported as percentage of control. Selenium compounds alone did not interfere with the MTT method described here (data not shown).

Assessment of glutathione and hydroperoxide content and lipid peroxidation

Glutathione content was measured as nonprotein thiols according to a method previously described (25). After treatment with different concentrations (10, 30, and 100 µM) of MeHg for 30 min, 300 µL 10% trichloroacetic acid was added to the samples (300 µL). After centrifugation (4000 *g* at 4°C for 10 min), the protein pellet was discarded and free thiol groups were determined in the clear supernatant (which was neutralized with 0.1 M NaOH) by the method of Ellman (25). The total hydroperoxide content was assessed using the xylenol orange method (24) that allows the detection of hydrogen peroxide as well as lipid hydroperoxides. Briefly, samples were incubated in the medium described above for 60 min at 25 ± 1°C in the presence or absence of MeHg and/or selenium compounds. Then, the xylenol orange reagent, containing 0.25 mM $Fe(NH_4)_2(SO_4)_2$, 0.25 mM xylenol orange, and 110 mM perchloric acid, was added to the incubation medium. After 30 min, absorbance was recorded at 560 nm and compared to a hydrogen/cumene peroxide standard curve. Selenium compounds alone did not interfere with the method described here (data not shown).

The lipid peroxidation end-products were determined by the TBARS assay originally described by Ohkawa et al. (26). After 60 min of incubation as described for the xylenol orange method, samples were incubated with 0.45 M acetic acid/HCl buffer, pH 3.4, 0.28% thiobarbituric acid, 1.2% SDS, and thereafter at 95°C for 60 min to promote color reaction, measured at 532 nm. Malondialdehyde (0 to 3 nmol/mL) was used as a standard. Protein concentration was determined by the method of Bradford (27) using bovine serum albumin as standard.

Assessment of glutathione peroxidase (GPx)-like activity of organodiselenides

The GPx-like activity of the organoselenium compounds was determined using the coupled assay described by Wendel (28), which indirectly monitors the consumption of NADPH at 340 nm. The GPx/GPx-like compounds use GSH to reduce tert-butylhydroperoxide, producing GSSG, which is readily reduced to GSH by excess glutathione reductase (GR), thus consuming NADPH.

Statistical analysis

Statistically significant differences among groups were analyzed by one-way ANOVA followed by the Duncan multiple range test when appropriate. Differences were considered to be statistically significant when $P < 0.05$.

Results

MeHg induces oxidative stress and reduction of mitochondrial metabolic activity

The toxicity of MeHg has been demonstrated in *in vitro* and *in vivo* models and is generally associated with increased oxidative stress, disruption of oxidative defenses by inhibition of antioxidant enzymes as well as reduction

of GSH levels in the cell (15,16). Moreover, mitochondria are an important cellular target for MeHg toxicity (17). In the present study, as shown in Figure 2A, using the MTT reduction test, MeHg decreased the mitochondrial activity in a concentration-dependent manner. This effect was significantly different from control at concentrations as low as 10 µM and a reduction of about 50% in cell viability was verified at the highest concentration (100 µM). This effect was followed by a concentration-dependent decrease in mitochondrial GSH levels (Figure 2B). In parallel, a significant increase in total-hydroperoxide production (Figure 2C) and increased lipid peroxidation were observed with 100 µM MeHg (Figure 2D). The following experiments were performed using 100 µM MeHg, which affected all parameters analyzed.

Protective effects of organoselenium compounds against MeHg-induced mitochondrial oxidative stress

We investigated the potential protective effects of organoselenium compounds against the decrease in mitochondrial activity promoted by MeHg, using the MTT reduction assay. As observed in Figure 3A, DD at concentrations of 30 and 100 µM was able to partially and totally reverse the effect of 100 µM MeHg on mitochondrial activity, respectively. Only the highest concentration of DFD (100 µM) caused a slight reversal of the MeHg-induced reduction of mitochondrial activity (Figure 3B), while CLD and MD did not prevent the effects of MeHg on mitochondrial function (Figure 3C and D).

Considering the significant decrease in GSH levels promoted by MeHg treatment, we investigated whether co-incubation of MeHg in the presence of organoselenium compounds would be able to modulate the decrease in GSH levels caused by this metal. As observed in Figure 4 (A-D), none of the compounds tested was able to protect against the depletion of GSH levels caused by MeHg. In fact, when MD and MeHg were co-administered (Figure 4D), the decrease of GSH levels was greater than in the presence of MeHg alone, although this effect was not statistically significant (P = 0.08).

The antioxidant potential of the different organoselenium compounds against lipid peroxidation induced by MeHg was investigated by determining TBARS levels. As observed in Figure 5A, DD totally blocked the increase in lipid peroxidation induced by MeHg at a concentration as low as 10

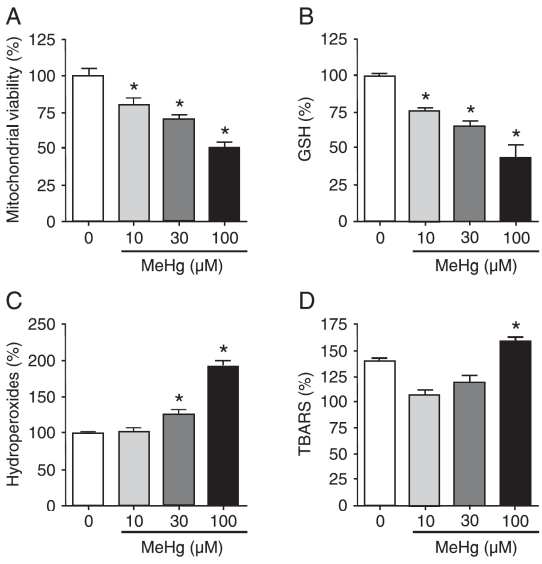


Figure 2. Effects of MeHg on brain mitochondria. Mouse brain mitochondrial-enriched fractions were isolated and incubated with different concentrations (0, 10, 30, and 100 µM) of MeHg for 30 min (MTT test and GSH measurement) or 60 min (total-hydroperoxide and TBARS content). Data are reported as means ± SD of percent of control for 4-6 separate assays carried out in duplicate. *P < 0.05 compared to control (without MeHg; ANOVA and Duncan *post hoc* test). *A*, Mitochondrial activity; *B*, GSH levels; *C*, total-hydroperoxides content; *D*, production of thiobarbituric acid reactive substances (TBARS).

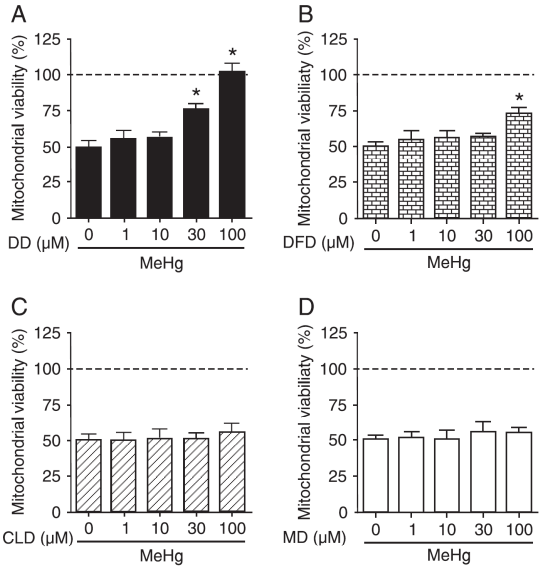


Figure 3. Effects of organoselenium compounds on MeHg-induced decrease in mitochondrial activity. Mouse brain mitochondrial-enriched fractions were incubated with 100 µM MeHg in the presence or absence of different concentrations (1-100 µM) of diphenyl diselenide (DD; *A*); 3'3-ditrifluoromethyldiphenyl diselenide (DFD; *B*); *p*-chloro-diphenyl diselenide (CLD; *C*), and *p*-methoxy-diphenyl diselenide (MD; *D*). Data are reported as means ± SD of percent of control for 4-6 separate assays carried out in duplicate. *P < 0.05 compared to MeHg alone (ANOVA and Duncan *post hoc* test). The dashed line indicates the control without MeHg (100%).

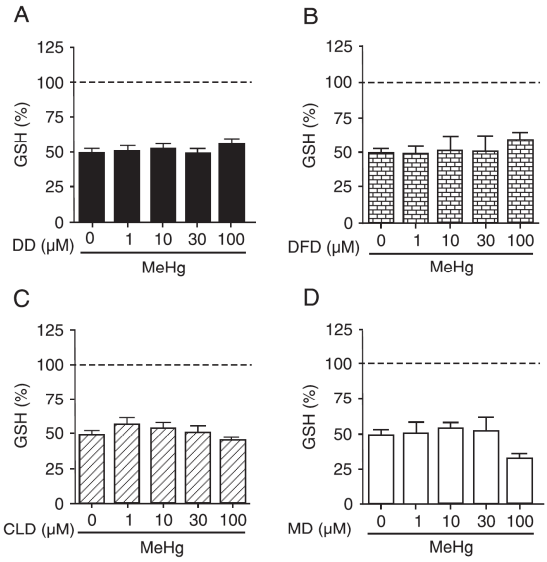


Figure 4. Effects of organoselenium compounds on MeHg-induced decrease in reduced glutathione (GSH) levels. Mouse brain mitochondrial-enriched fractions were incubated with 100 µM MeHg in the presence or absence of different concentrations (1-100 µM) of diphenyl diselenide (DD; *A*); 3'3-ditrifluoromethyldiphenyl diselenide (DFD; *B*); *p*-chloro-diphenyl diselenide (CLD; *C*), and *p*-methoxy-diphenyl diselenide (MD; *D*). Data are reported as means ± SD of percent of control for 4-6 separate assays carried out in duplicate. The dashed line indicates the control without MeHg (100%).

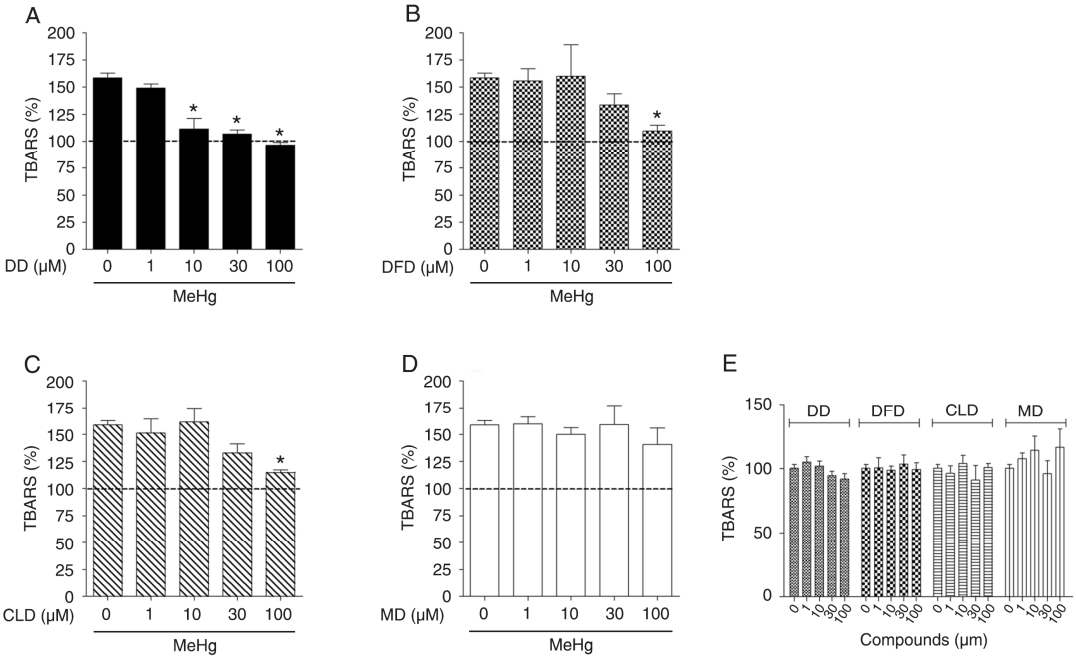


Figure 5. Effects of organoselenium compounds on MeHg-induced increase in the content of thiobarbituric acid reactive species (TBARS) levels. Mouse brain mitochondrial-enriched fractions were incubated with 100 µM MeHg in the presence or absence of increasing concentrations (1-100 µM) of diphenyl diselenide (DD; *A*); 3'3-ditrifluoromethyldiphenyl diselenide (DFD; *B*); *p*-chloro-diphenyl diselenide (CLD; *C*), and *p*-methoxy-diphenyl diselenide (MD; *D*). Effect of compounds alone on TBARS induction (*E*). Data are reported as means ± SD of percent of control for 4-6 separate assays carried out in duplicate. *P < 0.05 compared to MeHg alone (ANOVA and Duncan *post hoc* test). The dashed line indicates the control without MeHg (100%).

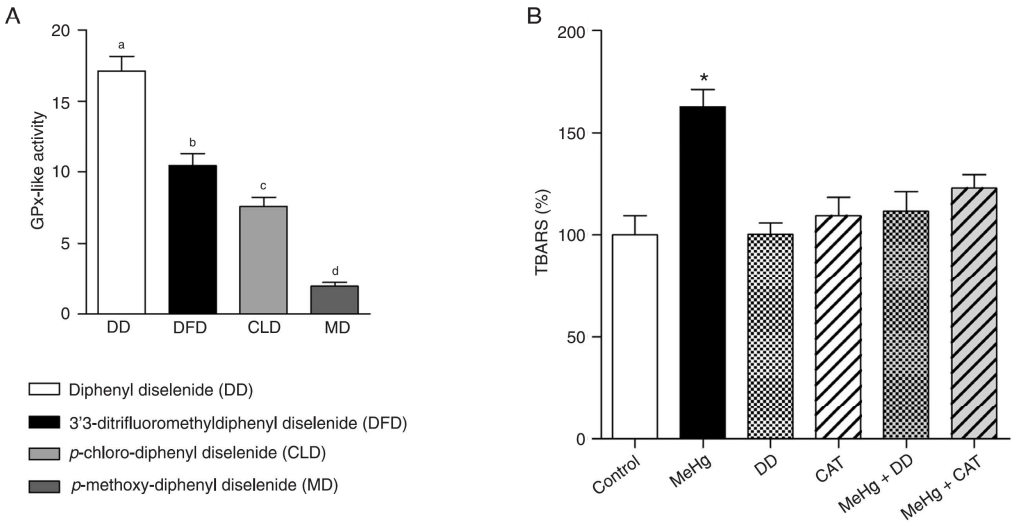


Figure 6. *A*, Glutathione peroxidase (GPx)-like activity of organodiselenides. A solution of each compound was added to cuvettes and GPx activity was determined as described in the Material and Methods section. Small letters indicate statistical differences between compounds, considering GPx-like activity ($P < 0.05$; ANOVA and Duncan *post hoc test*). *B*, Effect of DD and catalase (CAT) on MeHg-induced lipid peroxidation (thiobarbituric acid reactive species, TBARS). *$P < 0.05$ compared to control and to all other groups tested (ANOVA and Duncan *post hoc test*).

μM. However, DFD and CLD (Figure 5B and C) blocked the increase in lipid peroxidation promoted by MeHg only at the highest concentration (100 μM), while MD did not demonstrate a protective potential against lipid peroxidation induced by MeHg (Figure 5D). There was no significant TBARS induction by compounds alone (Figure 5E).

Glutathione peroxidase-like activity of organoselenium compounds

In a previous report (24), our group demonstrated that H_2O_2 generation represents a relevant event in MeHg-mediated oxidative stress in mouse brain mitochondria. GPx (EC 1.11.1.9) is a main cellular antioxidant responsible for the removal of peroxides in the brain (29). Thus, considering that GPx-like activity of organoselenium compounds is potentially involved in their antioxidant properties, we investigated the *in vitro* GPx-like activity of each compound. As observed in Figure 6A, DD demonstrated a higher GPx-like activity when compared to the other compounds tested. The order of magnitude for GPx-like activity was DD > DFD > CLD > MD. In parallel experiments, in order to confirm whether the anti-peroxidative activity of DD is linked to its peroxide removal ability, we incubated mouse brain mitochondrial-enriched fractions with MeHg in the presence or absence of DD and catalase, an enzyme involved in the clearance of H_2O_2. As shown in Figure 6B, MeHg-induced lipid peroxidation was completely reversed by catalase (200 U) as well as 100 μM DD, confirming that removal of peroxides is an important mechanism responsible for the protective effects of DD in our study model.

Discussion

In the present study, we used isolated brain mitochondria as a model to investigate MeHg toxicity, since this organelle represents a major target for MeHg in cells and plays a pivotal role in the initiation of biochemical cascades that lead to cell death (30,31). The effects of MeHg on mitochondrial function are associated with loss of the regular organization of the cristae (32) and dissipation of mitochondrial membrane potential (18,17,33).

The acute treatment of mitochondrial-enriched fractions from mouse brain with MeHg caused a decrease in mitochondrial activity, in agreement with previously reported results for kidney, brain and striatal mitochondrial fractions (17,24,34). This effect occurred in parallel to an increase in lipid peroxidation and GSH depletion. The relationship between ROS formation and mitochondrial damage after MeHg exposure is not fully understood. ROS can cause oxidative damage to mitochondria, leading to compromised mitochondrial function (35,36). On the other hand, ROS can also be produced by the mitochondria via leakage of electrons from the electron transport chain to molecular O_2, forming superoxide anion radicals ($O_2^{\cdot-}$). The $O_2^{\cdot-}$ is converted to H_2O_2 by the mitochondrial enzyme manganese superoxide dismutase (MnSOD) (37). Although this process occurs normally at a low rate in intact mitochondria, $O_2^{\cdot-}$ production can be dramatically increased if mitochondria

are challenged by toxicants (38). It was previously demonstrated that treatment with MeHg causes an increase in H_2O_2 generation as well inhibition of GPx activity (15,16,39), which could be responsible in part for the increase in lipid peroxidation observed in our model. We confirmed the participation of H_2O_2 formation in MeHg toxicity when we incubated the brain mitochondria with catalase. This H_2O_2 detoxifying enzyme was able to ameliorate the increase in lipid peroxidation promoted by MeHg, which points to an involvement of H_2O_2 in the lipid peroxidation promoted by MeHg and suggests that the GPx-like activity of DD is involved in the antioxidant effect against MeHg.

DD (30 and 100 µM) protected mouse brain mitochondria against MeHg toxicity by reversing the MeHg-induced loss of mitochondrial activity/viability. Among the novel organoselenium compounds tested here, only DFD partially reversed the effect of MeHg on mitochondrial activity at the highest concentration (100 µM). The co-treatment with DD completely blocked TBARS production by MeHg. This effect was observed from 10 µM up to 100 µM of this compound. The novel organodiselenides DFD and CLD were able to reverse the increase in lipid peroxidation promoted by MeHg only at 100 µM, emphasizing a higher efficiency of DD as a protective antioxidant, as demonstrated in previous studies (6-8).

GPx mimetic compounds can degrade hydroperoxides, consuming thiol reserves. Such ability confers to these compounds the capacity of protecting cells against oxidative stress conditions (7). The GPx-like activity of DD was significantly higher than the activity of DFD, CLD and MD, an effect possibly related to the higher antioxidant and protective effects of this compound. This result was confirmed by the fact that incubation of samples with catalase, which removes H_2O_2, avoided TBARS formation induced by MeHg exposure. These data suggest that the protective and antioxidant actions of DD are linked to its ability to remove peroxides. MeHg is known to increase H_2O_2 formation by mitochondria (24,34). In this regard, the GPx mimetic activity of DD may represent a promising tool against the cytotoxic effects of this environmental neurotoxin. The lack of protective effect of the organodiselenides DFD, MD and CLD may be related to their lower peroxidase-like activity compared to DD. A recent study from our group has shown the central role of GPx in the toxicity of MeHg (40). In that study, we showed that MeHg was able to decrease GPx activity in cell and animal models. In addition, the inhibition of GPx activity with mercaptosuccinic acid increased cell susceptibility to the toxic effects of methylmercury. On this basis, it seems plausible that DD, which showed higher thiol peroxidase activity than the other three substituted diselenides tested here, had the most prominent protective effects against methylmercury-induced oxidative stress and loss of mitochondrial activity *in vitro*. In addition to the antioxidant properties of selenium compounds, the ability to bind Hg ions may represent an important mechanism for cytoprotection. In fact, a recent study from our group showed that DD is able to remove Hg from tissues (22), reinforcing the therapeutic potential of selenium compounds against Hg intoxication.

The data reported here reinforce the antioxidant and protective potential of DD when comparing to other substituted organodiselenides. Our findings support the fact that compounds with glutathione peroxidase-like activity are potent blockers of mercurial-induced neurotoxic actions. In addition, our data indicate that depending on the chemical substitutions made on DD, its GPx-like activity may be impaired, which is crucial for the protective capacity of the compound. Considering that oxidative stress has been implicated in MeHg toxicity and that there are no effective treatments available to counteract the toxic effects of MeHg, the use of DD may represent an important therapeutic approach.

Acknowledgments

Research supported by a CNPq grant to T. Posser, a FAPERGS grant to J.L. Franco, CNPq grants to D.F. Meinerz and M.T. de Paula (#140030/2011-5 and #556081/2010-2, respectively), a FAPERGS-PRONEX grant to J.B.T. Rocha, CNPq and FAPESC grants to M. Farina and A.L. Dafre, and FINEP research grants “Rede Instituto Brasileiro de Neurociência (IBN-Net)”.

References

1. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. *Nature* 2000; 408: 239-247.
2. Mugesh G, Singh H. Synthetic organoselenium compounds as antioxidants: glutathione peroxidase activity. *Chem Soc Rev* 2000; 29: 347-357.
3. Arteel GE, Sies H. The biochemistry of selenium and the glutathione system. *Environ Toxicol Pharmacol* 2011; 10: 153-158.
4. Paulmier C. *Selenium reagents and intermediates in organic synthesis*. Oxford: Pergamon Press; 1986.
5. Nogueira CW, Zeni G, Rocha JB. Organoselenium and organotellurium compounds: toxicology and pharmacology. *Chem Rev* 2004; 104: 6255-6285.
6. Farina M, Frizzo ME, Soares FA, Schwalm FD, Dietrich MO, Zeni G, et al. Ebselen protects against methylmercury-induced inhibition of glutamate uptake by cortical slices from adult mice. *Toxicol Lett* 2003; 144: 351-357.
7. Posser T, Franco JL, dos Santos DA, Rigon AP, Farina M, Dafre AL, et al. Diphenyl diselenide confers neuroprotection against hydrogen peroxide toxicity in hippocampal slices. *Brain Res* 2008; 1199: 138-147.
8. Posser T, Moretto MB, Dafre AL, Farina M, da Rocha JB,

Nogueira CW, et al. Antioxidant effect of diphenyl diselenide against sodium nitroprusside (SNP) induced lipid peroxidation in human platelets and erythrocyte membranes: an *in vitro* evaluation. *Chem Biol Interact* 2006; 164: 126-135.
9. Clarkson TW, Magos L, Myers GJ. The toxicology of mercury - current exposures and clinical manifestations. *N Engl J Med* 2003; 349: 1731-1737.
10. Malm O. Gold mining as a source of mercury exposure in the Brazilian Amazon. *Environ Res* 1998; 77: 73-78.
11. Myers GJ, Davidson PW, Strain JJ. Nutrient and methyl mercury exposure from consuming fish. *J Nutr* 2007; 137: 2805-2808.
12. Aschner M, Syversen T, Souza DO, Rocha JB, Farina M. Involvement of glutamate and reactive oxygen species in methylmercury neurotoxicity. *Braz J Med Biol Res* 2007; 40: 285-291.
13. Ou YC, White CC, Krejsa CM, Ponce RA, Kavanagh TJ, Faustman EM. The role of intracellular glutathione in methylmercury-induced toxicity in embryonic neuronal cells. *Neurotoxicology* 1999; 20: 793-804.
14. Shanker G, Syversen T, Aschner JL, Aschner M. Modulatory effect of glutathione status and antioxidants on methylmercury-induced free radical formation in primary cultures of cerebral astrocytes. *Brain Res Mol Brain Res* 2005; 137: 11-22.
15. Farina M, Cereser V, Portela LV, Mendez A, Porciúncula LO, Fornaguera J, et al. Methylmercury increases S100B content in rat cerebrospinal fluid. *Environ Toxicol Pharmacol* 2005; 19: 249-253.
16. Mori N, Yasutake A, Hirayama K. Comparative study of activities in reactive oxygen species production/defense system in mitochondria of rat brain and liver, and their susceptibility to methylmercury toxicity. *Arch Toxicol* 2007; 81: 769-776.
17. Dreiem A, Gertz CC, Seegal RF. The effects of methylmercury on mitochondrial function and reactive oxygen species formation in rat striatal synaptosomes are age-dependent. *Toxicol Sci* 2005; 87: 156-162.
18. Araragi S, Kondoh M, Kawase M, Saito S, Higashimoto M, Sato M. Mercuric chloride induces apoptosis via a mitochondrial-dependent pathway in human leukemia cells. *Toxicology* 2003; 184: 1-9.
19. Shenker BJ, Guo TL, Insung O, Shapiro IM. Induction of apoptosis in human T-cells by methyl mercury: temporal relationship between mitochondrial dysfunction and loss of reductive reserve. *Toxicol Appl Pharmacol* 1999; 157: 23-35.
20. Tchounwou PB, Ayensu WK, Ninashvili N, Sutton D. Environmental exposure to mercury and its toxicopathologic implications for public health. *Environ Toxicol* 2003; 18: 149-175.
21. Moretto MB, Funchal C, Santos AQ, Gottfried C, Boff B, Zeni G, et al. Ebselen protects glutamate uptake inhibition caused by methyl mercury but does not by Hg^{2+}. *Toxicology* 2005; 214: 57-66.
22. de Freitas AS, Funck VR, Rotta MS, Bohrer D, Morschbacher V, Puntel RL, et al. Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury-induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice. *Brain Res Bull* 2009; 79: 77-84.
23. Roos DH, Puntel RL, Santos MM, Souza DO, Farina M, Nogueira CW, et al. Guanosine and synthetic organoselenium compounds modulate methylmercury-induced oxidative stress in rat brain cortical slices: involvement of oxidative stress and glutamatergic system. *Toxicol In Vitro* 2009; 23: 302-307.
24. Franco JL, Braga HC, Stringari J, Missau FC, Posser T, Mendes BG, et al. Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. *Chem Res Toxicol* 2007; 20: 1919-1926.
25. Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959; 82: 70-77.
26. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95: 351-358.
27. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248-254.
28. Wendel A. Glutathione peroxidase. *Methods Enzymol* 1981; 77: 325-333.
29. Dringen R, Pawlowski PG, Hirrlinger J. Peroxide detoxification by brain cells. *J Neurosci Res* 2005; 79: 157-165.
30. Aschner M, Syversen T. Methylmercury: recent advances in the understanding of its neurotoxicity. *Ther Drug Monit* 2005; 27: 278-283.
31. Atchison WD, Hare MF. Mechanisms of methylmercury-induced neurotoxicity. *FASEB J* 1994; 8: 622-629.
32. O'Kusky J. Methylmercury poisoning of the developing nervous system: morphological changes in neuronal mitochondria. *Acta Neuropathol* 1983; 61: 116-122.
33. Stavrovskaya IG, Kristal BS. The powerhouse takes control of the cell: is the mitochondrial permeability transition a viable therapeutic target against neuronal dysfunction and death? *Free Radic Biol Med* 2005; 38: 687-697.
34. Lund BO, Miller DM, Woods JS. Studies on Hg(II)-induced H_2O_2 formation and oxidative stress *in vivo* and *in vitro* in rat kidney mitochondria. *Biochem Pharmacol* 1993; 45: 2017-2024.
35. Galindo MF, Jordan J, Gonzalez-Garcia C, Cena V. Reactive oxygen species induce swelling and cytochrome c release but not transmembrane depolarization in isolated rat brain mitochondria. *Br J Pharmacol* 2003; 139: 797-804.
36. Radi R, Cassina A, Hodara R, Quijano C, Castro L. Peroxynitrite reactions and formation in mitochondria. *Free Radic Biol Med* 2002; 33: 1451-1464.
37. Shanker G, Aschner JL, Syversen T, Aschner M. Free radical formation in cerebral cortical astrocytes in culture induced by methylmercury. *Brain Res Mol Brain Res* 2004; 128: 48-57.
38. Halliwell B, Gutteridge JMC. *Free radicals in biology and medicine*. New York: Oxford University Press; 1999.
39. Carvalho MC, Franco JL, Ghizoni H, Kobus K, Nazari EM, Rocha JB, et al. Effects of 2,3-dimercapto-1-propanesulfonic acid (DMPS) on methylmercury-induced locomotor deficits and cerebellar toxicity in mice. *Toxicology* 2007; 239: 195-203.
40. Franco JL, Posser T, Dunkley PR, Dickson PW, Mattos JJ, Martins R, et al. Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. *Free Radic Biol Med* 2009; 47: 449-457.

2.2 Manuscrito 1:

DIPHENYL DISELENIDE PROTECTS AGAINST METHYLMERCURY - INDUCED INHIBITION OF THIOREDOXIN REDUCTASE AND GLUTATHIONE PEROXIDASE IN HUMAN NEUROBLASTOMA CELLS: A COMPARISON WITH EBSELEN.

DAIANE F. MEINERZ; VASCO BRANCO; MICHAEL ASCHNER; CRISTINA CARVALHO; JOÃO BATISTA T. DA ROCHA

(Manuscrito submetido à revista Chemical research in Toxicology)

Diphenyl diselenide protects against methylmercury - induced inhibition of thioredoxin reductase and glutathione peroxidase in human neuroblastoma cells: a comparison with ebselen.

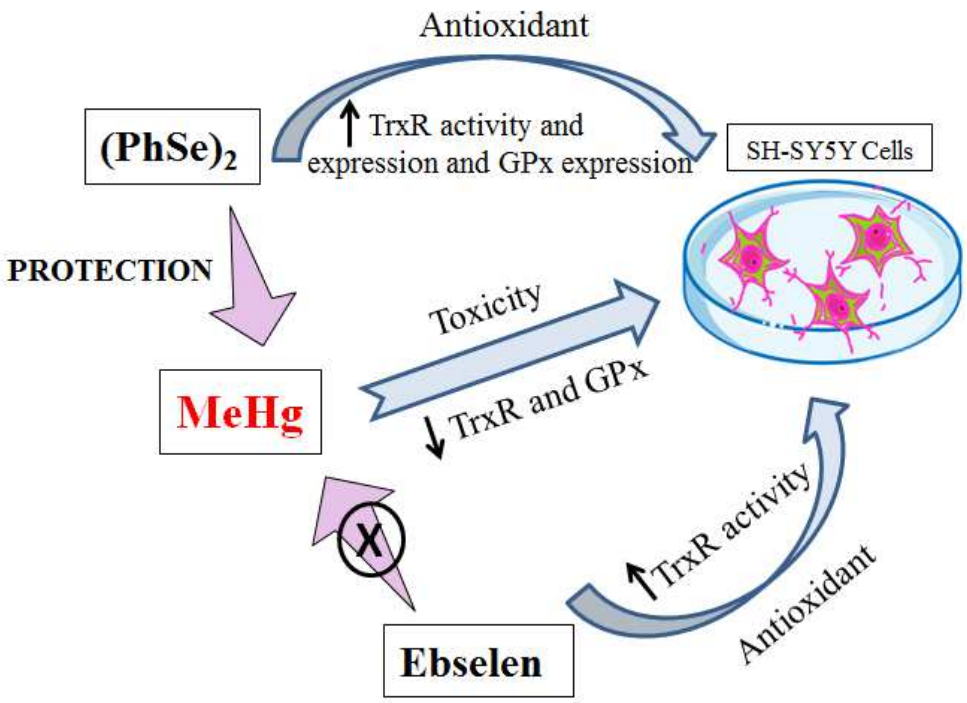
Daiane F. Meinerz [#]; Vasco Branco [‡]; Michael Aschner [$]; Cristina Carvalho [‡]; João Batista T. da Rocha [#]*

[#] Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900, Santa Maria RS, Brazil;

[‡] Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto 1649-003 Lisboa, Portugal.

[$]Department of Molecular Pharmacology; Albert Einstein College of Medicine, Forchheimer 209; 1300 Morris Park Avenue, Bronx, NY 10461, U.S.A.

TABLE OF CONTENTS (TOC) GRAPHIC



ABSTRACT

Exposure to methylmercury (MeHg), an important environmental toxicant, may lead to serious health risks, damaging various organs and predominantly affecting the brain function. This effect may be related to the inhibition of important selenoenzymes, such as glutathione peroxidase (GPx) and thioredoxin reductase (TrxR). Experimental studies have shown that selenocompounds play an important role as cellular detoxifiers and protective agents against the harmful effects of mercury. The present study investigated the mechanisms by which $(PhSe)_2$ and ebselen interfered with the interaction of mercury (MeHg) and selenoenzymes (TrxR and GPx), using an experimental model of human neuroblastoma cells (SH-SY5Y).Our results established that $(PhSe)_2$ and ebselen increased the activity of TrxR, but only $(PhSe)_2$ caused a significant increase of TrxR expression. In contrast, MeHg inhibited TrxR activity and expression even at low doses. Coexposure to selenocompounds and MeHg showed a protective effect of $(PhSe)_2$ on both the activity and expression of TrxR. However, ebselen failed to induce TrxR expression. When the selenoenzyme GPx was evaluated, selenocompounds did not alter its activity, but $(PhSe)_2$, when added to cultures alone, increased the expression of GPx, whereas MeHg inhibited the activity of GPx. Among the selenocompounds only $(PhSe)_2$ protected against MeHg's effects on GPx activity. Taken together, these results indicate a potential use for ebselen and $(PhSe)_2$ against MeHg toxicity. Furthermore, for the first time, we have demonstrated that $(PhSe)_2$ caused a more pronounced upregulation of TrxR than ebselen in neuroblastoma cells, likely reflecting be an important molecular mechanism involved in the antioxidant properties of this compound.

Key works: Diphenyl diselenide, Ebselen, Methylmercury, neuroblastoma cells, Thioredoxin reducatese and Glatathione Peroxidase.

INTRODUCTION

Mercury (Hg) is one of the most harmful metals in the environment. Exposure to this element, particularly in the form of methylmercury (MeHg), which is present in variable amounts in fish and seafood, can cause serious health risks.[1-3] MeHg readily crosses the blood brain barrier and disrupts normal brain function.[2,4] In addition, MeHg can also lead to severe damage to immune and cardiovascular systems,[5-7] kidney [8] and induce genetic damage.[9]

Exposure to MeHg has been associated with oxidative stress *in vivo*[10] and *in vitro*[11,12] and it can alter the antioxidant defense system by inhibiting sulfhydryl (-SH) and selenohydryl (-SeH) containing proteins.[2,13-16] In this context, studies suggest that the activity of selenoenzymes, such as glutathione peroxidase (GPx), might be negatively affected by MeHg.[17,18] Furthermore, thioredoxin reductase (TxrR), a high priority selenoenzyme, is particularly sensitive to mercury compounds both upon *in vitro*[15,19] and *in vivo* exposure.[14,16] Branco et al. showed *in vivo* exposure to MeHg profoundly affected TrxR activity both in brain and liver of fish (*Diplodus cervinus)*, corroborating the vulnerability of TrxR to this organomercury compound.[20]

As an integral component of selenoproteins, selenium is an important essential trace element with several biological functions.[21,22] Selenoenzymes, such as GPx and TrxR serve as potent antioxidants and inorganic selenium (Se) has been reported to antagonize the toxicity of several heavy metals, including mercury.[23] To counteract or minimize Hg-induced toxicity, selenium compounds can act by improving antioxidant defenses[2,24-26] and/ or by inducing changes in the distribution, deposition and excretion of Hg.[2,24,27] Nevertheless, there is a dearth of information on the molecular mechanism(s) involved in the protection afforded by organic forms of selenium against the toxicity of MeHg .[24,27]

Diaryl diselenides and ebselen are synthetic organoselenium compounds, with antioxidant properties.[28-30] Both compounds have been shown to possess thiol peroxidase-like activity and neuroprotective properties in experimental models of toxicity both *in vitro* and *in vivo*. [24,31-35] Notably, both diphenyl diselenide [$(PhSe)_2$] and ebselen have been reported to protect against MeHg *in vivo* and *in vitro* neurotoxicity, [24,36-38] yet the mechanism(s) associated with their efficacy has yet to be recognized. We have postulated that ebselen and $(PhSe)_2$ can be reduced to selenol intermediates (either via a direct interaction with thiols or indirectly via NADPH mediated TrxR reduction), which subsequently bind to the -Hg moiety in the MeHg molecule.[2,24] However, recent reports have indicated that ebselen and $(PhSe)_2$

can have antioxidant properties by stimulating the transcription of antioxidant genes, increasing the synthesis of selenoenzymes with antioxidant properties.[39-41] Therefore, the objective of the present study was to determine whether the beneficial properties of $(PhSe)_2$ and ebselen in mitigating MeHg-induced neurotoxicity are associated with increased synthesis of selenoenzymes. Accordingly, the present study investigated the mechanisms by which $(PhSe)_2$ and ebselen interfered with the interaction of mercury (MeHg) and selenoenzymes (TrxR and GPx), using an experimental model of human neuroblastoma cells (SH-SY5Y).

MATERIALS AND METHODS

Chemicals. Selenium compounds (Diphenyl diselenide and Ebselen) (Figure 1), MeHg and other laboratory chemicals used in the experiments were purchased from Sigma- Aldrich (St. Louis, MO, USA). Cell culture reagents were obtained from Gibco-Invitrogen. TrxR and GPx 1 antibody were obtained from Santa Cruz Biotechnology (CA, USA).

Maintenance and treatment of cell line. Human neuroblastoma cells (SH-SY5Y) (a kind gift from Profª. Dora Brites, Faculdade de Farmácia Universidade de Lisboa) were grown in medium consisting of a 1:1 mixture of DMEM and F-12, supplemented with 10% fetal bovine serum, 5% of a Pen-Strep mixture (all from Gibco- Invitrogen). Cells were grown in a humidified incubator at 37º C and 5% CO_2.

Cellular viability assay. Cells were plated in 96-well plates ($4x10^3$ cells/well) and allowed to attach for 24 h after which different concentrations of MeHg (0, 0.1, 0.5, 1, 5, 10 µM) or $(PhSe)_2$ and ebselen (1, 5, 10 and 25 µM) were added to the plates. Cell viability was determined at the time of addition (0h) and after 24 hours of exposure to each compound with the MTT assay. MTT (3[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; Sigma) was added to the plates at a final concentration of 400 µg/mL per well, followed by incubation a 37° C for 4 h. After incubation the medium was removed and the formazan crystals formed during the MTT reduction by viable cells dissolved in a 4:1 DMSO/Glycine buffer (pH 10.5 – 0.1M). Viability was then assessed by measuring formazan absorption at 550 nm. Results are expressed as percentage of viable cell (% of control).

Cell culture and treatment for the assessment of enzymatic activities. The SH-SY5Y cells were plated (1×10^6) in Petri dishes, incubated at 37°C and allowed to reach 70-80% confluence. Fresh medium was then added to the cells with the following concentrations of various compounds: $(PhSe)_2$ (0.1, 0.5, 1, 2.5 and 5 µM); ebselen (0.1, 0.5, 1, 2.5 and 5 µM); MeHg (0.1, 0.5, 1, 2.5 and 5 µM) and co-exposure to $(PhSe)_2$ or ebselen (0.5, 1, 2.5 and 5 µM) and MeHg (1 µM).

After 24 h incubation, the cells were harvested from plates and lysed in a buffer containing: 25 mM Tris (pH 7.5), 0.1 M NaCl, 2.5 mM EDTA, 2.5mM EGTA, 0.5% Triton X-100, 20 mM β glycerolphosphate, 1 mM Na_3VO_4, 20mM NaF, and 10mM $Na_2P_2O_7$. Cells were then in accordance with requisite protocols, as described below.

Protein determination. The protein content was determined by the method of Bradford et al.[42] Briefly, 1 µL supernatant was incubated with Coomassie dye (Bio-Rad; diluted 5 times) in 96-well plates and read at 595 nm in a microplate reader (Zenyth 3100, Anthos Labtec Instruments). Bovine serum albumin was used as standard.

Measurement of glutathione peroxidase activity. The activity of cytosolic seleno-dependent glutathione peroxidase 1 (GPx) was measured according to the method by Esworthy and coauthors.[43] Forty µg of protein from whole cell lysates were mixed in 96-well plates with sodium phosphate buffer (50 mM, pH 7.0), GSH (10 mM), NADPH (2 mM), sodium azide (1.125 mM), and GR (100 U/mL). A 5 mM solution of H_2O_2 was used as the substrate. The oxidized glutathione (GSSG) resulting from H_2O_2 degradation by GPx is reduced to GSH in the presence of GR and NADPH. The decrease in NADPH is monitored at 340 nm for 5 min at 30 ºC and used to calculate GPx activity. The activity was expressed relative to control activity (relative GPx activity- % of control, standardized to protein levels).

Determination of TrxR activity. TrxR activity was determined with the insulin endpoint assay[44] for complex biological samples. For TrxR activity determination, 30 µg of soluble protein samples were incubated in 96-well plates with 0.3 mM insulin, 720 µM NADPH, 2.5 mM EDTA, and 3 µM fully reduced human Trx (IMCO Corp., Sweden) in 85 mM Hepes buffer (pH 7.6), for 20 min at room temperature. Control wells containing the same reagents,

but excluding Trx addition, were prepared in parallel. After the incubation period, 250 µL of a 1 mM DTNB solution in 6 M guanidine hydrochloride (pH 8.0) were added to each well and absorbance was measured in a microplate reader (Zenyth 3100, Anthos Labtec Instruments) at 412 nm. TrxR activity was quantified as the difference in absorbance between the Trx containing well and the control well. The activity was expressed relative to control activity (relative TrxR activity- % of control, standardized to protein levels).

Western blot analysis. TrxR1 and GPx1 expression was analyzed by Western blot after separation of lysates (50 µg of protein) by SDS-PAGE on a 12 % Bis-Tris gel with MES running buffer (Invitrogen). For TrxR1, the following antibodies were used: rabbit anti-human TrxR (sc-20147; Santa Cruz Biotechnology), rabbit anti-human GAPDH (sc-25118; Santa Cruz Biotechnology) and goat anti-rabbit IgG-HRP (sc-2004; Santa Cruz Biotechnology). For GPx1 the antibodies utilized were: anti GPx1 goat polyclonal IgG (sc-22145) and donkey anti-goat IgG-HRP (sc-2020) (Santa Cruz Biotechnology). Western blots shown are representative of two independent experiments.

Statistical analysis. All values are calculated as mean ± standard error (SEM), and expressed as percentage of control. Differences between the groups were analyzed by one or two-way ANOVA, followed by Dunnett's multiple comparison test. P values < 0.05 were considered statistically significant.

RESULTS

Cell viability. After 24 hours of exposure, cell viability was decreased by ~50% in the presence of 5-10 µM of MeHg. In contrast, 25 µM of $(PhSe)_2$ and ebselen caused only a significant, yet much smaller decrease in cell viability (~15%) (Figure 2).

Thioredoxin reductase activity. Thioredoxin reductase activity in human neuroblastoma cells was significantly decreased by MeHg exposure. The inhibitory effect was concentration-dependent and observed from 0.5 ($p<0.05$) to 5 µM of MeHg. At 5 µM MeHg the inhibitory

effect was ~80% ($p<0.0001$) (Figure 3A). MeHg (1 µM) caused 50% inhibiton in TrxR activity. Accordingly, this concentration was chosen for the co-exposure assay with the selenium compounds.

Both $(PhSe)_2$ and ebselen caused a significant increase in TrxR activity at high exposure concentrations (2.5 and 5 µM) when compared to control. Notably, TrxR activity was more effectively increased by $(PhSe)_2$ than ebselen, corresponding to a 4-fold increase at the highest tested concentration (Figure 3B), whereas ebselen increased TrxR activity by only 1.5 fold (Figure 3C).

Co-treatment of SH-SY5Y cells with $(PhSe)_2$ (2.5 and 5 µM)) blunted the inhibitory effect of MeHg (1µM), and at 5 µM(PhSe)2 significantly increased TxrR activity to levels higher than controls (Figure 4A). In contrast, ebselen did not reverse the inhibitory effect of MeHg on TrxR activity (Figure 4B).

Glutathione peroxidase activity. As depicted in Figure 5A, MeHg at concentrations of 1 µM to 5 µM caused a small, yet significant ($p<0.01$) decrease in GPx activity in neuroblastoma cells, albeit it the effect was not concentration-dependent. In contrast, selenium compounds did not alter the activity of this selenoenzyme (Figure 5B and 5C).

Diphenyl diselenide (5 µM), blunted the inhibitory effect of MeHg on GPx activity (Figure 6 A). However, co-exposure to ebselen with MeHg failed to modify the inhibitory effect of MeHg on GPx activity (Figure 6 B).

Western blot. As shown in Figure 7, $(PhSe)_2$ induced an increase in TrxR protein expression in neuroblastoma cells. This effect was concentration-dependent. Diphenyl diselenide also slightly increased the expression of GPx at lower concentrations. In contrast, ebselen did not change the expression of TrxR, but increased GPx1 protein expression upon co-treatment with MeHg.

DISCUSSION

In the present study, we demonstrated that exposure of human neuroblastoma cells (SH-SY5Y) to MeHg was associated with cell mortality, corroborating analogous effects in human cervical carcinoma HeLa cells and human hepatoma cells (HepG2).[15,26] Cell death is likely triggered by the ability of MeHg to induce oxidative stress.[2,4] Herein, we investigated the mechanisms that may mediate this effect, taking advantage of antioxidant organoselenium compounds.

Considering that the toxicity of MeHg is associated with the generation of reactive oxygen species (ROS), mainly by inhibiting sulfhydryl (-SH) and selenohydryl (-SeH) containing proteins, such as selenoezymes,[2,13-15] we have used the activity and protein expression of the selenoenzymes TrxR and GPx as endpoints of MeHg neurotoxicity. These enzymes are known important molecular targets of MeHg toxicity.[14,15,17,19,24]

MeHg caused a significant inhibition of both TrxR and GPx activity, possibly related to a reduction in enzymes synthesis and/or a direct interaction of MeHg with the selenol groups of these enzymes.[2] Earlier studies have indicated that MeHg can inhibit TrxR and GPx derived from various tissues and experimental models.[14,16,20,45] Here, we observed that the sensitivity of TrxR to MeHg was higher than that of GPx. This likely reflects the presence of the selenocysteine (Sec) residue in close proximity to three Cys residues in the open C-terminus of mammalian TrxR[15,46] *vs.* the GPx active site, where the Sec residue is in close proximity to Gln and Trp residues.[47] This accessibility, and the ability of the TrxR to bind electrophilic agents,[48] such as mercury compounds, increases significantly the reactivity of MeHg with the active site of TrxR.[45]

As a corollary to the above, it has been previously hypothesized that antioxidants might be useful tools to mitigate MeHg toxicity,[2,10,11,49,50] since there is no effective treatment to completely counteract its ill effects. Accordingly, selenium compounds with antioxidant properties might offer efficacious treatment against injury caused by MeHg as demonstrated here and in other studies.[2,24,27,51] The protective effect of the selenium compounds, when cotreated with MeHg, might be related to the potential effects of these compounds to scavenge hydroxyl, superoxide and peroxyl radicals.[27,52] In this context, a study by Li et al. reported that in humans exposed to Hg, daily supplementation with selenium-enriched yeast for three months decreased the urinary levels of biomarkers of oxidative stress, such as malondialdehyde and 8-hydroxy- 2-deoxyguanosine. In addition, in the same study the

authors demonstrated another important property of selenium compounds in counteracting Hg toxicity, showing an increase in the excretion of Hg after supplementation of volunteers with selenomethionine.[27]

Moreover, considering that TrxR is an important target of MeHg [14-16,19,20,26,45] $(PhSe)_2$, as an antioxidant organoselenium compound, may be considered an efficacious compound in protecting against MeHg toxicity. Our findings showed a significant increase in TrxR activity and expression when neuroblastoma cells were treated with $(PhSe)_2$ alone. Furthermore, $(PhSe)_2$ when co-administered with MeHg fully reversed the inhibitory effect of MeHg on TrxR activity. The molecular mechanism(s) involved in the protection afforded by $(PhSe)_2$ likely reflect its ability to induce the synthesis of TrxR[41]. Furthermore, $(PhSe)_2$ may also be transformed into its selenol intermediate and bind to MeHg with high affinity.[24,53] In contrast, ebselen, another important organoselenium compound with marked antioxidant properties, had only a modest protective effect against MeHg. The weak efficcacy of ebselen in counteracting the toxicity of MeHg may be related to its weak effect as inducer of TrxR, despite its documented ability to decrease the toxicity of MeHg both in rats and mice.[2,36,37,54]

Treatment of neuroblastoma cells with ebselen and $(PhSe)_2$ did not increase the total GPx activity, but $(PhSe)_2$ treatment alone increased the expression of this selenoenzyme. $(PhSe)_2$ and ebselen are mimetics of GPx activity[30,39,53] and exposure of endothelial cells to these compounds has been shown to increase in the expression of GPx 4 and the total GPx activity.[39] However, as demonstrated in our study, $(PhSe)_2$ induced a significantly higher increase in both cellular GPx expression when compared to ebselen.[39]

Moreover, $(PhSe)_2$ and ebselen can be reduced to selenol intermediates both after a direct reaction with thiol groups[55] or via a NADPH-dependent reduction catalyzed by TrxR reductase.[56,57] Of particular significance to our results, is the observation that $(PhSe)_2$ serves as a better mimetic of GPx,[39,53,58] and it is also a better substrate for TrxR than ebselen.[53,59] Taken together, the protective effect of $(PhSe)_2$ and ebselen against MeHg toxicity may be associated with their antioxidant properties (for instance, as a mimetics of GPx). However, the up-regulation of TrxR may also be involved in direct neuroprotective effect of $(PhSe)_2$ observed here.

In conclusion, our results support the potential use of selenocompounds, such as $(PhSe)_2$ against MeHg toxicity and other xenobiotic TrxR/Trx inhibitors. Furthermore, we have demonstrated for the first time that $(PhSe)_2$ caused up-regulation of TrxR in neuroblastoma cells, reflecting an important molecular mechanism in the antioxidant properties of this compound.

AUTHOR INFORMATION

Corresponding author

*E-mail: jbtrocha@yahoo.com.br

Funding

This study was partly financed by the MERTOX project (PTDC/QUI-BIQ/117281/2010) and by iMed.ULisboa through FCT's strategic project: PEst-OE/SAU/UI4013/2011 and CNPq. Daiane F. Meinerz is financed by a fellowship (BEX 18480/12-8) from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Vasco Branco is financed by a postdoctoral fellowship (SFRH/BPD/85219/2012) from Fundação para a Ciência e Tecnologia (www.fct.mctes.pt).

Notes

The authors declare no competing financial interest.

ABREVIATIONS

Hg, mercury; MeHg, methylmercury; Se, selenium; $(PhSe)_2$, Diphenyl diselenide; TrxR, Thioredoxine reductase; GPx, Glutathione peroxidase; Sec, selenocysteine.

REFERENCES

(1) Clarkson, T. W., and Magos, L. (2006) The toxicology of mercury and its chemical compounds. *Crit. Rev. Toxicol. Sep.* 36(8), 609-62.

(2) Farina, M., Aschner M., and Rocha, J.B.T. (2011) Oxidative stress in MeHg-induced neurotoxicity. *Toxicol. Appl. Pharmacol.* 256, 405–417.

(3) Burger, J., Gochfeld, M., Jeitner, C., Donio, M., and Pittfield, T. (2014) Sushi consumption rates and mercury levels in sushi: Ethnic and demographic differences in exposure *J. of Risk Res.*, 17(8), 981-997.

(4) Aschner, M., Syversen, T., Souza, D.O., Rocha, J.B., and Farina, M. (2007) Involvement of glutamate and reactive oxygen species in methylmercury neurotoxicity. *Braz. J. Med. Biol. Res.* 40(3), 285–291.

(5) Moszczynski, P., Rutowski, J., Słowinski, S., and Bem, S. (2008) Immunological effects of occupational exposure to metallic mercury in the population of T-cells and NK-cells. *Analyst* 123, 99–103.

(6) Virtanen, K.J., Rissanen, T.H., Voutilainen, S., and Tuomainen, T.P. (2007) Mercury as a risk factor for cardiovascular diseases. *J. Nutr. Biochem.* 18, 75–85.

(7) Moreira, E.L.G., and Farina, M. (2014) An unsolved puzzle: The complex interplay between methylmercury and fish oil-derived fatty acids within the cardiovascular system. *Toxicol. Res.* 3 (5), 300-310.

(8) Rutowski, J., Moszczynski, P., Bem, S., and Szewczyk, A. (1998) Efficacy of urine determination of early renal damage markers for nephrotoxicity monitoring during occupational exposure to mercury vapours. *Med. Pracy* 49, 129–135.

(9) Grotto, D., Barcelos, G.R.M., Valentini, J., Antunes, J.M.G., Angeli, J.P.F., Garcia, S.C., and Barbosa, F. Jr. (2009) Low levels of methylmercury induce DNA damage in rats: protective effects of selenium. *Arch. Toxicol.* 83, 249–254.

(10) Franco, J. L., Braga, H. C., Stringari, J., Missau, F. C., Posser, T., and Mendes, B. G. (2007) Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. *Chem. Res. Toxicol.* 20, 1919-1926.

(11) Lu, T.H., Hsieh, S.Y., Yen, C.C., Wu, H.C., Chen, K.L., Hung, D.Z., Chen, C.H., Wu, C.C., Su, Y.C., Chen, Y.W., Liu, S.H., and Huang, C.F. (2011) Involvement of oxidative

stress-mediated ERK1/2 and p38 activation regulated mitochondria-dependent apoptotic signals in methylmercury-induced neuronal cell injury. *Toxicol. Lett.* 204, 71-80.

(12) Sarafian, T.A. (1999) Methylmercuty-induced generation of free radical: biological implications. *Met. Ions. Biol*. Syst. 36, 415–444.

(13) Mori, N., Yasutake, A., and Hirayama, K. (2007) Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. *Arch. Toxicol.* 81, 769–776.

(14) Wagner, C., Sudati, J.H., Nogueira, C.W., and Rocha, J.B. (2010) In vivo and in vitro inhibition of mice thioredoxin reductase by methylmercury. *Biometals.* 23(6), 1171-7.

(15) Carvalho, C. M. L., Chew, E., Hashemy, S. I., Lu, J., and Holmgren, A. (2008) Inhibition of human thioredoxin system: a molecular mechanism of mercury toxicity. *J. Biol. Chem.* 283, 11913–11923.

(16) Dalla Corte, C.L., Wagner, C., Sudati J.H., Comparsi, B., Leite, G.O., Busanello A., Soares, F.A., Aschner, M., and Rocha. J.B. (2013) Effects of diphenyl diselenide on methylmercury toxicity in rats. *Biomed Res. Int.* 2013, 983821.

(17) Franco, J.L., Posser, T., Dunkley, P.R., Dickson, P.W., Mattos, J.J., Martins, R., Bainy, A.C.D., Marques, M.R., Dafre, A.L., and Farina, M. (2009) Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. *Free Radic. Biol. Med.* 47, 449–457.

(18) Glaser, V., Nazari, E.M., Müller, Y.M.R., Feksa, L., Wannmacher, C.M.D., Rocha, J. B.T., Bem, A.F., Farina, M., and Latini, A. (2010) Effects of inorganic selenium administration in methylmercury-induced neurotoxicity in mouse cerebral cortex. *Int. J. Dev. Neurosci.* 28, 631–637.

(19) Carvalho, C. M. L., Lu, J., Zhang, X., Arnér, E. S. J., and Holmgren, A. (2011) Effects of selenite and chelating agents on mammalian thioredoxin reductase inhibited by mercury: implications for treatment of mercury poisoning. *FASEB J.* 25, 370–381.

(20) Branco, V., Canário, J., Holmgren, A., and Carvalho, C. (2011) Inhibition of the thioredoxin system in the brain and liver of zebra-seabreams exposed to waterborne methylmercury. *Toxicol. Appl. Pharmacol.* 251, 95–103.

(21) Lobanov, A.V.; Hatfield, D.L.; and Gladyshev, V.N. (2009) Eukaryotic selenoproteins and selenoproteomes. *Biochim. Biophys. Acta.* 1790(11), 1424-8.

(22) Shchedrina, V.A., Zhang, Y., Labunskyy, V.M., Hatfield, D.L., and Gladyshev, V.N. (2010) Structure-function relations, physiological roles, and evolution of mammalian ER-resident selenoproteins. *Antioxid. Redox Signal*. 12(7), 839-49.

(23) Falnoga, I., and Tusek-Znidaric, M. (2007) Selenium-mercury interactions in man and animals. *Biol. Trace Elem. Res.* 119, 212–220.

(24) Freitas, A.S., Funck, V.R., dos Santos Rotta, M., Bohrera, D., Morschbachera, V., Puntel, R.L., Nogueira, C.W., Farina, M., Aschner, M., and Teixeira Rocha, J.B. (2009) Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury-induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice. *Brain Res. Bull.* 79, 77–84.

(25) Kalender, S., Uzun, F.G., Demir, F., Uzunhisarcıklı, M., and Aslanturk, A. (2013) Mercuric chloride-induced testicular toxicity in rats and the protective role of sodium selenite and vitamin E. Food Chem. *Toxicol.* 55, 456–462.

(26) Branco, V., Santos, A.G., Gonçalves, J., Lu J., Holmgren, A., and Carvalho, C. (2014) Mitochondrial thioredoxin reductase inhibition,selenium status, and Nrf-2 activation are determinant factors modulating the toxicity of mercury compounds. *Free Radic. Biol. Med.* 73, 95–105.

(27) Li, Y.F.; Dong, Z.; Chen, C.; Li, B.; Gao, Y.; Qu, L.; Wang, T.; Fu, X.; Zhao, Y.; and Chai, Z. (2012) Organic Selenium Supplementation Increases Mercury Excretion and Decreases Oxidative Damage in Long-Term Mercury-Exposed Residents from Wanshan, China. *Environ. Sci. Technol.* 46,11313−11318.

(28) Mugesh, G., Dumont, W.W., and Sies, H. (2001) Chemistry of biologically important synthetic organoselenium compounds. *Chem. Rev.* 101, 2125–2179.

(29) Nogueira, C.W., Zeni, G., and Rocha, J.B. (2004) Organoselenium and organotellurium compounds: pharmacology and toxicology. *Chem. Rev.* 104, 6255–6286.

(30) Muller, A., Cadenas, E., Graf, P., and Sies, H. (1984) A novel biologically active seleno-organic compound-I. Glutathione peroxidase-like activity in vitro and antioxidant capacity of PZ 51 (Ebselen). *Biochem. Pharmacol.* 33 (20), 3235–3239.

(31) Burger, M.E., Fachinetto, R., Wagner, C., Perottoni, J., Pereira, R.P., Zeni G., and Rocha, J. B. (2006) Effects of diphenyl–diselenide on orofacial dyskinesia model in rats. *Brain Res. Bull.* 70,165–170.

(32) Moretto, M.B., Lermen, C.L., Morsch, V.M., Bohrer, D., Ineu, R.P., Silva, A.C., Balz, D., and Schetinger, M.R. (2004) Effect of subchronic treatment withmercury chloride on NTP- Dase 50-nucleotidase and acetylcholinesterase from cerebral cortex of rats, *J. Trace Elem. Med. Biol.* 17, 255–260.

(33) Moretto, M.B., Funchal, C., Zeni, G., Pessoa-Pureur, R., and Rocha, J.B. (2005) Selenium compounds prevent the effects of methylmercury on the in vitro phosphorylation of cytoskeletal proteins in cerebral cortex of young rats. *Toxicol. Sci.* 85, 639–646.

(34) Moretto, M.B., Boff, B., Franco, J., Posser, T., Roessler, T.M., Souza, D.O., Nogueira, C.W., Wofchuk, S., and Rocha, J.B.T. (2007) Ca-45(2+) influx in rat brain: effect of diorganylchalco- genides compounds. *Toxicol. Sci.* 99, 566–571.

(35) Dalla Corte, C.L., Bastos, L.L., Dobrachinski, F., Rocha, J.B., and Soares, F. A. (2012) The combination of organoselenium compounds and guanosine prevents glutamate-induced oxidative stress in different regions of rat brains. *Brain Res.* 1430, 101-11.

(36) Farina, M., Frizzo, M.E., Soares, F.A., Schwalm, F.D., Dietrich, M.O., Zeni, G., Rocha, J.B., and Souza, D.O. (2003a) Ebselen protects against methylmercury-induced inhibition of glutamate uptake by cortical slices from adult mice. *Toxicol. Lett.* 144(3), 351–357.

(37) Farina, M., Dahm, K.C., Schwalm, F.D., Brusque, A.M., Frizzo, M.E., Zeni, G., Souza, D.O., and Rocha, J.B. (2003b) Methylmercury increases glutamate release from brain synaptosomes and glutamate uptake by cortical slices from suckling rat pups: modulatory effect of ebselen. *Toxicol. Sci.* 73(1), 135–140.

(38) Roos, D.H., Puntel, R.L., Santos, M.M., Souza, D.O., Farina, M., Nogueira, C.W., Aschner, M., Burger, M.E., Barbosa, N.B., and Rocha, J.B. (2009) Guanosine and synthetic organoselenium compounds modulate methylmercury-induced oxidative stress in rat brain cortical slices: involvement of oxidative stress and glutamatergic system. *Toxicol. In Vitro.* 23(2), 302-7.

(39) de Bem, A.F., Fiuza, B., Calcerrada, P., Brito, P.M., Peluffo, G., Dinis, T.C., Trujillo, M., Rocha, J.B., Radi, R., and Almeida, L.M. (2013) Protective effect of diphenyl diselenide against peroxynitrite-mediated endothelial cell death: a comparison with ebselen. *Nitric Oxide.* 31, 20-30.

(40) Sakurai, T., Kanayama, M., Shibata, T., Itoh, K., Kobayashi. A., Yamamoto, M., and Uchida, K. (2006) Ebselen, a seleno-organic antioxidant, as an electrophile. *Chem. Res. Toxicol.* 19(9), 1196-204.

(41) Zhang, G., Nitteranon, V., Guo, S., Qiu, P., Wu, X., Li F., Xiao, H., Hu, Q., and Parkin, K. L. (2013) Organoselenium Compounds Modulate Extracellular Redox by Induction of Extracellular Cysteine and Cell Surface Thioredoxin Reductase. *Chem. Res. Toxicol*. 26, 456−464.

(42) Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.

(43) Esworthy, R.S., Chu, F.F., and Doroshow, J.H. (1999) Analysis of glutathione-related enzymes. In: Costa, L.G., Hodgson, E., Lawrence, D.A., Reed, D.J., GreenLee, W.F. (Eds.) *Curr. Protocol. Toxicol.* New York: Wiley, USA. 7.1.1-7.1.32.

(44) Arnér, E. S. J., and Holmgren, A. (1999) Measurement of thioredoxin and thioredoxin reductase. In: Costa, L.G., Hodgson, E., Lawrence, D.A., Reed, D.J., GreenLee, W.F. (Eds.). *Curr. Protoc. Toxicol*. New York: Wiley, USA. 7.4.1-7.4.14.

(45) Branco, V., Canário, J., Lu, J., Holmgren, A., and Carvalho, C. (2012) Mercury and selenium interaction in vivo: effects on thioredoxin reductase and glutathione peroxidase. *Free Radic. Biol. Med.* 52(4), 781-93.

(46) Zhong, L., Arnér, E. S. J., and Holmgren, A. (2000) Structure and mechanism of mammalian thioredoxin reductase: the active site is a redox-active selenothiol/selenenylsulfide Formed from the conserved cysteine-selenocysteine sequence. *Proc. Natl. Acad. Sci. U. S. A.* 97, 5854–5859.

(47) Toppo, S., Flohé, L., Ursini, F., Vanin, S., and Maiorino, M. (2009) Catalytic mechanisms and specificities of glutathione peroxidases: variations of a basic scheme. *Biochim. Biophys. Acta* 1790, 1486–1500.

(48) Papp, L.V.; Lu, J., Holmgren, A., and Khanna, K. K. (2007) From selenium to selenoproteins: synthesis, identity and their role in human health. *Antioxid. Redox Signal.* 9, 775–806.

(49) Barcelos, G.F.M., Grotto, D., Serpeloni, J.M., Angeli, J.P.F., Rocha, B.A., Souza, V.C.O., Vicentini, J.T., Emanuelli, T., Bastos, J.K., Antunes, L.M.G., Knasmüller, S., and

Barbosa, Jr. F. (2011) Protective properties of quercetin against DNA damage and oxidative stress induced by methylmercury in rats. *Arch. Toxicol.* 85, 1151–1157.

(50) Liu, W., Xu, Z., Yang, T., Deng, Y., Xu, B., Feng, S., and Li, Y. (2014) The protective role of tea polyphenols against methylmercury-induced neurotoxic effects in rat cerebral cortex via inhibition of oxidative stress. *Free Radic. Res.* 48(8), 849-63.

(51) Cordero-Herrera, I., Cuello, S., Goya, L., Madrid, Y., Bravo, L., Cámara, C., and Ramos, S. (2013) Molecular mechanisms involved in the protective effect of selenocystine against methylmercury-induced cell death in human HepG2 cells. *Food Chem. Toxicol.* 59, 554-63.

(52) Cuello, S., Ramos, S., Mateos, R., Martin, M.A., Madrid, Y., Camara, C., Bravo, L., and Goya, L. (2007) Selenium methylselenocysteine protects human hepatoma HepG2 cells against oxidative stress induced by tert-butylhydroperoxide. *Anal. Bioanal. Chem.* 389, 2167–2178.

(53) Freitas, A.S., Prestes, A.S., Wagner, C., Sudati, J.H., Alves, D., Porciúncula, L.O., Kade, I.J., and Rocha, J.B.T. (2010) Reduction of Diphenyl Diselenide and Analogs by Mammalian Thioredoxin Reductase Is Independent of Their Gluthathione Peroxidase-Like Activity: A Possible Novel Pathway for Their Antioxidant Activity. *Molecules* 15(11), 7699-7714.

(54) Yin, Z., Lee, E., Ni, M., Jiang, H., Milatovic, D., Rongzhu, L., Farina, M., Rocha, J.B.T., and Aschner, M. (2011) Methylmercury-induced alterations in astrocyte functions are attenuated by ebselen. *NeuroToxicology*, 32 (3): 291-299.

(55) Nogueira, CW, and Rocha, JB. (2011) Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. *Arch. Toxicol.* 85(11), 1313-59.

(56) Zhao, R., and Holmgren, A. (2002a) A novel antioxidant mechanism of ebselen involving ebselen diselenide, a substrate of mammalian thioredoxin and thioredoxin reductase. *J. Biol. Chem.* 277(42), 39456-62.

(57) Zhao, R., Masayasu, H., and Holmgren, A. (2002b) Ebselen: a substrate for human thioredoxin reductase strongly stimulating its hydroperoxide reductase activity and a superfast thioredoxin oxidant. *Proc. Natl. Acad. Sci. U. S. A.* 99(13), 8579-84.

(58) Wilson, S.R., Zucker, P.A., Huang, R.R.C., and Spector, A. (1989) Development of synthetic compounds with glutathione peroxidase activity. *J. Am. Chem. Soc.* 111, 5936–5939.

(59) Freitas, A. S., and Rocha, J. B. (2011) Diphenyl diselenide and analogs are substrates of cerebral rat thioredoxin reductase: a pathway for their neuroprotective effects. *Neurosci Lett.* 503(1), 1-5.

LEGENDS TO FIGURES

Figure 1. Chemical structure of the selenium compounds diphenyl diselenide (a) and ebselen (b).

Figure 2. Effect of MeHg, $(PhSe)_2$ and ebselen on viability in SH-SY5Y cells. Data are expressed as percentage of control and the values are mean ± S.E.M. of 4 independent experiments. * $P < 0.05$, and **** $P < 0.0001$ vs. control.

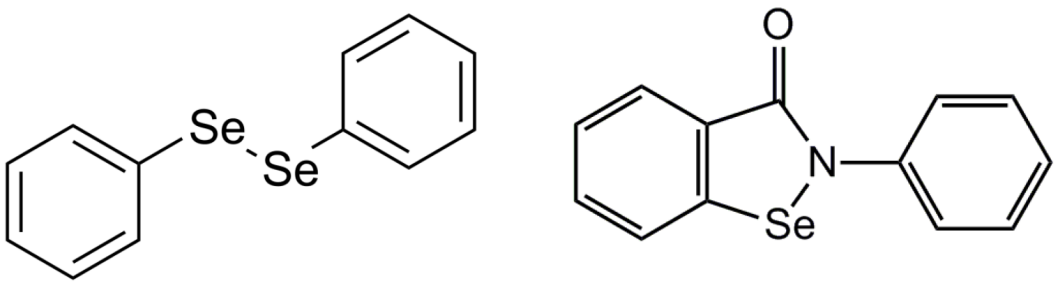
Figure 3. Effect of MeHg (A), $(PhSe)_2$ (B) and Ebselen (C) on TrxR activity in SH-SY5Y cells. Data are expressed as percentage activity relative to control and the values are mean ± S.E.M. of 4-5 independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ vs. control.

Figure 4. Effect of co- treatment with $(PhSe)_2$ and MeHg (A) and co-treatment with ebselen and MeHg (B) on TrxR activity in SH-SY5Y cells. Data are expressed as percentage activity relative to control and the values are mean ± S.E.M. of 4-5 independent experiments. ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ vs. control; $^{\#\#\#\#}$ $P < 0.0001$ vs. MeHg.

Figure 5. Effect of MeHg (A), $(PhSe)_2$ (B) and Ebselen (C) on GPx activity in SH-SY5Y cells. Data are expressed as percentage activity relative to control and the values are mean ± S.E.M. of 4-5 independent experiments. * $P < 0.05$, ** $P < 0.01$ vs. control.

Figure 6. Effect of co- treatment with $(PhSe)_2$ and MeHg (A) and co- treatment with ebselen and MeHg (B) on GPx activity in SH-SY5Y cells. Data are expressed as percentage activity relative to control and the values are mean ±S.E.M. of 4-5 independent experiments. $^{\#}$ $P < 0.05$ vs. MeHg.

Figure 7. Effect of treatment with $(PhSe)_2$ and ebselen alone or in co-treatment with MeHg on TrxR and GPx expression by western blot analysis in SH-SY5Y cells. Equal loading of western blots was verified with GAPDH expression and represent one of two experiments. (P.S.: The values of selenium compounds are expressed in µM; MeHg was used only at a concentration of 1µM).



(A) **(B)**

Figure 1.

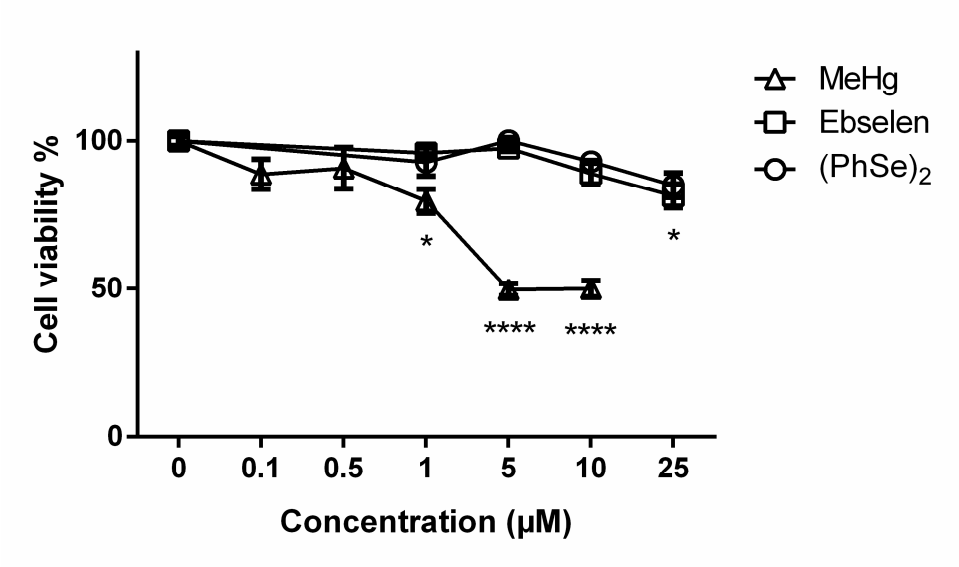

Cell viability %
100
50
0
0
0.1
0.5
1
5
10
25
Concentration (μM)
*
****
****
*
MeHg
Ebselen
(PhSe)2

Figure 2.

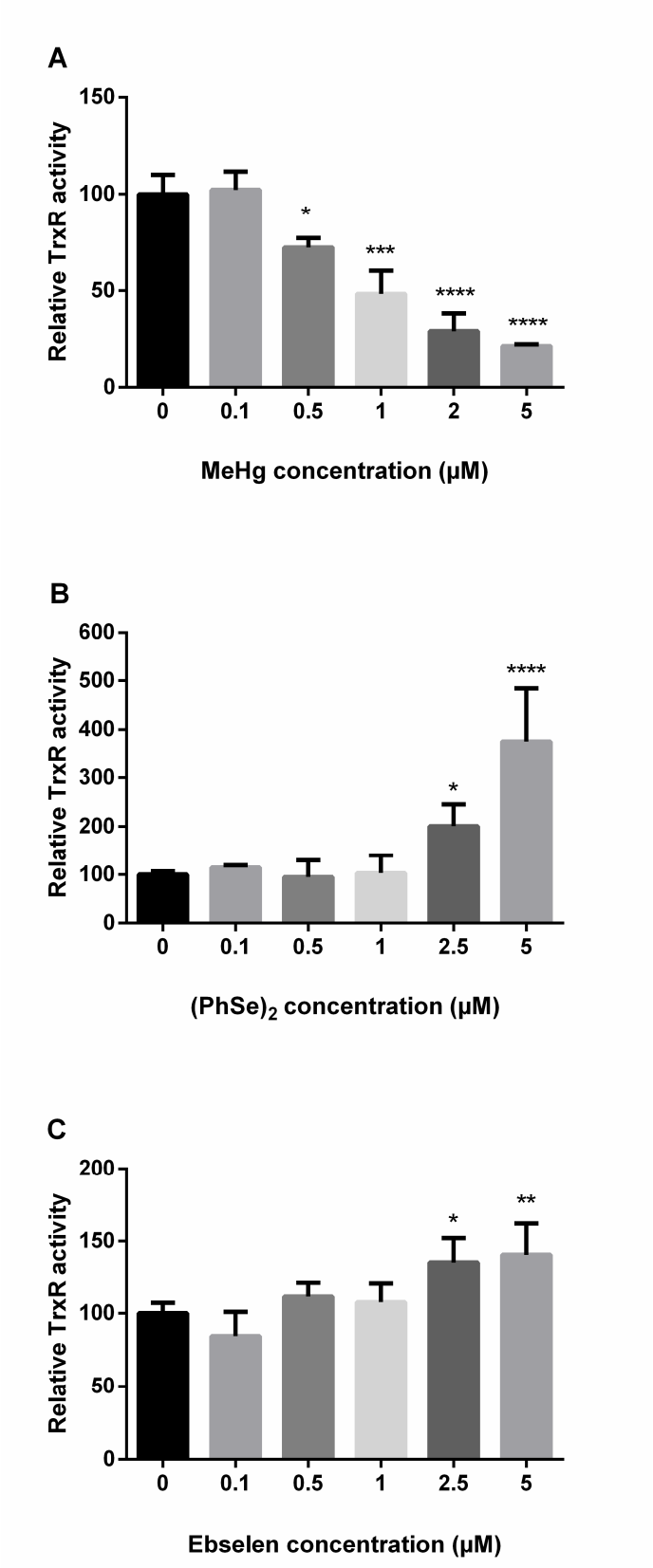

A
Relative TrxR activity
150
100
50
0
*
***
****
****
0
0.1
0.5
1
2
5
MeHg concentration (μM)
B
Relative TrxR activity
600
500
400
300
200
100
0
*
****
0
0.1
0.5
1
2.5
5
(PhSe)2 concentration (μM)
C
Relative TrxR activity
200
150
100
50
0
*
**
0
0.1
0.5
1
2.5
5
Ebselen concentration (μM)

Figure 3.

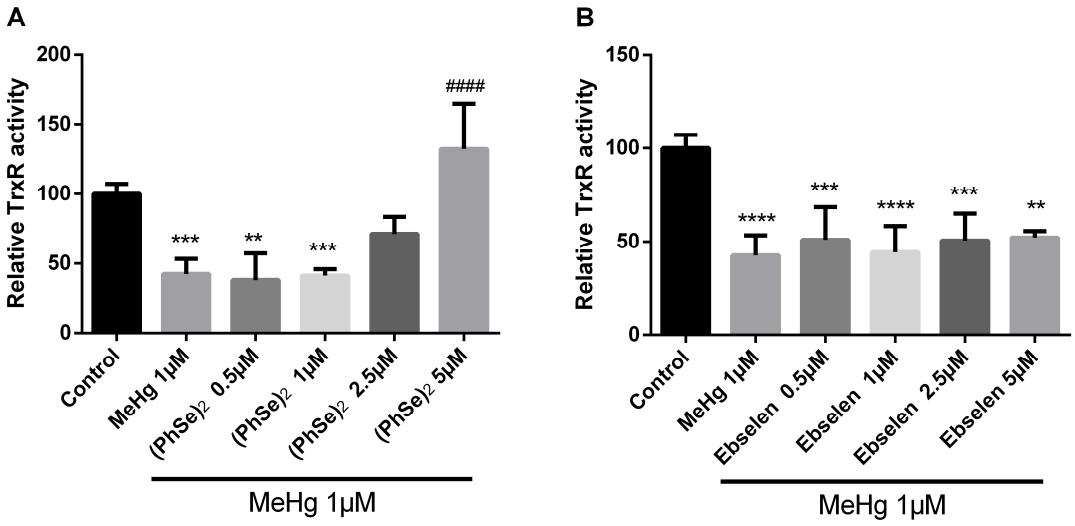

A
Relative TrxR activity
200
150
100
50
0
***
**
***
####
Control
MeHg 1μM
(PhSe)2 0.5μM
(PhSe)2 1μM
(PhSe)2 2.5μM
(PhSe)2 5μM
MeHg 1μM
B
Relative TrxR activity
150
100
50
0
****
***
****
***
**
Control
MeHg 1μM
Ebselen 0.5μM
Ebselen 1μM
Ebselen 2.5μM
Ebselen 5μM
MeHg 1μM

Figure 4.

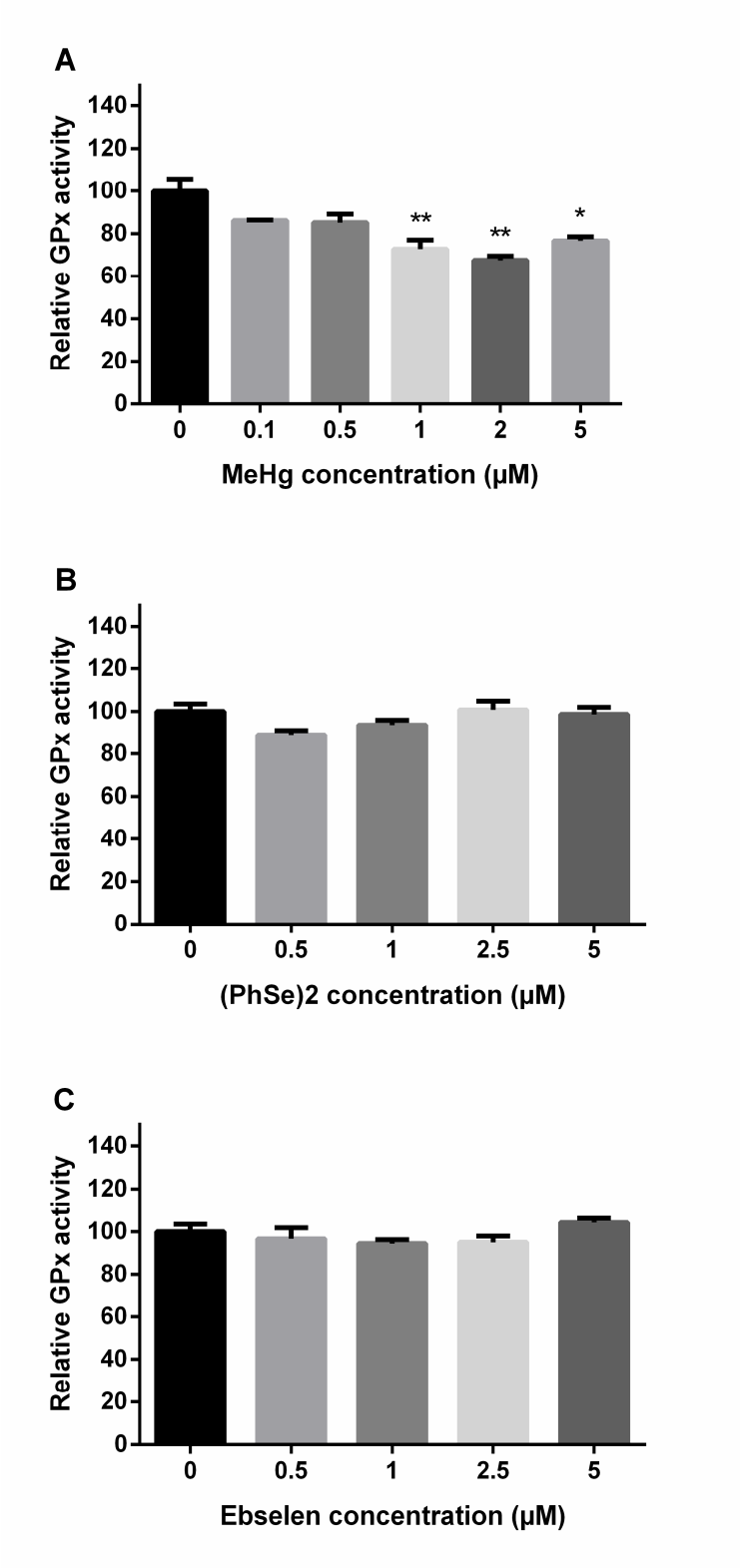

A
Relative GPx activity
140
120
100
80
60
40
20
0
**
**
*
0
0.1
0.5
1
2
5
MeHg concentration (µM)
B
Relative GPx activity
140
120
100
80
60
40
20
0
0
0.5
1
2.5
5
(PhSe)2 concentration (µM)
C
Relative GPx activity
140
120
100
80
60
40
20
0
0
0.5
1
2.5
5
Ebselen concentration (µM)

Figure 5.

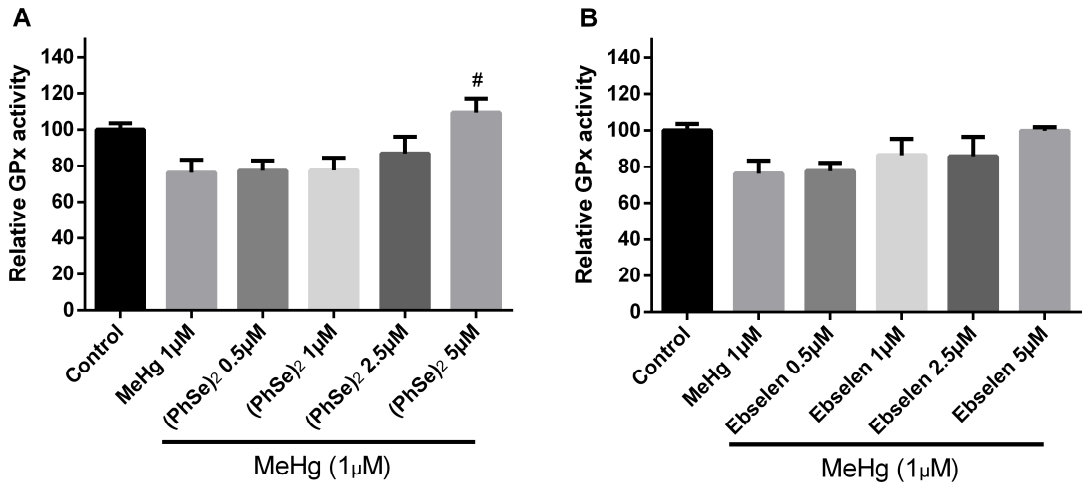

A
Relative GPx activity
140
120
100
80
60
40
20
0
#
Control
MeHg 1μM
(PhSe)2 0.5μM
(PhSe)2 1μM
(PhSe)2 2.5μM
(PhSe)2 5μM
MeHg (1μM)
B
Relative GPx activity
140
120
100
80
60
40
20
0
Control
MeHg 1μM
Ebselen 0.5μM
Ebselen 1μM
Ebselen 2.5μM
Ebselen 5μM
MeHg (1μM)

Figure 6.

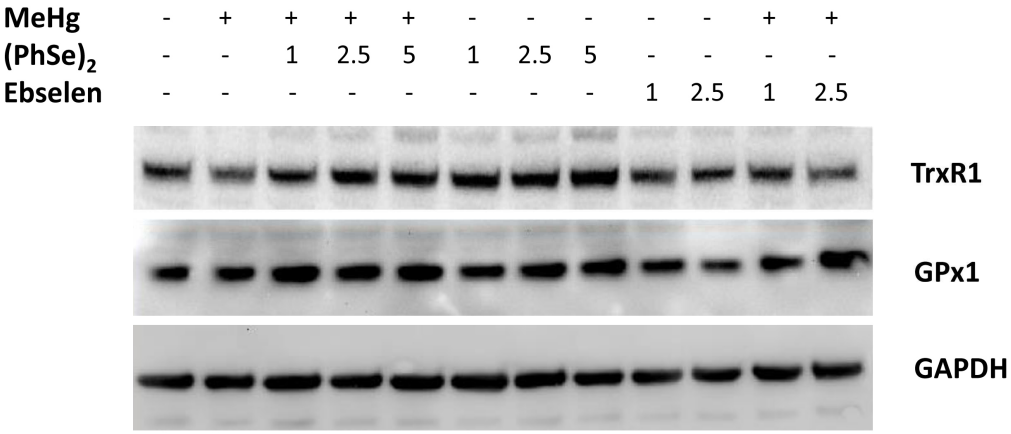


Figure 7.

2.3 Manuscrito 2:

PROTECTIVE EFFECT OF DIPHENYL DISELENIDE AGAINST METHYLMERCURY TOXICITY IN HUMAN LEUKOCYTES *IN VITRO*.

DAIANE F.MEINERZ; DIONES C. BUENO; JEFERSON L. FRANCO; JOÃO BATISTA TEIXEIRA DA ROCHA

(Resultados complementares)

Protective effect of diphenyl diselenide against methylmercury toxicity in human leukocytes *in vitro*.

Meinerz D.F.[1]; Bueno D.C.[1]; Franco, J.L.[2]; Rocha JBT[1].

[1] Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria RS, Brazil, 97105-900;

[2]Campus São Gabriel, Universidade Federal do Pampa, São Gabriel, RS, Brazil, 97300-000.

INTRODUCTION

Methylmercury (MeHg), the organic form of mercury, is among the most potentially toxic species to which human populations are exposed, both at high levels through poisonings and at lower levels through consumption of fish and other seafood (Clarkson and Magos, 2006).

Among the mechanisms proposed to explain the toxicity of MeHg, oxidative stress plays a crucial role demonstrated in *in vivo* and *in vitro* studies (Ascher and Syversen 2005; Franco et al. 2007; Meinerz et al. 2011), in which MeHg induces the generation of reactive oxygen species (ROS) (Sarafian, 1999) and alters the antioxidant defense system of cells by inhibiting their sulphydryl groups [–SH] and selenohydryl groups [-SeH], compromising the expression and activity of thiol and selenoenzymes (Mori et al. 2007; Branco et al., 2011; 2014). These ROS generated, such as hydroxyl radical ($^{\cdot}OH$), superoxide anion ($O_2^{-\cdot}$), and hydrogen peroxide, may also attacks the DNA molecule and frequently cause oxidative DNA damage and altered intracellular redox status (Stohs and Bagchi, 1995; Joshi et al. 2014). On the other hand, selenium (Se) acts as an antagonist to the toxic effects of many heavy metals, including Hg (Yoneda and Suzuki 1997; Falnoga and Tusek-Znidaric 2007).

Moreover, selenium is a fundamental component of the living cells of a variety of organisms with antioxidant properties (Bock et al. 1991; Nogueira et al. 2004; Freitas et al. 2009), and it is necessary for the expression of selenoenzymes, such as glutathione peroxidase (GPx) (Flohe et al. 1973) and thioredoxin reductase (TrxR) (Holmgren, 1989). In this context, the interest in compounds containing selenium has increased in the past three decades, mainly due to their biological activities (Nogueira et al. 2004).

Organoselenium compounds, such as $(PhSe)_2$ - the simplest of the synthetic diaryl diselenides - are known as pharmacologically active compounds, exhibiting antioxidant, anti-inflammatory, neuroprotective, and antimutagenic properties (Nogueira et al. 2004; Rossato et a. 2002; Nogueira and Rocha, 2011; Farina et al. 2003). At low concentrations, these compounds protect cells against the insults generated by ROS production, depleting H_2O_2 via their GPx mimic activity (Mugesh and Singh, 2000). Based on this, it appears that $(PhSe)_2$ could behave as a potent protective compound in models of toxicity.

In this regard, the purpose of the present study was evaluated whether human leukocytes *in vitro* exposed to low levels of MeHg are subject to toxicity. Moreover, we evaluated the possible protective effect of $(PhSe)_2$, an potent antioxidant compound, to

counteract the toxicity caused by MeHg related to cell viability, DNA damage and changes in gene expression (TrxR, GPx and NFE2L2) in the same exposed cells.

MATERIALS AND METHODS

Chemicals

Diphenyl diselenide, methylmercury, Trypan's Blue, dextran, tungstosilicic acid and Triton X-100 were obtained from Sigma- Aldrich (St. Louis, MO). All the other reagents were purchased from standard chemical suppliers.

Sample Preparation

Leukocytes were isolated from heparinized venous blood obtained from healthy volunteers (4 volunteers for each methodology). The protocol of study was reviewed and approved by the appropriate institutional review board from Guidelines of the Committee of UFSM (0089.0.243.000-07).

Briefly, 2 mL of dextran 5% (dissolved in Phosphate Buffer Saline 1%) was added to 8 mL of blood. The tube was gently mixed and left to stand at room temperature for 45 minutes. Afterwards, the supernatant was centrifuged (480 ×g, 10 min) and plasma was discarded. The pellet was washed with erythrocyte lysis solution (NH_4Cl 150 mM; $NaHCO_3$ 10 mM; EDTA 1 mM) and centrifuged (480 ×g, 2 min). The supernatant was discarded and the pellet was washed twice with 1mL erythrocyte lysis solution. After the second centrifugation, the pellet was suspended in 2 mL Hank's buffer solution (KCl 5.4 mM; Na_2HPO_4 0.3 mM; KH_2PO_4 0.4 mM; $NaHCO_3$ 4.2 mM; $MgCl_2$ 0.5 mM; NaCl 122.6 mM; D-glicose 10 mM, Tris- HCl 10 mM; $CaCl_2$ 1.3 mM; pH 7.4). The concentration of leukocytes was adjusted to 2000 cells/μL.

Leukocytes Exposure to compounds

Leukocytes were exposed to $(PhSe)_2$, or MeHg at 1, 5, and 10 μM or they are co-treated with $(PhSe)_2$ and MeHg at the same concentrations and as control (vehicle) was used

an equal volume of ethanol (final concentration of 0.2%) during 3 hours at 37 °C. Positive control group was treated with hydrogen peroxide (H_2O_2) 2 mM and sodium azide 1 mM.

Trypan's Blue Exclusion Test

Trypan's Blue exclusion test was performed according to Mischel and Shiingi (1980). After 3 hours of incubation, 50 μL of Trypan's Blue 0.4% was mixed with 50 μL of leukocytes and left to stand at room temperature for 5 minutes. Cell viability was determined microscopically (400 x magnification) and expressed as number of viable cells divided by the total number of cells multiplied by 100.

Comet Assay

Comet Assay was performed according to Collins (2004) with some modifications. After three hours of incubation, 15 μL of the sample was mixed with 90μL of low melting point agarose 0.75% and placed in a slide pre covered with agarose 1%. A coverslip was added and the samples were left to solidify at 4°C. The coverslips were removed and the slides were placed on a lysis solution (NaCl 2.5 M; EDTA 100 mM; Tris-HCl 8 mM; Triton X-100 1%; pH 10-10.5) during 24 hours at 4 °C. Afterwards, the slides were incubated in an electrophoresis solution (NaOH 300 mM; EDTA 1 mM; pH 13.5) for 20 minutes at 4°C and the electrophoresis was performed (25 V; 300 mA; 7 W) for 20 minutes. All the steps were performed in the dark until this moment. After electrophoresis, the slides were washed in a neutralizing solution (Tris-HCl 400 mM; pH7.5) three times, washed with distilled water, and left to dry. The slides were rehydrated and fixed (Trichloroacetic acid 15%; ZnSO4 5%; glycerol 5%), washed thrice with distilled water, and left to dry. Afterwards, the rehydrated slides were stained (Na_2CO_3 5%; NH_4NO_3 0.1%; $AgNO_3$ 0.1%; $H_4[W_{12}SiO_{40}]$ 0.25%; formaldehyde 0.15%). The slides were immersed in acetic acid 1%, washed, and left to dry.

One hundred cells randomly selected were analyzed in each sample according to tail size and intensity in five classes. The damage score for each cell can range between 0 (no damage) and 4 (maximum damage), according to Figure 1. Damage index (DI) was defined as follows: DI = $1n1 + 2n2 + 3n3 + 4n4$, where $n1$ represents the number of cells with damage level 1, $n2$ represents the number of cells with damage level 2, $n3$ represents the number of cells with damage level 3, and $n4$ represents the number of cells with damage level 4. At least two different individuals analyzed the slides under blind conditions.

Real time RT-PCR analysis of mRNA

After incubation time, leukocytes were immediately centrifuged at 480 *x* g during 5 minutes. The buffer was discarded and the leukocytes were homogenized in 1 mL of TriZOL®.

Total RNA extraction was performed according to manufacturer's instructions (Invitrogen, USA). RNA samples were dissolved in 20 µL of water, and the quality/integrity of RNA was confirmed in agarose 1% gel stained with ethidium bromide 0.5 µg/mL and visualized under ultraviolet light. RNA samples were treated with DNase I according to manufacturer's instructions and reverse transcripted in cDNA molecules using M-MLVRT reverse transcriptase enzyme according to manufacturer's instructions (Invitrogen, USA).

Gene expression analysis was performed through real-time Polymerase Chain Reaction (PCR). The reaction mixture for PCR reaction contains: 10 µL diluted 1:100 cDNA or water as negative control; 0.2 µM primers; 0.2 mM deoxiribonucleotide triphosphates; 1.5-3 mM $MgCl_2$; 1x PCR buffer; 0.05 U/µL *Taq* DNA Polymerase; and 0.01x SYBR Green. The reaction was submitted to the following cycling conditions: *Taq* DNA Polymerase activation at 95°C for 3 min, followed by 40 cycles of 15s at 95°C (denaturation), 15s at 58-62°C (annealing), and 15s at 72°C (extension). Threshold levels were manually determined using StepOne Software v2.0 (Applied Biosystems, NY). SYBR Green fluorescence and Cq values generation were analyzed using StepOne Software v2.0 (Applied Biosystems, NY).

The analyzed genes were TRXR1, GPX4 and NFE2L2. The reference genes were GAPDH and RPL30. Primers were designed using Primer3 or BLAST software based on published sequences in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) as shown in Table 1. Results are expressed as relative expression, which will be calculated using $2^{-\Delta\Delta Cq}$ formula (Livak and Schmittgen, 2001).

Statistical Analysis

Statistical analyses were performed using analysis of variance (ANOVA) followed by Newman- Keuls multiple test when appropriate. The results are expressed as mean ± SEM for three to four independent replicates. The difference was considered significant when $P < 0.05$.

RESULTS

Cell viability

Diphenyl diselenide did not cause any significant toxic effects on leukocytes at concentrations tested (1, 5 and 10 µM) when compared to the control. However, MeHg at concentrations from 5µM caused a significant decrease in cell viability when compared to the control. Furthermore, at the highest concentration tested, the cell viability was about 35% ($P < 0.001$) (Figure 2).

Diphenyl diselenide was able to protect against the toxic effect of MeHg at concentrations of 1 and 5 µM when co-exposed to 5 µM of MeHg, and at concentrations of 5 and 10µM when co-exposed to 10µM of MeHg, avoiding significantly the cell mortality (Figure 3).

The positive control (H_2O_2 2 mM and sodium azide 1 mM) caused a significant decrease in cell viability (approximately 33%) when compared to control (Fig. 3, $P < 0.001$).

DNA damage

Figure 4 shows the effects of $(PhSe)_2$ and/ or MeHg on DNA damage in human leukocytes *in vitro*. As expected, H_2O_2 and azide used as a positive control caused a significant increase in the damage index values when compared to the control ($P < 0.001$). Incubation of human leukocytes with $(PhSe)_2$ until 10 μM did not show significant increase in the damage index. However, the MeHg even at lowest concentration tested, induced a significant increase in the DNA damage when compared to control ($P<0.01$), suggesting a possible induction of DNA double-strand breaks.

Nevertheless, when the leukocytes where co-exposed to $(PhSe)_2$ and MeHg, the selenium compound had an effective activity in atenuate the DNA damage caused by the mercury toxic compound, at two concentrations tested (Figure 4).

Levels of mRNA expression

Fig. 5 shows the expression of TrxR1, GPx 4 and NFE2L2 in human leukocytes treated with $(PhSe)_2$ and and/or MeHg. Exposure of human leukocytes to $(PhSe)_2$, alone or co-treated with MeHg 5µM, caused a significant increase in TrxR1 expression when compared to control ($P < 0.05$, Figure 5A). On the other hand, the treatment only with MeHg did not change the expression of TrxR1.

The GPx 4 expression did not show any significant difference among the treatments (Figure 5 B). In contrast, when the NFE2L2 expression was evaluated, only the exposition of leukocytes to MeHg was able to increase significantly its expression ($P < 0.05$) (Figure 5C). But the selenium compound tested, did not modify the expression of NFE2L2 (Figure 5 C).

5 C).

REFERENCES

Ascher, M., and Syversen, T. (2005) Methylmercury: recent advances in the understanding of its neurotoxicity. Ther. Drug. Monit. 27, 278–283.

Bock, A., Forchhammer, K., Heider, J., Leinfelder W., Sawers G., Veprek B., and Zinoni F. (1991) Selenocysteine: the 21st amino acid. Mol. Microbiol. 5 (3), 515– 520.

Branco, V., Canário, J., Holmgren, A., and Carvalho, C. (2011) Inhibition of the thioredoxin system in the brain and liver of zebra-seabreams exposed to waterborne methylmercury. Toxicol. Appl. Pharmacol. 251, 95–103.

Branco, V., Santos, A.G., Gonçalves, J., Lu J., Holmgren, A., and Carvalho, C. (2014) Mitochondrial thioredoxin reductase inhibition, selenium status, and Nrf-2 activation are determinant factors modulating the toxicity of mercury compounds. Free Radic. Biol. Med. 73, 95–105.

Clarkson, T. W., and Magos, L. (2006) The toxicology of mercury and its chemical compounds. Crit. Rev. Toxicol. 36, 609–662.

Collins, A. R. (2004) The comet assay for DNA damage and repair: principles, applications, and limitations. Molecular Biotechnology 26 (3), 249–261.

Freitas, A. S., Funck, V. R., Rotta, M. dos S., Bohrer, D., Morschbacher, V., Puntel, R. L., Nogueira, C.W., Farina, M., Aschner, M., and Rocha J. B. (2009) Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury-induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice. Brain Res. Bull. 79 (1), 77–84.

Falnoga, I., and Tusek-Znidaric, M. (2007) Selenium-mercury interactions in man and animals. Biol. Trace Elem. Res. 119, 212–220

Farina, M., Frizzo, M. E. S., and Soares F. A. A. et al. (2003) Ebselen protects against methylmercury-induced inhibition of glutamate uptake by cortical slices from adult mice. Toxicol. Lett. 144 (3), 351–357.

Flohe, L., Gunzler, W.A., and Schock H.H. (1973) Glutathione peroxidase: a selenoenzyme. FEBS Lett. 32 (1), 132–134.

Franco, J. L., Braga, H. C., Stringari, J., Missau, F.C., Posser, T., Mendes, B.G., Leal, R. B., Santos, A. R. S., Dafre, A. L., Pizzolatti, M. G., and Farina, M. (2007) Comparative study of activities in reactive oxygen species production/defense system in mitochondria of rat brain and liver, and their susceptibility to methylmercury toxicity. Chem. Res. Toxicol. 20, 1919–1926.

Joshi, D., Kumar, M. D., Kumar, S. A., and Sangeeta, S. (2014) Reversal of methylmercury-induced oxidative stress, lipid peroxidation, and DNA damage by the treatment of N-acetyl cysteine: a protective approach. J. Environ Pathol. Toxicol. Oncol. 33(2), 167-82.

Livak, K. J., and Schmittgen, T. D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 25(4), 402-8.

Meinerz, D. F., de Paula, M. T., Comparsi, B., Silva, M. U., Schmitz, A. E., Braga, H. C., Taube, P. S., Braga, A. L., Rocha, J. B., Dafre, A. L., Farina, M., Franco, J. L., and Posser, T. (2011) Protective effects of organoselenium compounds against methylmercury-induced oxidative stress in mouse brain mitochondrial-enriched fractions. Braz. J. Med. Biol. Res. 44(11), 1156-63.

Mischell, B. B., and Shiingi, S. M. (1980) Selected Methods in Cellular Immunology, W.H. Freeman & Co. Oxford, UK. 10, 43.

Mori, N., Yasutake, A., and Hirayama, K. (2007) Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. Arch. Toxicol. 81, 769–776

Mugesh, G., and Singh H. B. (2000) Synthetic organoselenium compounds as antioxidants: glutathione peroxidase activity. Chemical Society Reviews 29(5), 347–357.

Nogueira, C. W., and Rocha J. B. T. (2011) Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds, Archives of Toxicology 85(11), 1313–1359.

Nogueira, C. W., Zeni, G., and Rocha, J. B. (2004) Organoselenium and organotellurium compounds: toxicology and pharmacology. Chem. Rev. 104(12), 6255–6285.

Rossato, J. I., Ketzer, L. A., Centurião, F. B. et al. (2002) Antioxidant properties of new chalcogenides against lipid peroxidation in rat brain. Neurochem. Res., 27 (4), 297–303.

Sarafian, T. A. (1999) Methylmercuty-induced generation of free radical: biological implications. Met. Ions. Biol. Syst. 36, 415–444

Stohs, S. J., and Bagchi, D. (1995) Oxidative mechanisms in the toxicity of metal ions. Free Radic. Biol. Med. 18, 321–336

Yoneda, S., and Suzuki, K. T. (1997) Equimolar Hg–Se complex binds to selenoprotein P. Biochem. Biophys. Res. Commun. 231, 7–11.

Table 1. Primers used to amplify target gene fragments.

Name of gene	Symbol	Reference sequence	Primer sequence (5'-3')	Amplicon
Tiorredoxina Redutase 1	TXNRD1	NM_001093771	GCATCAAGCAGCTTTGTTAGG	95pb
		NM_001093771	TCATTCTGTCCCAATCATGC	
Glutationa Peroxidase 4	GPX4	NM_001039847	AGATCCAACCCAAGGGCAAG	72 pb
		NM_001039847	GACGGTGTCCAAACTTGGTG	
Nuclear factor	NFE2L2	NM_001145412	AACCAGTGGATCTGCCAACT	134pb
(erythroid-derived 2)-like 2		NM_001145412	ACGTAGCCGAAGAAACCTCATT	
Glyceraldehyde-3-phosphate	GAPDH	NM_001256799	TTCGTCATGGGTGTGAACC	112pb
dehydrogenase		NM_001256799	AGTTGTCATGGATGACCTTGG	
Ribosomal protein L30	RPL30	NM_000989	GATGATCAGACAAGGCAAAGC	105pb
		NM_000989	ACACCAGTTTTAGCCAACATAGC	

LEGENDS

Figure 1. Damage levels considered for analysis in Comet Assay. Level 5 was excluded from our evaluation. This figure is based on Santos et al. (2009).

Figure 2. Effect of $(PhSe)_2$ or MeHg on cell viability of human leukocytes *in vitro.* Data are expressed as means ± SEM of four independent experiments. * P <0.05 and *** P < 0.001 vs control.

Figure 3. Effect of co-exposure to $(PhSe)_2$ and MeHg on cell viability of human leukocytes *in vitro.* Data are expressed as means ± SEM of four independent experiments. * P <0.05 and *** P < 0.001 vs control; $^{\$}$ P < 0.05 vs MeHg 5µM; $^{\#\#\#}$ P < 0.001 vs MeHg 10µM. (The dotted line represents the control (100%) and PC: positive control).

Figure 4. Damage index (DI) of human leukocytes exposed to $(PhSe)_2$ and/ or MeHg. Data are expressed as means ± SEM of four independent experiments. ** P <0.01 and *** P < 0.001 vs control; $^{\$}$ P < 0.05 vs MeHg 5µM; $^{\#\#\#}$ P < 0.001 vs MeHg 10µM. (Positive control had a DI of 388 ± 4.94).

Figure 5. Effect of $(PhSe)_2$ and/ or MeHg treatment on human leukocytes relative mRNA expression of TrxR 1 (A), GPx 4 (B) and NFE2L2 (C). Values are the means ± SEM of n = 3 independent experiments performed in triplicates. * P < 0.05 vs control.

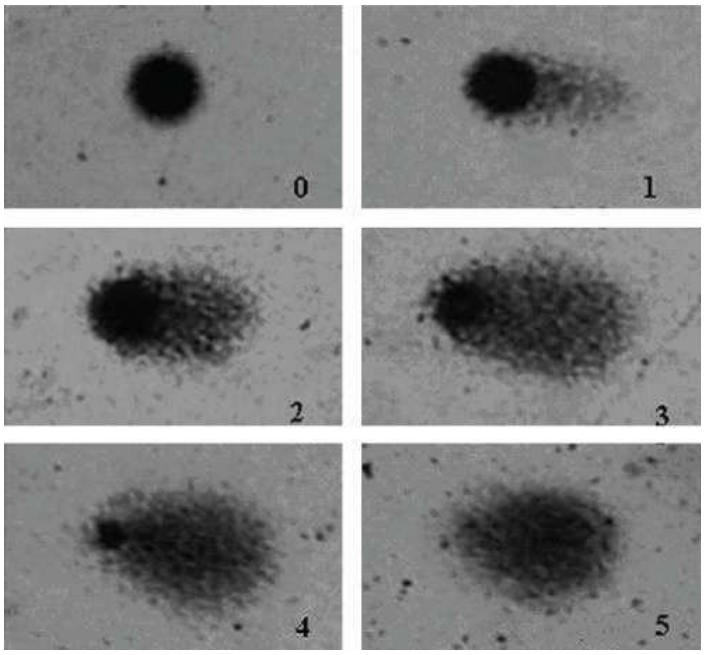


Figure 1.

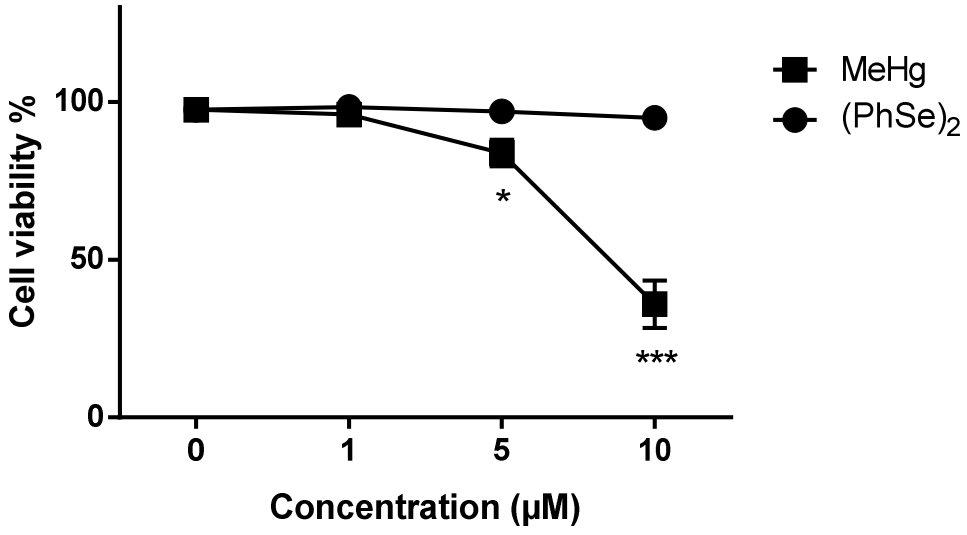


Figure 2.

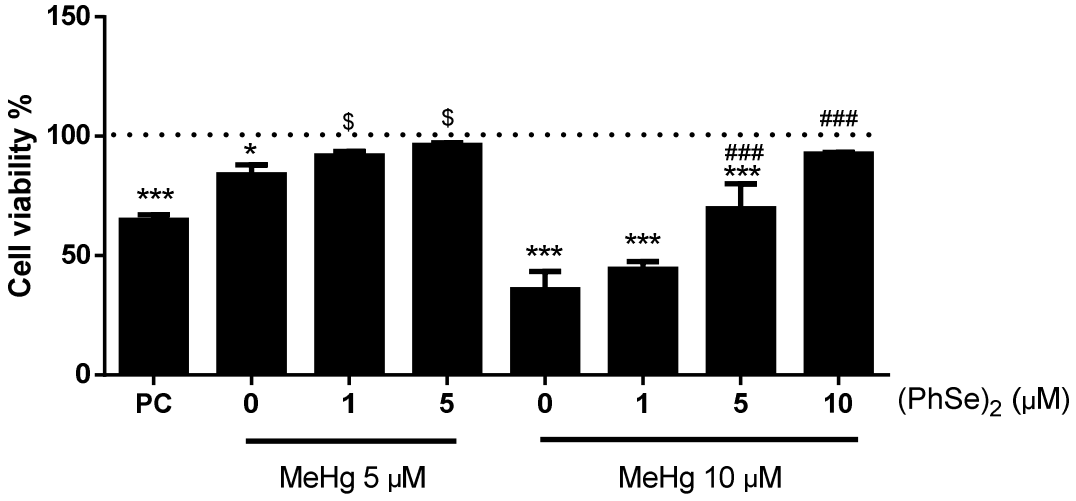

150
100
50
0
Cell viability %
***
*
$
$
***
***
###
***
###
PC
0
1
5
0
1
5
10
(PhSe)2 (µM)
MeHg 5 µM
MeHg 10 µM

Figure 3.

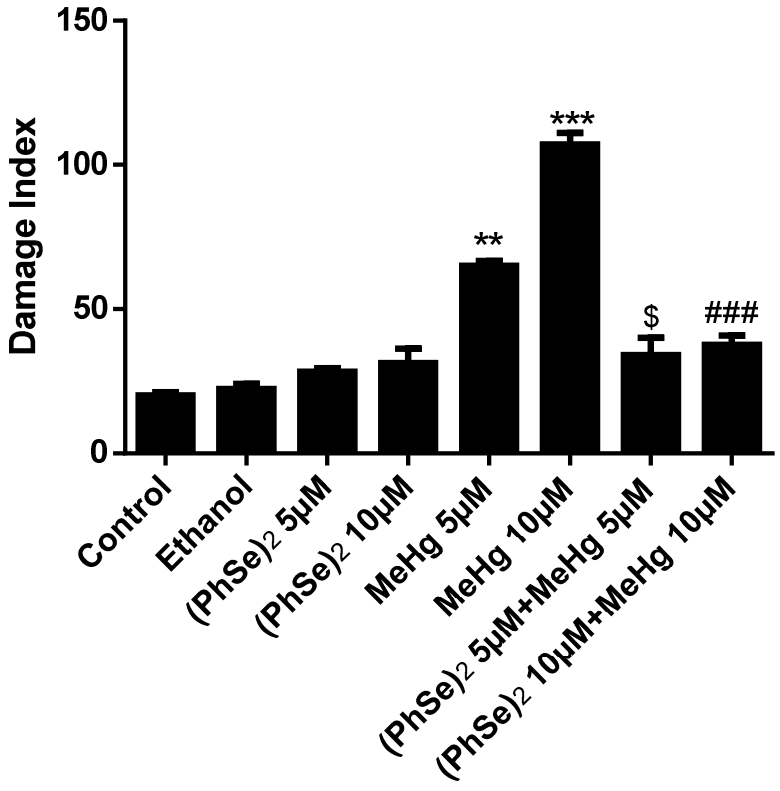

150
100
50
0
Damage Index
**
***
$
###
Control
Ethanol
(PhSe)2 5μM
(PhSe)2 10μM
MeHg 5μM
MeHg 10μM
(PhSe)2 5μM+MeHg 5μM
(PhSe)2 10μM+MeHg 10μM

Figure 4.

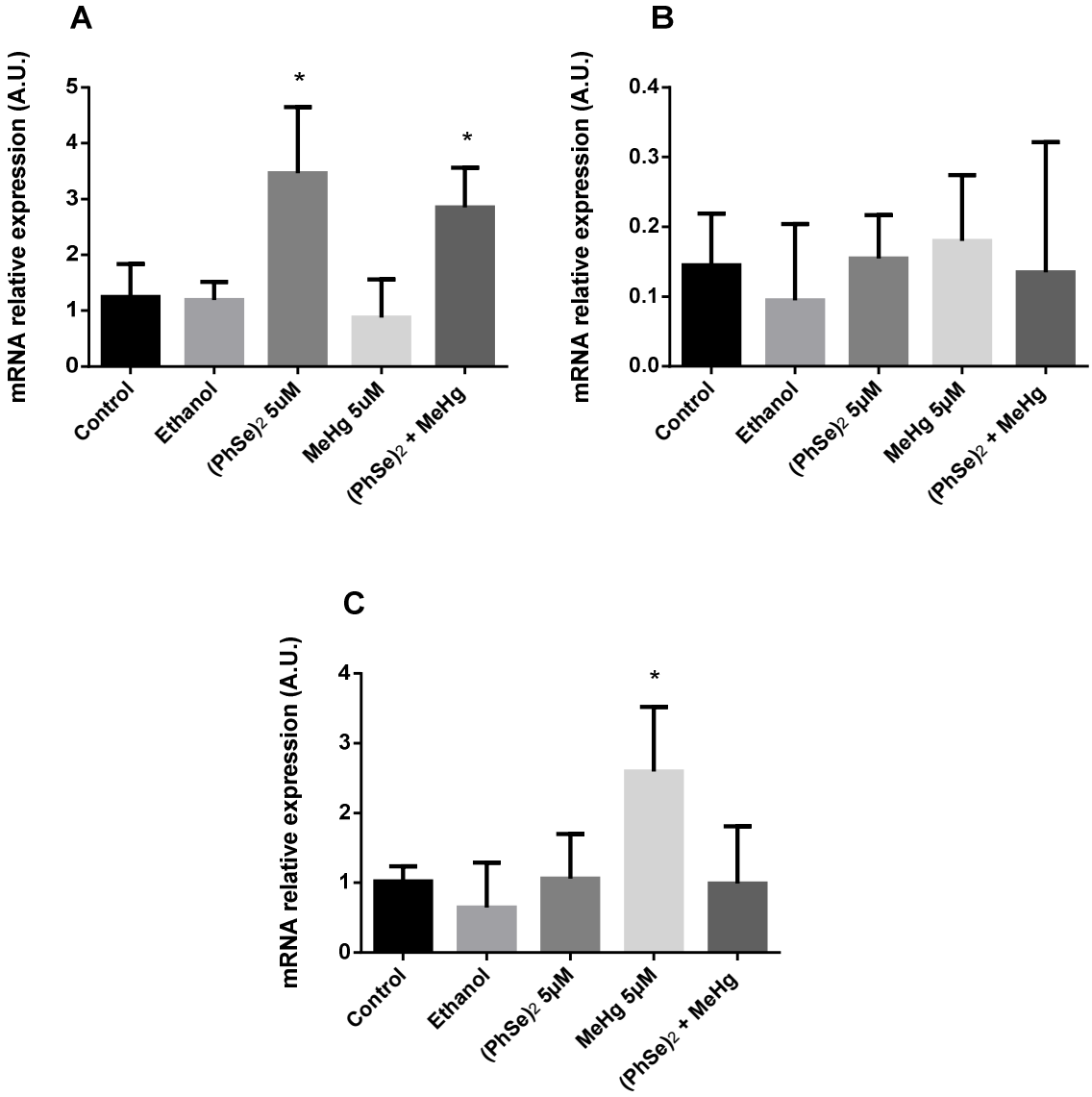

A
mRNA relative expression (A.U.)
5
4
3
2
1
0
*
*
Control
Ethanol
(PhSe)2 5uM
MeHg 5uM
(PhSe)2 + MeHg
B
mRNA relative expression (A.U.)
0.4
0.3
0.2
0.1
0.0
Control
Ethanol
(PhSe)2 5µM
MeHg 5µM
(PhSe)2 + MeHg
C
mRNA relative expression (A.U.)
4
3
2
1
0
*
Control
Ethanol
(PhSe)2 5µM
MeHg 5µM
(PhSe)2 + MeHg

Figure 5.

3. DISCUSSÃO

O MeHg, principal forma orgânica de mercúrio encontrada na natureza, é um conhecido poluente ambiental (CLARKSON *et al*., 2003). A toxicidade do MeHg em humanos está relacionada, principalmente, ao consumo de peixes e frutos do mar que acumulam este composto, o qual acarreta sérios riscos à saúde (BURGER *et al*., 2014). No Brasil, comunidades ribeirinhas localizadas próximas a áreas de garimpo, e que se alimentam quase que exclusivamente de peixes, sofrem exposição crônica a níveis relativamente elevados de MeHg em sua dieta (PINHEIRO *et al*., 2003). Pela facilidade em atravessar a barreira hemato-encefálica, o MeHg causa alterações cerebrais modificando as suas funções normais (FARINA *et al*., 2011; ASCHNER *et al*., 2007). Este composto também pode ser responsável por danos ao sistema imune e cardiovascular (MOSZCZYNSKI *et al*., 2008, VIRTANEN *et al*., 2007, MOREIRA & FARINA, 2014), aos rins e indução de danos ao material genético (RUTOWSKI *et al*., 1998; GROTTO *et al*., 2009). Assim, a exposição humana ao MeHg é um importante problema de saúde pública, em nível mundial, tornando relevante estudos que buscam a elucidação dos mecanismos celulares e moleculares pelos quais o MeHg exerce seus efeitos tóxicos, bem como possíveis compostos que possam ser utilizados no desenvolvimento de novas abordagens terapêuticas para os casos de intoxicação por este organometal.

O presente estudo demonstrou inicialmente, que entre os principais mecanismos pelos quais o MeHg exerce seus efeitos tóxicos está o aumento de espécies reativas (ou estresse oxidativo), o que pode ser visto aqui como desencadeador de um aumento da peroxidação lipídica, conteúdo de hidroperóxidos e uma significativa diminuição na atividade mitocondrial. Além disso, também foi demonstrado neste estudo uma depleção significativa de tióis na mitocôndria causada pelo MeHg (artigo 1). Deste modo, a mitocôndria é a organela que representa o maior alvo para a toxicidade do MeHg nas células e desempenha um papel central no início de cascatas bioquímicas que levam a morte celular (ASCHNER & SYVERSEN, 2005; ATCHISON *et al*., 1994). Ainda, o MeHg pode alterar o funcionamento mitocondrial por inibir enzimas tiólicas incluindo as dos complexos da cadeia respiratória (GLASER *et al*., 2010). A ação da toxicidade do MeHg realmente pode estar associada com o aumento de estresse oxidativo nessa organela. Essa produção excessiva de ERO pode exacerbar a toxicidade do MeHg, o qual ataca outros centros nucleofílicos na mitocôndria e em outros compartimentos celulares (FARINA *et al*., 2011). Neste estudo confirmamos a

participação da formação de peróxido de hidrogênio (H_2O_2) pelo MeHg quando as mitocôndrias foram incubadas com a catalase e MeHg, a qual reduziu significativamente a produção de TBARS induzida pelo MeHg.

Para verificar mecanismos de neurotoxicidade causados pelo MeHg, sendo esta uma importante ação deste metal, este composto foi testado em células SH-SY5Y (neuroblastoma humano). Neste modelo este composto orgânico de mercúrio foi capaz de inibir a atividade de importantes selenoenzimas, principalmente a TrxR, mas também a GPx em menor grau, o qual ocorreu mesmo em concentrações muito baixas (manuscrito 1). A alta afinidade do MeHg a grupos tióis (como os presentes na cisteína), bem como ao selenol (como o presente na selenocisteína), presentes nos sítios ativos de inúmeras enzimas (ex. selenoenzimas) provavelmente é o responsável pela inibição observada na atividade da TrxR e da GPx (FARINA *et al*., 2011). No entanto, a maior afinidade e interação com a TrxR, observada neste estudo deve-se provavelmente à presença do resíduo de Sec em estreita proximidade com três resíduos de cisteína (Cys) na porção C terminal livre da TrxR de mamíferos quando comparada ao sítio ativo da GPx, onde o resíduo de Sec está em proximidade com resíduos de glutamina (Gln) e triptofano (Trp). Essa acessibilidade, e a capacidade da TrxR em se ligar com agentes eletrofílicos como o Hg, aumenta significativamente a reatividade do MeHg com o sítio ativo da TrxR (BRANCO *et al*., 2014).

Além disso, sendo o cérebro um importante alvo do MeHg, este estudo mostra que este composto parece inibir com maior intensidade a TrxR e GPx de cultura de células que representam um modelo de função neuronal do que outros tecidos, como mostrado em estudos com células de hepatoma (HepG2) ou câncer cervical (HeLa) (BRANCO *et al*., 2014; CARVALHO et al., 2008). O que demonstra uma maior sensibilidade de células cerebrais quando comparadas com os demais tecidos.

O MeHg foi posteriormente testado em células sanguíneas humanas (leucócitos isolados) (manuscrito 2), outro importante alvo da sua toxicidade, e causou uma significativa morte celular e um aumento significativo dos níveis de dano no DNA de leucócitos humanos quando tratados *in vitro*. Este dano causado ao DNA pelo MeHg é provavelmente uma consequência da diminuição de antioxidantes celulares, como GSH e selenoenzimas e do aumento de ERO geradas pelo MeHg, as quais interagem com as moléculas do DNA levando a um dano oxidativo e quebras na molécula, ou ainda, o MeHg pode causar uma inibição no sistema de reparo por ligar-se a região sulfidrila das enzimas de reparo (GROTTO *et al*., 2009). Não obstante, o MeHg causou um aumento significativo da expressão gênica do fator de transcrição Nrf2, observado através dos níveis de expressão do mRNA para este transcrito.

Este resultado pode estar diretamente relacionado à ativação do Nrf2 como resposta ao estresse oxidativo gerado pela toxicidade do MeHg (YOSHIDA *et al*., 2014) podendo aumentar, assim, também a expressão deste fator. Basicamente, o Nrf2 é um fator de transcrição que regula a expressão de uma bateria de genes no combate ao estresse oxidativo e eletrofílico, induzido por químicos exógenos e seus metabólitos. Sob condições normais o Nrf2 é mantido no citoplasma ligado a proteína Keap1, no entanto, sob condições de estresse ele é translocado para o núcleo onde se liga ao elemento de resposta antioxidante (ARE) na região promotora de muitos genes antioxidantes e inicia a transcrição dos mesmos (incluindo GPx e TrxR) (NO *et al.*, 2014).

Ni e colaboradores (2010) observaram ainda que uma exposição de células da micróglia ao MeHg aumentou muito os níveis proteicos de Nrf2, mesmo em baixas concentrações (até 5µM) e em um curto período de tempo (10min). Essas células respondem rapidamente ao MeHg super regulando o Nrf2 como mediador de uma resposta antioxidante (NI *et al*., 2010).

Considerando todos esses efeitos de resposta a toxicidade induzida pelo MeHg, torna-se importante a pesquisa de agentes antioxidantes, com potencial em degradar/reduzir espécies reativas ou interagir com o MeHg com o intuito de reduzir seus efeitos tóxicos, os quais podem ser considerados uma classe terapêutica promissora na toxicidade causada por este composto orgânico de mercúrio (FRANCO *et al*., 2007; FARINA *et al*., 2011; LIU *et al*., 2014).

Um bom exemplo de composto antioxidante é o selênio, um elemento traço essencial, (COMBS & COMBS, 1984) encontrado em diferentes formas orgânicas e inorgânicas na natureza e que exerce funções de grande importância no organismo, principalmente como aminoácido selenocisteína, o qual está presente no sítio ativo de importantes selenoproteínas como a GPx e a TrxR (FLOHÉ *et al*., 1973; ARNER & HOLMGREN, 2000), as quais desempenham um papel fundamental como antioxidantes agindo no equilíbrio redox celular. O selênio também é capaz de interagir com metais pesados (incluindo o mercúrio) atenuando sua toxicidade (FALNOGA & TUSEK-ZNIDARIC, 2007).

Compostos sintéticos de selênio têm sido utilizados em inúmeros estudos e têm demonstrado potencial atividade protetora. Este potencial terapêutico e antioxidante deve-se principalmente a atividade GPx-like destes compostos, tornando-os capazes de degradar peróxidos através da formação de um intermediário selenol a partir da oxidação de tióis (ex: GSH), e também a atividade como substrato da TrxR, o qual, pela oxidação de NADPH, será reduzido pela TrxR a um intermediário selenol (FREITAS *et al*., 2010). No presente estudo

foram utilizados diferentes compostos sintéticos orgânicos de selênio e a maioria deles demonstrou algum efeito protetor como pode ser visto com mais detalhes adiante.

Inicialmente, avaliou-se o efeito protetor do $(PhSe)_2$ a análogos (3'3-disseleneto de ditrifuorometildifenil; p-cloro-disseleneto de difenila; p-metoxi-disseleneto de difenila) frente ao estresse oxidativo induzido por MeHg em frações mitocondriais cerebrais enriquecidas (artigo 1). Assim, demonstrou-se que os compostos $(PhSe)_2$ e 3'3-disseleneto de ditrifuorometildifenil foram capazes de proteger da redução na atividade mitocondrial causada pelo MeHg e do aumento de TBARS, o qual o p-cloro-disseleneto de difenila também mostrou-se efetivo. Além disso, o $(PhSe)_2$, composto com melhor atividade protetora neste estudo, foi capaz de detoxificar o H_2O_2 gerado pelo MeHg, sendo demonstrado através de um estudo comparativo com a catalase, enzima responsável pela degradação desta espécie, o que também pode ser explicado pela atividade mimética a GPx apresentada por este composto (reduzindo peróxidos). Na verdade, acredita-se que as atividades protetoras apresentadas por estes compostos de selênio neste estudo, estão diretamente relacionadas à ordem crescente da atividade GPx-like dos mesmos. Considerando que o composto (p-metoxi-disseleneto de difenila) com menor atividade mimética a GPx não foi capaz de proteger em nenhum modelo testado da toxicidade causada pelo MeHg.

De fato, de acordo com Glaser e colaboradores (2013), o $(PhSe)_2$ demonstra atividade de proteção à mitocôndria, especialmente em ensaios nos quais o agente tóxico é acumulado dentro da mesma. O $(PhSe)_2$ parece também ser capaz de reduzir a incorporação mitocondrial de MeHg, consequentemente contrabalançando a toxicidade mitocondrial. Ainda, estes autores sugerem um efeito neuroprotetor do $(PhSe)_2$, o qual pode estar relacionado a atividade de proteção à mitocôndria.

Percebendo o maior potencial antioxidante e protetor do $(PhSe)_2$ demonstrado no primeiro estudo, utilizou-se então, em uma segunda análise, com foco para células do sistema nervoso, onde o $(PhSe)_2$ foi comparado com o potencial protetor do ebselen, outro importante composto orgânico de selênio com propriedades antioxidantes que já vem sendo utilizado na clínica. Estes compostos foram testados quanto aos mecanismos pelos quais os mesmos interferem na interação do MeHg e selenoenzimas (TrxR e GPx), bem como a ação isolada destes compostos de selênio na atividade e expressão destas selenoenzimas (manuscrito 1). Foi possível observar mais uma vez em destaque, com melhor atividade protetora, o efeito do $(PhSe)_2$ em comparação ao ebselen, mesmo ambos não terem causado nenhuma toxicidade em concentrações consideravelmente altas. O $(PhSe)_2$ causou um aumento significativo e bastante alto da atividade e expressão da TrxR e foi capaz de proteger da inibição causada pelo MeHg.

O mecanismo molecular envolvido na proteção proporcionada pela $(PhSe)_2$ provavelmente reflete a sua capacidade para induzir a síntese de TrxR (ZHANG *et al*., 2013). Além disso, $(PhSe)_2$ também pode ser transformado no seu intermediário selenol, o qual liga-se ao metilmercúrio com alta afinidade. Esta atividade antioxidante também foi demonstrada pelo ebselen, aumento da atividade da TrxR, mas com apenas uma modesta proteção ao MeHg.

O $(PhSe)_2$ também causou um aumento da expressão da GPx, o qual foi maior quando comparado ao efeito do ebselen, o que mais uma vez pode ser explicado pela melhor atividade mimética a GPx do $(PhSe)_2$ (WILSON *et al*., 1989; FREITAS *et al*., 2010) e também por ser um melhor substrato para a TrxR do que o ebselen (FREITAS *et al*., 2010; FREITAS & ROCHA, 2011). Assim, este potencial protetor dos compostos pode estar diretamente associado às propriedades antioxidantes dos mesmos. Este estudo mostrou, a super regulação da TrxR causada pelo $(PhSe)_2$, em neuroblastomas, o que reflete um importante mecanismo molecular na atividade antioxidante deste composto.

Este significativo aumento da atividade e expressão da TrxR pelo $(PhSe)_2$ é importante, pois pode evitar ou diminuir uma inibição da TrxR pelo MeHg. E, ainda, mesmo que o MeHg consiga inibir parcial ou totalmente esta enzima, a presença do $(PhSe)_2$, provavelmente fará com que esta seja sintetizada em maior proporção para agir frente a toxicidade induzida pelo MeHg e substituir aquelas inibidas pelo mesmo. Além disso, a formação de um intermediário selenol direta ou indiretamente pelo $(PhSe)_2$ permite que este intermediário, por apresentar um alto potencial nucleofílico, complexe-se ao MeHg evitando que o mesmo interaja com importantes agentes antioxidantes celulares (inclusive selenoenzimas) impedindo, assim, o aumento de estresse oxidativo. Como o MeHg diminui a capacidade antioxidante celular, por depletar tióis ou selenóis, é de suma importância um tratamento que retome estas atividades, como, por exemplo, foi demonstrado pelo $(PhSe)_2$,o qual também reduz peróxidos através da mesma atividade GPx-like.

Tendo em vista a proteção desempenhada pelo $(PhSe)_2$ frente a inibição de selenoenzimas, muito melhor que o ebselen (manuscrito 1), e considerando que este composto parece ser menos tóxico que os outros compostos sintéticos de selênio (NOGUEIRA *et al*., 2004; NOGUEIRA & ROCHA, 2011; MUGESH *et al*., 2001), no manuscrito 2 focou-se apenas no potencial antioxidante do $(PhSe)_2$ com o objetivo de verificar principalmente o papel protetor deste composto em relação a danos genéticos causados pelo MeHg, tanto em relação a dano direto ao DNA ou em alteração de expressão de enzimas antioxidantes (já verificadas como alvo no estudo anterior), e do Nrf2, um fator responsável pela via de resposta antioxidante. Utilizou-se então um modelo já padronizado em nosso laboratório e que

também é considerado um alvo para o MeHg, células sanguíneas humanas *in vitro*. A atividade protetora do $(PhSe)_2$ ocorreu mesmo em baixas doses. Este composto de selênio foi capaz de evitar a morte celular e o dano no DNA causada pelo MeHg, provavelmente i) pela habilidade do mesmo em formar um complexo inerte e estável com o MeHg (FREITAS *et al.*, 2009), impedindo que este organometal interaja negativamente com as macromoléculas celulares ou ainda, ii) por levar a uma super regulação da enzima antioxidante TrxR, que demonstrou um aumento significativo da expressão quando exposta ao $(PhSe)_2$ mesmo na presença do agente tóxico. Esta enzima é capaz de degradar espécies reativas como, H_2O_2 e peroxinitrito (ARNÉR & HOLMGREN, 2000) e reduzir consequentemente a oxidação do DNA pelas mesmas.

Como observado nos dois últimos manuscritos, o $(PhSe)_2$ causou um aumento siginificativo na expressão da TrxR, o que pode ser explicado pela formação de ácido selenídrico a partir do $(PhSe)_2$, forma inorgânica de selênio, que pode ser convertido a selenocisteína e incorporada a selenoenzimas como a TrxR (PINHEIRO *et al*., 2009). Sendo assim, este composto pode agir positivamente, também frente a outros agentes tóxicos, onde a inibição de selenoenzimas como a TrxR estão envolvidas.

Em conjunto, os resultados obtidos neste estudo reforçam o papel central do estresse oxidativo na toxicidade do MeHg e a selenoezima TrxR como alvo molecular principal deste composto. Além disso, os resultados indicam que os compostos orgânicos de selênio, em especial o $(PhSe)_2$, exercem um importante papel protetor frente a toxicidade deste organometal. A reversão da inibição da enzima TrxR causada pelo $(PhSe)_2$, parece ser um importante mecanismo envolvido na proteção contra os efeitos deletérios causados pela exposição ao MeHg, um agente tóxico ambiental.

4. CONCLUSÕES

De acordo com os resultados apresentados nesta tese podemos sugerir que:

- O MeHg foi capaz de causar danos mitocondriais, a partir de geração de espécies reativas de oxigênio que levam ao aumento da peroxidação lipídica e disfunção em mitocôndrias cerebrais;

- O MeHg inibiu as selenoenzimas GPx e TrxR (expressão e atividade), sendo esta última o principal alvo deste composto; o qual pode estar ligado ao aumento de espécies reativas que levam a danos no DNA e morte celular;

- Os compostos de selênio protegem contra a toxicidade *in vitro* do MeHg. E esta proteção parece estar diretamente relacionada ao grau de capacidade antioxidante destes compostos;

- O $(PhSe)_2$ foi o composto que apresentou melhor atividade protetora contra os danos causados pelo MeHg em comparação com os demais compostos testados. Este foi capaz de proteger contra danos mitocôndrias, inibição de atividade e expressão de selenoenzimas (TrxR e GPx) e danos no DNA causados pelo MeHg, o que está relacionado principalmente a sua alta atividade mimética a GPx ou a sua ação como substrato da TrxR.

- O $(PhSe)_2$ levou a uma super-regulação (aumento da expressão e atividade) da enzima TrxR, um importante mecanismo molecular em relação a atividade antioxidante deste composto. Sendo assim, um importante agente que pode ser utilizado para detoxificação dos efeitos tóxicos do MeHg.

5. PERSPECTIVAS

- Investigar se o $(PhSe)_2$, composto com melhor atividade antioxidante neste estudo, é capaz de proteger dos efeitos do MeHg em um modelo de pós-exposição, o que seria significativo na reversão da toxicidade do MeHg;

- Estudar com maior detalhes a interação $(PhSe)_2$ e MeHg para melhor entender os efeitos deste complexo em modelos biológicos;

- Investigar outros genes alvos da toxicidade do MeHg e como os compostos sintéticos de selênio podem modular a toxicidade deste organometal;

- Invetigar o efeito direto do MeHg sobre a enzima TrxR (isolada) e como os compostos sintéticos de selênio agem frente as alterações causadas nesta selenoenzima;

- Avaliar a interação dos compostos orgânicos de selênio e do MeHg com as selenoezimas (TrxR e GPx) através de técnicas de modelagem molecular.

DEMAIS TRABALHOS REALIZADOS DURANTE O DOUTORADO RELACIONADOS À TESE

1. Meinerz, D.F.; Ecker, A.; Rocha, J.B.T. Effect of resveratrol on the toxicity induced by methylmercury in a *Drosophila melanogaster* model. (MANUSCRITO)

2. Meinerz, D. F.; Allebrandt, J.; Mariano, D.O.C.; WACZUK, E. P.; SOARES, F. A.; HASSAN, W.; ROCHA, J. B. T. Differential genotoxicity of diphenyl diselenide $(PhSe)_2$ and diphenyl ditelluride $(PhTe)_2$. PeerJ v. 2, p. e290, 2014.

3. Meinerz, D. F ; Comparsi, B. ; Allebrandt, J. ; Mariano, D. O. ; Santos, D. B; Zemolin, A. P.; Farina, M. ; Dafre, A.L. ; Rocha, J.B. ; Posser, T. ; Franco, J. L. Sub-acute administration of (S)-dimethyl 2-(3-(phenyltellanyl) propanamido) succinate induces toxicity and oxidative stress in mice: unexpected effects of N-acetylcysteine. SpringerPlus v. 2, p. 182, 2013.

4. Caeran D. B.; Meinerz, D. F. ; Allebrandt, J. ; Waczuk, E. P.; Santos, D. B. ; Mariano, D. O. C.; Rocha, J. B.T. Cytotoxicity and Genotoxicity Evaluation of Organochalcogens in Human Leucocytes: A Comparative Study between Ebselen, Diphenyl Diselenide, and Diphenyl Ditelluride. BioMed Research International v. 2013, p. 1-6, 2013.

5. Zemolin, A.P.P.; Meinerz, D.F.; de Paula, M.T. ; Mariano, D.O.C. ; Rocha, J.B.T.; Pereira, A.B.; Posser, T.; Franco, J.L. Evidences for a role of glutathione peroxidase 4 (GPx4) in methylmercury induced neurotoxicity in vivo. Toxicology (Amsterdam) v. 302, p. 60-67, 2012.

REFERÊNCIAS BIBLIOGRÁFICAS

ALI, S. F; LEBEL, C. P; BONDY, S. C. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. **Neurotoxicology,** v. 13, p. 637-648, 1992.

ARNÉR, E.S. AND HOLMGREN, A. Physiological functions of thioredoxin and thioredoxin reductase. **Eur. J. Biochem.**, v. 267, p. 6102–6109, 2000.

ASCHNER, M.; SYVERSEN, T. Methylmercury: recent advances in the understanding of its neurotoxicity. **Ther. Drug Monit.**, v. 27, p. 278-283, 2005.

ASCHNER, M.; SYVERSEN, T.; SOUZA, D.O.; ROCHA, J.B.; FARINA, M. Involvement of glutamate and reactive oxygen species in methylmercury neurotoxicity. **Braz. J. Med. Biol. Res**., v. 40, p. 285–291, 2007.

ATCHISON, W. D.; HARE, M. F. Mechanisms of methylmercury induced neurotoxicity. **FASEB J**., v. 8, p. 622-629, 1994.

BAKIR, F.; DAMLUJI, S.F.; AMIN-ZAKI, L.; MURTADHA, M.; KHALIDI, A.; AL-RAWI, N.Y.; TIKRITI, S.; DAHAHIR, H.I.; CLARKSON, T.W.; SMITH, J.C.; DOHERTY, R.A. Methylmercury poisoning in Iraq. **Science,** v.181, p. 230–241, 1973.

BRANCO, V.; SANTOS, A.G.; GONÇALVES, J.; LU, J.; HOLMGREN, A.; CARVALHO, C. Mitochondrial thioredoxin reductase inhibition,selenium status, and Nrf-2 activation are determinant factors modulating the toxicity of mercury compounds. **Free Radic. Biol. Med.,** v. 73, p. 95-105, 2014.

BRANCO,V.; CANÁRIO, J.; LU, J.; HOLMGREN, A.; CARVALHO C. Mercury and selenium interaction *in vivo*: Effects on thioredoxin reductase and glutathione peroxidase. **Free Radic. Biol. Med.**, v. 52, p. 781–793, 2012.

BURGER, J.; GOCHFELD, M.; JEITNER, C.; DONIO, M.; PITTFIELD, T. Sushi consumption rates and mercury levels in sushi: Ethnic and demographic differences in exposure. **J. of Risk Res.,** v. 17, n. 8, p. 981-997, 2014.

CARVALHO, C. M. L.; CHEW, E.; HASHEMY, S. I.; LU, J.; HOLMGREN, A. Inhibition of human thioredoxin system: a molecular mechanism of mercury toxicity. **J. Biol. Chem.,** v. 283, p. 11913-11923, 2008.

CARVALHO, C. M. L.; LU, J.; ZHANG, X.; ARNÉR, E. S. J.; HOLMGREN, A. Effects of selenite and chelating agents on mammalian thioredoxin reductase inhibited by mercury: implications for treatment of mercury poisoning, **FASEB J**., v. 25, p. 370-381, 2011.

CECCATELLI, S.; DARÉ, E., MOORS, M. Methylmercury-induced neurotoxicity and apoptosis. **Chem. Biol. Interact.,** v. 188, p. 301-308, 2010.

CLARKSON, T. W.; MAGOS, L.; MYERS, G. J. The toxicology of mercury-current exposures and clinical manifestations. **N. Engl. J. Med.** v.349, p.1731–1737, 2003.

COMBS, G. F. JR; COMBS, S. B. The nutritional biochemistry of selenium. **Annu. rev. nut.,** v. 4, p. 257-280, 1984.

DE BEM, A. F.; PORTELLA, R. L.; COLPO, E.; DUARTE, M. M.; FREDIANE, A.; TAUBE, P. S.; NOGUEIRA, C. W.; FARINA, M.; DA SILVA, E. L.; TEIXEIRA ROCHA, J. B. Diphenyl diselenide decreases serum levels of total cholesterol and tissue oxidative stress in cholesterol-fed rabbits. **Basic Clin. Pharmacol. Toxicol.,** v. 105, p. 17-23, 2009.

DE LORGERIL, M; SALEN P. Selenium and antioxidant defenses as major mediators in the development of chronic heart failure. **Heart Fail Review,** v. 11, p. 13-17, 2006.

DIAZ, D.; KREJSA, C.M.; WHITE, C.C.; KEENER, C.L.; FARIN, F.M.; KAVANAGH, T,J. Tissue specific changes in the expression of glutamate–cysteine ligase mRNAs in mice exposed to methylmercury. **Toxicol. Lett**., v. 122 p. 119–129, 2001.

FALNOGA, I.; TUSEK-ZNIDARIC, M. Selenium-mercury interactions in man and animals. **Biol. Trace Elem. Res.,** v.119, p. 212-220, 2007.

FARINA, M.; ASCHNER M., ROCHA, J.B.T. Oxidative stress in MeHg-induced neurotoxicity. **Toxicol. Appl. Pharmacol.,** v. 256, p. 405-417, 2011.

FARINA, M.; ROCHA J. B. T.; ASCHNER, M. Mechanisms of methylmercury-induced neurotoxicity: Evidence from experimental studies. **Life Sciences,** v. 89, p. 555-563, 2011.

FARINA, M; FRIZZO, M.E; SOARES, F.A; SCHWALM, F.D; DIETRICH, M.O; ZENI, G;ROCHA J.B; SOUZA, D.O. Ebselen protects against methylmercury-induced inhibition of glutamate uptake by cortical slices from adult mice. **Toxicol. Lett.**, v. 144, p. 351-357, 2003.

FLOHÉ, L.; GUNZLER, W. A.; SCHOCK, H. H. Glutathione peroxidase: a selenoenzyme, **FEBS Lett.,** v. 32, n. 1, p. 132–134,1973.

FRANCO, J. L.; BRAGA, H. C.; STRINGARI, J.; MISSAU, F. C.; POSSER, T.; MENDES, B. G. Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. **Chem. Res. Toxicol.,** v. 20, p. 1919-1926, 2007.

FRANCO, J. L.; POSSER, T.; DUNKLEY, P. R.; DICKSON, P. W.; MATTOS, J. J.; MARTINS, R.; BAINY, A. C. D.; MARQUES, M. R.; DAFRE A.L.; FARINA, M. Methylmercury neurotoxicity is associated with inhibition of the antioxidant enzyme glutathione peroxidase. **Free Radic. Biol. Med.,** v. 47, p.449-457, 2009.

FREITAS, A. S.; FUNCK, V. R.; ROTTA, M. S.; BOHRER, D.; MÖRSCHBÄCHER, V.; PUNTEL, R.; NOGUEIRA, C. W.; FARINA, M.; ASCHNER, M.; ROCHA, J. B. T. Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury-induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice. **Brain Res. Bull.,** v. 79, p. 77-84, 2009.

FREITAS, A. S.; PRESTES, A. S.; WAGNER, C.; SUDATI, J. H.; ALVES, D.; PORCIÚNCULA, L. O.; KADE, I. J.; ROCHA, J. B. T. Reduction of Diphenyl Diselenide and Analogs by Mammalian Thioredoxin Reductase Is Independent of Their Gluthathione Peroxidase-Like Activity: A Possible Novel Pathway for Their Antioxidant Activity. **Molecules**, v. 15, n. 11, p. 7699-7714, 2010.

FREITAS, A. S.; ROCHA, J. B. Diphenyl diselenide and analogs are substrates of cerebral rat thioredoxin reductase: a pathway for their neuroprotective effects. **Neurosci Lett.,** v. 503, n. 1, p. 1-5, 2011.

FRESHNEY, I. Application of cell cultures to toxicology. **Cell. Biol. Toxicol**., v. 17 p. 213-230, 2001.

GERHARDSSON, L.; SKERFVING, S. Concepts on biological markers and biomonitoring for metal toxicity. In: Chang WL, Magos L, Suzuki T, editors. **Toxicology of metals**, Florida: CRC Lewis; p. 95–96, 1996.

GLASER, V.; LEIPNITZ, G.; STRALIOTTO, M. R.; OLIVEIRA, J.; DOS SANTOS, V. V.; WANNMACHER, C. M. ET AL. Oxidative stress-mediated inhibition of brain creatine kinase activity by methylmercury. **Neurotoxicology**, v. 31, n. 5, p.454-60, 2010.

GLASER, V.; NAZARI, E. M.; MÜLLER, Y. M. R.; FEKSA, L.; WANNMACHER, C. M. D.; ROCHA, J. B. T.; BEM, A. F.; FARINA, M.; LATINI, A. Effects of inorganic selenium administration in methylmercury-induced neurotoxicity in mouse cerebral cortex. **Int. J. Dev. Neurosci.,** v. 28, p. 631–637, 2010.

GLASER, V.; MORITZ, B.; SCHMITZ, A.; DAFRÉ, A. L.; NAZARI, E. M.; RAUH MÜLLER, Y. M.; FEKSA, L.; STRALIOTTOA, M. R.; DE BEM, A. F.; FARINA, M.; ROCHA, J. B. T.; LATINI, A. Protective effects of diphenyl diselenide in a mouse. **Chem. Biol. Interact.,** v. 206, p. 18-26, 2013.

GROTTO, D.; BARCELOS, G. R. M.; VALENTINI, J.; ANTUNES, J. M. G.; ANGELI, J.P.F.; GARCIA, S.C.; BARBOSA, F. JR. Low levels of methylmercury induce DNA damage in rats: protective effects of selenium. **Arch. Toxicol.,** v. 83, p. 249-254, 2009.

HORT, M. A.; STRALIOTTO, M. R.; NETTO, P. M.; DA ROCHA, J. B.; DE BEM, A. F.; RIBEIRO-DO-VALLE, R. M. Diphenyl diselenide effectively reduces atherosclerotic lesions in LDLr -/- mice by attenuation of oxidative stress and inflammation. **J Cardiovasc. Pharmacol.**, v. 58, p. 91-101, 2011.

LIU, W.; XU, Z.; YANG, T.; DENG, Y.; XU, B.; FENG, S.; LI, Y. The protective role of tea polyphenols against methylmercury-induced neurotoxic effects in rat cerebral cortex via inhibition of oxidative stress. **Free Radic. Res.,** v. 48, n. 8, p. 849-63, 2014.

MOREIRA, E. L. G.; FARINA, M. An unsolved puzzle: The complex interplay between methylmercury and fish oil-derived fatty acids within the cardiovascular system. **Toxicol. Res.,** v. 3, n. 5, p. 300-310, 2014.

MOSZCZYNSKI, P.; RUTOWSKI, J.; SŁOWINSKI, S.; BEM S. Immunological effects of occupational exposure to metallic mercury in the population of T-cells and NK-cells. **Analyst**, v. 123, p.99-103, 2008.

MUGESH, G.; DU MONT, W. W.; SIES, H. Chemistry of biologically important synthetic organoselenium compounds. **Chem. Rev.,** v. 101, n. 7, p. 2125-2179, 2001.

NI, M.; LI, X.; YIN, Z.; JIANG, H.; SIDORYK-WEGRZYNOWICZ, M.; MILATOVIC, D.; CAI, J.; ASCHNER, M. Methylmercury induces acute oxidative stress, altering Nrf2 protein level in primary microglial cells. **Toxicol. Sci.,** v. 116, n. 2, p. 590-603, 2010.

NO, J.H.; KIM, Y.B.; SONG, Y.S. Targeting nrf2 signaling to combat chemoresistance. **J. Cancer Prev.,** v. 19, n. 2, p. 111 – 117, 2014 .

NOGUEIRA, C. W.; ROCHA, J. B. T. Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. **Arch. Toxicol.**, v. 85, p. 1313-1359, 2011.

NOGUEIRA, C.W.; ZENI, G.; ROCHA, J.B.T. Organoselenium and organotellurium compounds: toxicology and pharmacology. **Chem. Ver.,** v. 104, p. 6255–6285, 2004.

PINHEIRO, M. C. N.; HARANDA, M.; YASODA, E.; NAKANISHI, J.; OIKAWA, T.; VIEIRA, J. L. F. Toxicological and epidemiological data on human exposure to mercury in the Tapajós River basin: 1994-1998. **Environ. Science**, v.10, p. 99-105, 2003.

PINHEIRO, M.C.N; DO NASCIMENTO, J.L.M.; SILVEIRA, L.C.L.; DA ROCHA, J.B.T.; ASCHNER M. Mercury and Selenium – A Review on Aspects Related to the Health of Human Populations in the Amazon. **Environ. Bioind.,** v. 4, p. 222-245, 2009.

RUTOWSKI, J.; MOSZCZYNSKI, P.; BEM, S.; SZEWCZYK, A. Efficacy of urine determination of early renal damage markers for nephrotoxicity monitoring during occupational exposure to mercury vapours. **Med. Pracy.,** v. 49, p. 129-135, 1998.

STEINBRENNER, H.; SIES, H. Protection against reactive oxygen species by selenoproteins. **Biochim. Biophys. Acta,** v. 1790, p. 1478-1485, 2009.

SUGIURA, Y.; HOJO, Y.; TAMAI, Y.; TANAKA, H. Selenium protection against mercury toxicity. Binding of Methylmercury by the selenohydryl- containing ligand. **J. Am. Chem. Soc.,** v 98, p.8 , 1976.

SUZUKI, K.T.; SASAKURA, C.; YONEDA, S. Binding sites for the (Hg–Se) complex on selenoprotein P. **Biochim. Biophys. Acta**., v. 1429, p. 102–112, 1998.

TAKEUCHI, T.; MORIKAWA, N.; MATSUMOTO, H.; SHIRAISHI, Y. A pathological study of Minamata disease in Japan. **Acta Neuropathol**., v.2, p.40–57, 1962.

USUKI, F.; YAMASHITA, A.; FUJIMURA, M. Post-transcriptional Defects of Antioxidant Selenoenzymes Cause Oxidative Stress under Methylmercury Exposure. **J. Biol. Chem.,** v. 286, p. 6641–6649, 2011.

VIRTANEN, K. J.; RISSANEN, T. H.; VOUTILAINEN, S.; TUOMAINEN, T. P. Mercury as a risk factor for cardiovascular diseases. **J. Nutr. Biochem.,** v. 18, p. 75-85; 2007.

WENDEL, A.; FAUSEL, M.; SAFAYHI, H.; TIEGS, G.; OTTER, R. A novel biologically active seleno-organic compound-II. Activity of PZ 51 in relation to glutathione peroxidase. **Biochem. Pharmacol.,** v. 33, p. 3241–3246, 1984.

WILSON, S. R.; ZUCKER, P. A.; HUANG, R. R. C.; SPECTOR, A. Development of synthetic compounds with glutathione peroxidase activity. **J. Am. Chem. Soc.,** v. 111, p. 5936-5939, 1989.

XIONG, S.; MARKESBERY, W.R.; SHAO, C.; LOVELL, M.A. Seleno-L-methionine protects against beta-amyloid and iron/hydrogen peroxide-mediated neuron death. **Antioxid. Redox Signal**., v.9, p.457–467, 2007.

YOSHIDA, E.; ABIKO, Y.; KUMAGAI, Y. Glutathione Adduct of Methylmercury Activates the Keap1-Nrf2 Pathway in SH-SY5Y Cells. **Chem. Res. Toxicol.,** 2014.

ZEMOLIN, A. P. P.; MEINERZ, D. F.; DE PAULA, M. T.; MARIANO, D. O. C.; ROCHA, J. B. T.; PEREIRA, A. B.; POSSER, T.; FRANCO, J. L. Evidences for a role of glutathione peroxidase 4 (GPx4) in methylmercury induced neurotoxicity in vivo. **Toxicology**, v.320, n.1, p. 60-67, 2012.

ZHANG, G.; NITTERANON, V.; GUO, S.; QIU, P.; WU, X.; LI, F.; XIAO, H.; HU, Q.; PARKIN, K. L. Organoselenium Compounds Modulate Extracellular Redox by Induction of Extracellular Cysteine and Cell Surface Thioredoxin Reductase. **Chem. Res. Toxicol.,** v. 26, p. 456-464, 2013.