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Review

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Naturally Occurring Genetic Variants of Human Acetylcholinesterase and Butyrylcholinesterase and Their Potential Impact on the Risk of Toxicity from Cholinesterase Inhibitors

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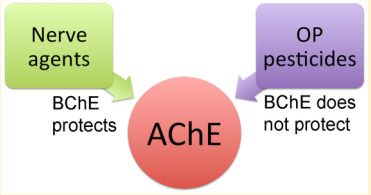
ABSTRACT: Acetylcholinesterase (AChE) is the physiologically important target for organophosphorus toxicants (OP) including nerve agents and pesticides. Butyrylcholinesterase (BChE) in blood serves as a bioscavenger that protects AChE in nerve synapses from inhibition by OP. Mass spectrometry methods can detect exposure to OP by measuring adducts on the active site serine of plasma BChE. Genetic variants of human AChE and BChE do exist, but loss of function mutations have been identified only in the BCHE gene. The most common AChE variant, His353Asn (H322N), also known as the Yt blood group antigen, has normal AChE activity. The most common BChE variant, Ala567Thr (A539T) or the K-variant in honor of Werner Kalow, has 33% reduced plasma BChE activity. The genetic variant most frequently associated with prolonged response to muscle relaxants, Asp98Gly (D70G) or atypical BChE, has reduced activity and reduced enzyme concentration. Early studies in young, healthy males, performed at a time when it was legal to test nerve agents in humans, showed that individuals responded differently to the same low dose of sarin with toxic symptoms ranging in severity from minimal to moderate. Additionally, animal studies indicated that BChE protects from toxicants that have a higher reactivity with AChE than with BChE (e.g., nerve agents) but not from toxicants that have a higher reactivity with BChE than with AChE (e.g., OP pesticides). As a corollary, we hypothesize that individuals with genetic variants of BChE may be at increased risk of toxicity from nerve agents but not from OP pesticides.



CONTENTS

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Table 1. Number of Mutations in Exons of Human ACHE and BCHE Genes

	HuACHE gene	HuBCHE gene
cytogenetic location	7q22.1	3q26.1
chromosome	7.100487615−100494594 reverse strand	3.165490692−165555260 reverse strand
# of exons	6	4
# of amino acids	31 signal peptide + 583 = 614 most abundant form	28 signal peptide + 574 = 602
accession number	P22303 isoform E4-E6; NP_056646 isoform E4-E5	P06276
# of missense mutations	137	229
# of homozygous missense	7	9
# of loss of function mutations	1 in glycolipid anchor exon 5; 2 splice acceptor mutations	34
# of synonymous mutations	141	64
# of homozygous LoF	0	0
pLI score[a]	1.0	0.0
most abundant missense mutation	His353Asn (H322N) = Yt blood group antigen	Ala567Thr (A539T) = K variant
frequency	1 out of 24 carry His353Asn	1 out of 5 carry Ala567Thr
SNP accession number	rs1799805	rs1803274

[a]pLI is the probability of a gene being tolerant to loss-of-function. A pLI score of 1.0 indicates intolerance of mutations that result in loss of function. A pLI score of 0.0 indicates tolerance of mutations that result in loss of function.[18]

1. INTRODUCTION

The Centers for Disease Control and Prevention (CDC) has a mandate to be prepared to analyze large numbers of samples in the event of a poisoning of the population by a wide range of chemical threat agents including nerve agents.[1] One of the current CDC methods to assess exposure to nerve agents uses mass spectrometry to analyze adducts on plasma butyrylcholinesterase (BChE).[2,3] BChE is a sensitive biomarker of exposure, capable of scavenging subclinical doses of nerve agents and other organophosphorus toxicants (OPs). A substantial proportion of the American and European population (35%) carries at least one mutated BChE allele.[4] One goal of this review is to evaluate difficulties that might arise for analysis of exposure when the plasma contains genetic variants of BChE.

Acute toxicity from exposure to cholinesterase inhibitors is attributed to the inhibition of acetylcholinesterase (AChE) activity at the neuromuscular junction and in the brain, resulting in depression of the respiratory and circulatory centers in the medulla, weakness of the muscles of respiration, and pulmonary edema.[5] Since toxicity correlates with depression of AChE activity rather than BChE activity, it would seem logical to focus on genetic variants of AChE. Though genetic variants of human AChE exist, deleterious mutations are rare and occur only in the heterozygous state.[6] Furthermore, AChE is not readily accessible for study because AChE is membrane bound to red blood cells (RBC).

A second goal of this review is to assess the effect of genetic variants of BChE on susceptibility to cholinesterase inhibitors. We examine the evidence for the common assumption that humans with BChE deficiency are at increased risk of toxicity from OP exposure. Hundreds of OP poisoning cases occur annually in Sri Lanka, India, and China, but their BChE genotype is unknown.[7] A single study did measure the BChE genotype in Brazilian farmers and correlated the BChE genotype with AChE activity levels in RBC as an indicator of pesticide exposure.[8] As of the year 2016, there is no conclusive evidence that individuals with BChE deficiency are at increased risk of toxicity from OP pesticides. Further studies are needed to make this link.

2. NATURALLY OCCURRING MUTATIONS IN THE HUMAN ACHE AND BCHE GENES

2.1. Mutations in the Human ACHE Gene. Gene sequences for 60,706 unrelated individuals are available online from the Exome Aggregation Consortium (ExAC) http://exac.broadinstitute.org/gene/ENSG00000114200. The database has a list of each observed mutation and its frequency. The subjects are European, American, African, and Asian. Humans have one ACHE gene on chromosome 7q22.1 spanning 7.4 kb. The database shows mutations in exons 2, 3, 4, 5, and 6 for ACHE but does not include upstream, untranslated exons. A summary of the number of mutations in human ACHE and BCHE exons can be found in Table 1.

The database shows 137 missense mutations and 141 synonymous mutations for AChE. The most abundant missense mutation, His353Asn (H322N), has an allele count of 4,910 out of 115,962 for a frequency of 0.04234. (The residue number in parentheses is for the secreted protein from which the signal peptide has been deleted.) This mutation defines the Yt blood group antigen but has no effect on AChE activity.[9,10]

The residues involved in activity and substrate interactions for AChE have been well characterized.[11−14] Missense mutations are absent from residues known to be important for AChE enzyme activity, with minimum exceptions. The catalytic triad residues, Ser234 (S203), Glu365 (E334), and His478 (H447) are conserved in the ExAC study with the exception of one His478 allele out of 111,128, mutated to His478Gln. The peripheral site residues Trp317 (W286), Asp105 (D74), Tyr372 (Y341), and Phe328 (F297) are conserved. The choline binding site Trp117 (W86) is conserved. Residues that constitute the oxyanion hole, Gly151 (G120), Gly153 (G122), and Ala235 (A204), are conserved with the exception of 2 alleles out of 116,198 for Ala235 that are mutated to Ala235Thr. The missense mutation Asp165His (D134H) destabilizes the AChE enzyme at temperatures higher than 31 °C.[6] This variant has normal catalytic activity with acetylthiocholine but is inhibited 2-times more slowly by paraoxon compared to wild-type AChE and is reactivated 4-times faster by the oxime 2-PAM. The frequency of the Asp165His mutant is so low that it was not found in the consortium study of 60,706 individuals.

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Table 2. Mutations in the Active Site Peptide FGESAGAAS of Human AChE and BChE

	sequence	mass $(M+H)^+$	frequency (%)	reference
AChE	FGESAGAAS	796.34	121,410 out of 121,412 (99.99)	Exac.broadinstitute.org
AChE	FGESTGAAS	826.35	1 out of 116,198 (0.0008)	Exac.broadinstitute.org
AChE	FGESAGATS	826.35	1 out of 115,840 (0.0008)	Exac.broadinstitute.org
BChE	FGESAGAAS	796.34	121,403 out of 121,412 (99.99)	Exac.broadinstitute.org
BChE	frameshift at F		1 out of 121,084 (0.0008)	Exac.broadinstitute.org
BChE	FGEGAGAAS	766.33	5 out of 121,110 (0.0041)	Exac.broadinstitute.org
BChE	FGESAEAAS	868.36	1 out of 121,108 (0.0008)	Exac.broadinstitute.org
BChE	FGESAGASS	812.34	2 out of 121,144 (0.0016)	Exac.broadinstitute.org
BChE	FGESVGAAS	824.37	unknown	[35]
BChE	FGESAGTAS	826.35	unknown	[36]
BChE	FGESAGAAP	806.36	unknown	[37]

The remarkable finding for the ACHE gene is that it is almost devoid of loss of function mutations. The ExAC database identified 2 splice acceptor mutations and one frameshift mutation in the ACHE gene. No loss of function mutations were found in the homozygous state. Only one allele in the set has a frameshift mutation, and that mutation is in the heterozygous state. The ACHE frameshift mutation Ser580-ArgfsTer70 is in exon 5 where it terminates translation. Exons 2, 3, 4, and 5 code for the glycolipid-anchored form of AChE bound to the red blood cell membrane.[15,16] This alternatively spliced form is called isoform E4-E5 in Table 1. AChE has no known function in the RBC. The AChE that functions in nerve impulse transmission is encoded by exons 2, 3, 4, and 6 and completely bypasses exon 5.[17] This form is called isoform E4-E6 in Table 1. The absence of loss of function mutations in the human ACHE gene spliced to exon 6, isoform E4-E6, is evidence for extreme selective constraint. It suggests that loss of a single ACHE copy results in embryonic lethality.[18] If this interpretation is correct, it is puzzling why ACHE knockout mice, with no AChE activity in any tissue, are born alive and live to adulthood, though they are weak, blind, suffer convulsions, and do not reproduce.[19] Heterozygous ACHE ± mice are healthy. Additionally, no disease has been attributed to a mutation in the human AChE enzyme.

2.2. Mutations in the Human BCHE Gene. Humans have one BCHE gene on chromosome 3q26.1 spanning 65 kb.[20] The human BCHE gene has 4 exons.[21] Mutation data for the 60,706 unrelated individuals in the ExAC report[18] are summarized in Table 1. In contrast to ACHE, the BCHE gene was observed to have 34 loss of function mutations, though none was in the homozygous state in the ExAC database. BCHE was assigned a pLI score of 0.0, indicating tolerance to loss of function mutations in the BCHE gene.[18] Tolerance means that having nonfunctional BChE is compatible with life. This conclusion is supported by the many reports of silent BChE in humans, where the individual has no BChE activity due to frameshift, splice junction, stop, or missense mutations. Humans homozygous for silent BChE are healthy, fertile, and live to old age.[22]

Table 1 shows 64 synonymous and 229 missense mutations in the BCHE gene. The missense mutations were in the heterozygous state. Missense mutations were found in residues of the catalytic triad of BCHE. Five alleles out of 121,110 had Ser226Gly (S198G) in the active site serine, a mutation that results in complete loss of BChE activity. One allele out of 121,174 had a mutation in His of the catalytic triad: His466Arg (H438R). There were no mutations in the catalytic triad residue Glu353 (E325). One BCHE allele out of 121,142 had the mutation Trp110Arg (W82R) at the choline binding site, a mutation expected to severely impair BChE activity.

The missense mutation Asp98Gly (D70G) is responsible for prolonged apnea in response to the muscle relaxants succinylcholine and mivacurium.[23] The mutation is cataloged as rs1799807 in the National Center for Biotechnology Information database. One out of 2500 individuals is homozygous for Asp98Gly. This is the atypical variant recognized by Kalow as having poor affinity for succinylcholine.[24] Individuals who are homozygous for atypical BChE are unable to breathe for 2 h from a dose of succinylcholine that paralyzes most people for 3 min. Other, less frequent mutations including those that produce silent BChE with no activity[25] are also associated with prolonged apnea in response to a muscle relaxant. These additional mutations and the case studies that report them have been recently reviewed.[4] A recent case study of six patients described new BChE mutations associated with prolonged apnea following administration of mivacurium.[26] BChE phenotype assays perfectly predict the atypical and fluoride variants.[27,28]

The most frequent missense mutation, Ala567Thr (A539T) the K-variant named in honor of Werner Kalow, is located in the tetramerization domain near the C-terminus of BChE.[29] Individuals homozygous for Ala567Thr have 33% lower BChE activity in plasma.[30] The recombinant K-variant has wild-type BChE activity and stability,[31] leading to the hypothesis that the reduced plasma activity is explained by an unknown mutation in a regulatory region rather than by the Ala567Thr mutation.

2.3. Naturally Occurring Mutations in the Active Site Peptide FGESAGAAS. The Netherlands Organisation for Applied Scientific Research (TNO) Prins Maurits Laboratory and the CDC have both developed sensitive mass spectrometry methods for detecting nerve agent and OP pesticide exposure by measuring adducts on the active site serine of BChE.[2,3,32−34] Similar methods are being developed for adducts on AChE. The active site peptide FGESAGAAS is derived by pepsin digestion of immunopurified BChE. Human AChE and BChE have the same FGESAGAAS sequence. Naturally occurring mutations in FGESAGAAS alter the mass of the parent ion and therefore would be missed in mass spectrometry methods set up to search for parent ion masses specific for the wild-type active site peptide. Table 2 lists naturally occurring missense mutations in the FGESAGAAS peptide in human AChE and BChE.

The frequency of AChE mutations in the FGESAGAAS peptide is 2 in 116,000 alleles. No homozygous missense mutations in AChE have been identified. The frequency of BChE mutations is slightly higher at 9 out of 121,000 alleles

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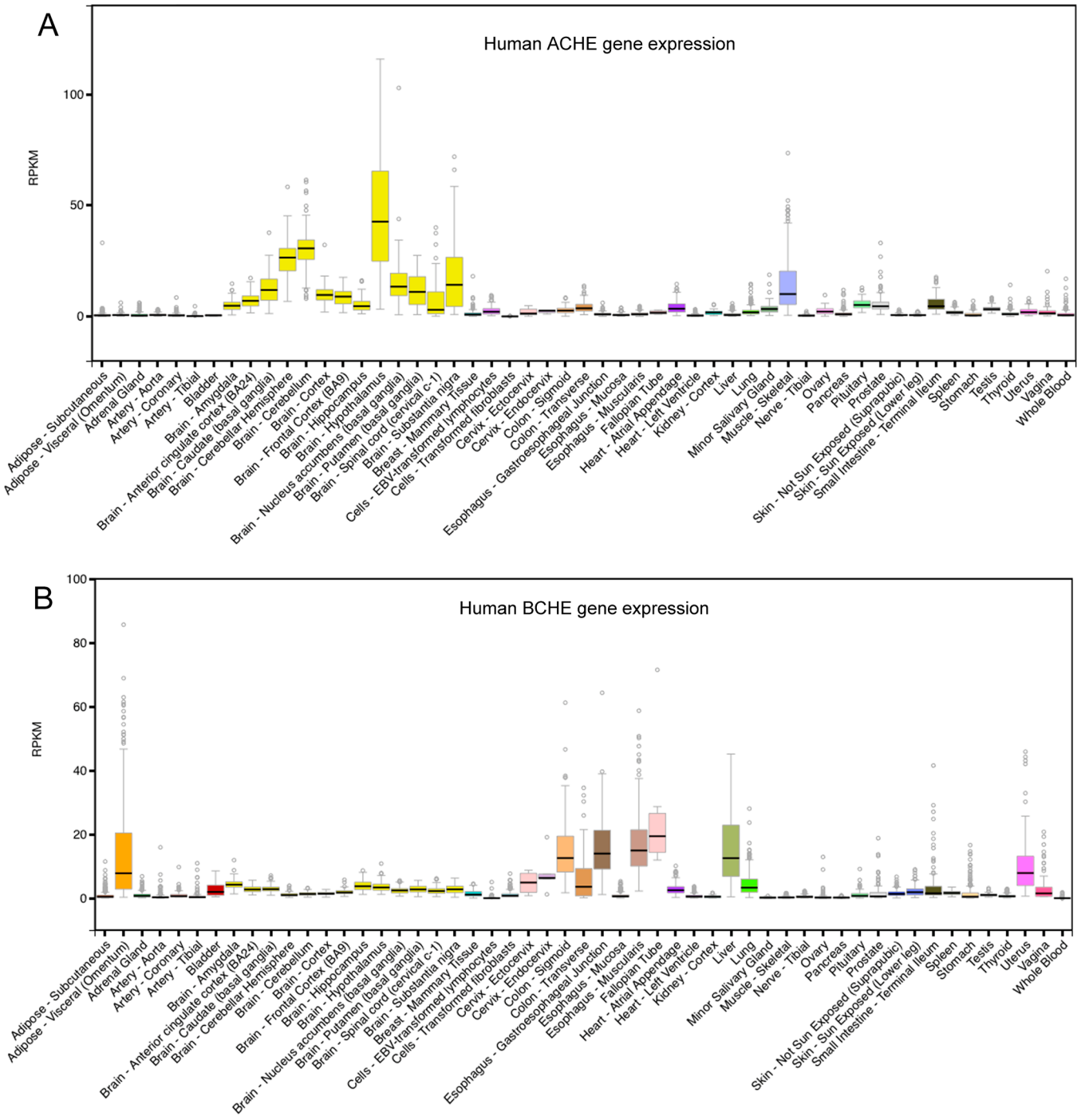

Figure 1. Expression of ACHE and BCHE genes in human tissues. RPKM, reads per kilobase of transcript per million mapped reads. Figures from GTEx Portal, version 6; www.gtexportal.org.

and includes mutations that result in the complete absence of BChE activity. The 60,706 individuals in the ExAC study population had no homozygous silent BChE, though inbred groups in Alaska and India include a high proportion of individuals who are completely deficient in BChE activity.[38,39] In a population of about 5,000 Eskimos in western Alaska, 39 persons completely lacked BChE activity, and 24 persons had very low (2−10% of normal) BChE activity.[39] A study of 490 persons belonging to the Vysya caste of Andhra Pradesh, India found 12 homozygous silent persons and 85 heterozygotes for a frequency of 0.1112 for the silent BChE allele.[38]

2.4. ACHE and BCHE Gene Expression in Human Tissues. The expression profiles for human ACHE and BCHE genes are markedly different. ACHE levels are highest in the brain, followed by skeletal muscle (Figure 1A), consistent with the function of AChE in nerve impulse transmission. Comparatively low levels of ACHE are expressed by other tissues. BCHE levels are highest in the liver, adipose-visceral, esophagus, colon, Fallopian tube, uterus, cervix, and lung (Figure 1B), consistent with the function of BChE in detoxication of poisons that are eaten or inhaled.

Whole body autoradiograms of mice injected intravenously with ^{3}H-soman showed that 5 min after administration, a high

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concentration of radioactivity was in the blood, heart, kidney, lung, nasal cavities, lacrymal glands, salivary glands, skin, and some striated muscles.[40] There was no radioactivity in the central nervous system. Interestingly, the tissue distribution of soman resembles that of BChE.

2.5. More BChE than AChE Enzyme in the Human Body. When enzyme activity levels are multiplied by the mass of the tissue, the highest quantity of AChE enzyme is in muscle, whereas the highest levels of BChE are in the plasma and liver. Overall, the adult human body has 10-times more BChE protein (680 nmol) than AChE protein (62 nmol).[22]

3. EVIDENCE THAT ACHE AND BCHE ARE TARGETS OF NERVE AGENT EXPOSURE

3.1. Similarities and Differences between Nerve Agents and OP Pesticides. Nerve agents and OP pesticides are structurally similar in that they are esters of phosphoric acid. OP pesticides that have a P═S bond (for example, parathion) are inactive as cholinesterase inhibitors until they are metabolically converted to the oxon (paraoxon). In contrast, nerve agents are ready cholinesterase inhibitors that require no activation. Exposure to nerve agents has immediate toxic consequences, whereas exposure to OP pesticides has delayed toxic consequences reflecting the time it takes the cytochrome P450 enzymes to replace the P═S bond with P═O. Nerve agents are selected for their toxicity to mammals, whereas OP pesticides are selected for their toxicity to pests. At the clinical level, the early symptoms of nerve agent and OP pesticide intoxication are identical, and treatment strategies for both types of poisoning are similar. The acute toxicity from nerve agents and OP pesticides is due to the inhibition of AChE activity, resulting in the accumulation of acetylcholine. The excess levels of acetylcholine overstimulate receptors, causing their dysfunction. Nerve agents and OP pesticides make a covalent bond with the active site serine of AChE and BChE. The added mass from the reaction with nerve agents is different from the added mass from the reaction with OP pesticides, making it possible to distinguish between these classes of poisons using mass spectrometry. A main difference between nerve agents and OP pesticides is the dose that inhibits AChE. Very low doses of nerve agents are inhibitory, whereas only high doses of OP pesticides are inhibitory. Thus, nerve agents are several orders of magnitude more acutely toxic than most OP pesticides.

3.2. Studies in Humans Exposed to Nerve Agents. Public Law 105−85 div. A, title X, section 1078 passed by the 105th US Congress in November 1997, restricted the use of human subjects for testing of chemical agents. Hundreds of young men had been tested with low doses of nerve agents in the years 1948 to 1967, though approval to publish findings was delayed about a decade. Many of the reports have never been published, but their contents are summarized in a chapter by Frederick Sidell.[41] Most studies quantified nerve agent exposure by measuring the depression of AChE activity in RBC, a surrogate for AChE activity in the neuromuscular junction and brain. Toxic symptoms were monitored, and it was found that the same low dose of nerve agent affected individuals differently.

One of the earliest studies in the open literature reported the effects of the nerve agent sarin administered orally to 10 volunteers.[42] The study established that AChE in RBC and BChE activity in plasma are irreversibly inhibited by nanomolar concentrations of sarin. Toxic symptoms correlated with the level of inhibition when the subject was first exposed, but AChE activity in RBC could be gradually depressed to near zero by repeated doses administered over a period of several days without symptoms necessarily ensuing. Symptoms usually disappeared before any restoration of RBC AChE or plasma BChE activity. Plasma BChE activity returned to normal in 40 days, but AChE activity in RBC took 92 days to return to its original value.

A study conducted in 1965 and approved for publication in 1974[43] measured the effects of small doses of the nerve agents VX and sarin administered to US Army enlisted men, 20−28 years old, who volunteered with no compensation. Thirty-four subjects were given VX intravenously, 32 subjects drank VX, and 23 subjects received sarin intravenously. Individual differences in response were noted. For example, of the 18 subjects who were given 1.5 μg/kg sarin intravenously, 11 had mild, transient symptoms of dizziness beginning within 1−2 min of injection and lasting 10−15 min. Four subjects had nausea after 1 h, and 6 additional subjects vomited. RBC AChE activity ranged from 9 to 33% of normal for the latter 6 subjects and 14−44% for the asymptomatic subjects, suggesting insignificant correlation between RBC AChE inhibition and toxic symptoms. It was found that VX was 3-times more potent than sarin; the dose that inhibited 50% of the RBC AChE was 1.1 μg/kg VX administered intravenously and 3 μg/kg sarin. Small doses of VX inhibited AChE in preference to BChE. RBC AChE activity spontaneously reactivated from inhibition by VX, and its reactivation rate was assisted by the administration of 2-pyridinium aldoxime methochloride (2-PAM) with a recovery of 70% of the initial AChE activity.

An accidental exposure case affected one man who inhaled a low dose of VX vapor in a military laboratory.[44] The subject exhibited early clinical signs of nerve agent intoxication including tightness in the chest, difficulty breathing, blurred vision, mild rhinorrhea, and eyelid muscle fasciculations. All symptoms disappeared within 24 h. Activity assays showed no depression of RBC AChE activity. However, the RBC fraction of whole blood collected on day 1 yielded 219.9 pg/mL of the fluoride analogue of VX, while the plasma fraction yielded 81.2 pg/mL.[45] Samples taken as late as 27 days following exposure yielded detectable quantities of the VX analogue[44,45] in the RBC fraction as well as in plasma. In this case, the fluoride reactivation method coupled to analysis by gas chromatography−mass spectrometry was clearly a more sensitive method for detecting VX exposure than AChE and BChE activity assays. AChE and BChE activity assays, though less sensitive indicators of exposure than mass spectrometry methods, are run because the enzyme activity assays are simple, quick, and relate to decades of literature.

The Aum Shinrikyo release of sarin in the Tokyo subway in 1995 poisoned 5,000 people and killed 12. Within 2 h of the event, doctors had correctly diagnosed nerve agent exposure.[46] Severely ill patients had reduced plasma BChE activity and the classic symptoms of nerve agent exposure. Reduced RBC AChE activity was noted in 34 out of 66 patients.[47] The speed with which physicians established the cause of illness saved lives because they knew to treat nerve agent intoxication with atropine and 2-PAM. In their publications, the Japanese researchers involved with the event[47,48] acknowledged the usefulness of previously published reports[42,49,50] describing the effects of sarin in man and treatment of nerve gas poisoning. Human studies with known doses of sarin had used only low doses that caused mild to moderate symptoms. Some of the

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victims in the Tokyo subway inhaled doses that resulted in coma and convulsions. The incident provided new information on survival from high doses of sarin and the consequent impairment of learning, memory, and personality change.[51,52] Following this event, scientists developed new methods to detect nerve agent exposure based on analysis of adducts on BChE, release of adducts with potassium fluoride, and analysis of metabolites in urine and serum.[33,53−55]

4. REACTION OF BCHE GENETIC VARIANTS WITH CHOLINESTERASE INHIBITORS

4.1. Effect of BChE Genotype on Reaction with Cholinesterase Inhibitors in Vitro. BChE with the single amino acid substitution Asp98Gly (D70G) is henceforth called atypical BChE to conform with 60 years of scientific literature. Individuals homozygous for atypical BChE have prolonged apnea of 2 h after a standard dose of succinylcholine or mivacurium.[24,56] In contrast, most people resume normal breathing after 3 min.

Substrates and inhibitors that carry a positive charge have a poor affinity for atypical BChE. The positively charged nerve agent VX inhibits atypical BChE 21-fold more slowly than VX inhibits wild-type BChE.[57] Atypical BChE is resistant to inhibition by the positively charged carbamate poison from the calabar bean, physostigmine, and the positively charged drugs neostigmine and physostigmine. Inhibition of 50% of the atypical BChE activity in human serum requires 20-fold higher concentrations of physostigmine and neostigmine than for wild-type BChE.[58] Rate constants for the inactivation of atypical BChE by physostigmine and pyridostigmine are 14- and 7-fold slower than that for wild-type BChE.[59] The muscle relaxant succinylcholine carries two positive charges; its IC_{50} is 100-fold higher for atypical BChE compared to that of wild-type BChE.[58]

Inhibitors that carry no charge are equally effective inhibitors of wild-type and atypical BChE. For example, the nerve agents sarin and soman inhibit wild-type and atypical BChE with the same rate constants.[57] Similarly, paraoxon, the active metabolite of the pesticide parathion, makes no distinction between wild-type and atypical BChE in rate of inhibition.[57,60]

Organophosphorus and carbamate inhibitors make a covalent bond with the active site serine of BChE. Carbamate-inhibited BChE spontaneously regains full activity with a half-life of about 2−3 h.[61,62] Organophosphorus nerve agents, having lost the leaving group during coupling to serine, lose a second group in a process called aging. The rate of aging is fast for soman (half-life 2 min) and relatively slow for sarin (half-life of 6 h) bound to wild-type BChE.[63,64] Soman-inhibited atypical BChE regained 16%, whereas soman-inhibited wild-type BChE regained 7% of its original activity following incubation with HI-6 oxime.[65] This result can be interpreted to mean that soman bound to atypical BChE ages more slowly. A parallel study for soman-inhibited human AChE showed that the D74N mutant, homologous to atypical BChE, aged 15-fold more slowly than soman-inhibited wild-type AChE.[66] The rate of aging of paraoxon-inhibited atypical BChE is 3-fold slower than the rate for paraoxon-inhibited wild-type BChE and 8-fold slower for the diisopropyl fluorophosphate adduct. Aged adducts are irreversibly inhibited, but unaged adducts can undergo spontaneous or oxime assisted reactivation. Slow aging of atypical BChE adducts means BChE activity can be regained.

The atypical and K-variant have decreased levels of BChE molecules in plasma, calculated from antibody-based assays.[30,67,68] The concentration of wild-type BChE in plasma is 4 mg/L (50 nM), while the concentration of homozygous atypical BChE in plasma is 70% of wild-type BChE.[67] The homozygous K-variant has 33% fewer BChE molecules per mL plasma compared to wild-type BChE.[30] Silent BChE variants resulting from frame shift, splice junction mutations, or stop codon insertions are associated with complete absence of BChE molecules, while silent variants resulting from missense mutations can have the protein but no BChE activity. Single nucleotide polymorphisms in BCHE introns are associated with 4.3−9.5% lower BChE activity.[69] Fewer active BChE molecules means reduced bioscavenger capacity, resulting in a larger dose of toxicant reaching the neuromuscular junction. Inhibition of AChE in the neuromuscular junction causes muscle weakness and respiratory failure.

In conclusion, wild-type BChE efficiently scavenges nerve agents. In contrast, atypical BChE is an inefficient bioscavenger of positively charged cholinesterase inhibitors. Individuals who carry the atypical BChE variant may suffer toxic effects from low doses of VX or neostigmine that cause no toxicity in persons with wild-type BChE. Genetic variants with abnormally low levels of BChE enzyme including the atypical, K-variant, and silent variants are predicted to be unusually sensitive to toxicity from low doses of nerve agents.

4.2. Effect of Cholinesterase Inhibitors on Humans with Rare BChE Genotypes. Only a few reports determined BChE genotype in individuals intoxicated by cholinesterase inhibitors. One Israeli soldier, homozygous for atypical BChE, suffered severe cholinergic symptoms following pyridostigmine prophylaxis during the 1991 Persian Gulf War.[59] A case-control study evaluated 144 veterans who had Gulf War Illness and 160 veterans who also served in the Persian Gulf War but had no symptoms.[70] Veterans used pyridostigmine bromide during the Gulf War as a protective measure against nerve agents. They also used cholinesterase-inhibiting pesticides and were possibly exposed to low levels of sarin and cyclosarin following demolition of an Iraqi compound near Khamisiyah. BChE activity levels and genotypes were compared in the 2 groups. Gulf War Illness risk was dramatically elevated for veterans with the less common BChE genotypes who reported taking pyridostigmine bromide pills during deployment.

Brazilian farmers who had no signs of poisoning but who treated their crops with organophosphorus and carbamate pesticides were screened for relative erythrocyte AChE levels.[8] Out of 134 farmers, 72 were classified as mildly poisoned based on RBC AChE activity. Plasma samples were phenotyped for BChE genetic variants. The less common BChE genotypes were more frequent in the mildly poisoned group than in the control group. It was concluded that individuals with nonusual BChE phenotypes seem to be predisposed to erythrocyte AChE inhibition. Whereas wild-type BChE protects AChE from inhibition by destroying pesticides, BChE variants are less efficient detoxifiers.

4.3. Inhibition of BChE Is Not an Adverse Effect. No signs of toxicity were seen in human volunteers administered chlorpyrifos orally at doses that inhibited plasma BChE activity 85%.[71] These doses of chlorpyrifos did not inhibit erythrocyte AChE activity. Plasma BChE activity is considered a biomarker of exposure but is an inappropriate end point for risk assessment.[72] Animal studies indicate that developmental effects on the fetus occur at chlorpyrifos doses that produce maternal toxicity and inhibit erythrocyte AChE activity but not at low doses that produce no maternal toxicity and no

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erythrocyte AChE inhibition.[73] These studies confirm that BChE is highly reactive with chlorpyrifos oxon, the toxic metabolite of chlorpyrifos, whereas AChE is resistant to inhibition, their rate constants being 3.8×10^6 M^{-1} min^{-1} for human AChE and 1.6×10^9 M^{-1} min^{-1} for human BChE.[74] The 3-orders of magnitude difference in chlorpyrifos oxon reactivity supports the conclusion that the toxic dose is much higher than the 50 nM concentration of plasma BChE, indicating that 50 nM BChE contributes little to no protection from toxicity. Individuals with genetic variants of BChE are not expected to be unusuallly sensitive to OP toxicity.

5. EVALUATION OF RISK OF OP TOXICITY

5.1. Polymorphism of Organophosphorus Pesticide-Metabolizing Genes. Howard et al. formulated a new approach to evaluate the risk of toxicity from OP by studying genetic variability in 30 genes involved in OP metabolism.[69] Gomez-Martin built on this idea by analyzing 10 polymorphic variants of seven genes involved in organophosphorus pesticide metabolism.[75] The goal was to identify individuals most at risk of adverse health effects from exposure. They defined risk as mutations in butyrylcholinesterase (BCHE), paraoxonase-1 (PON1), and cytochrome P450 enzymes (CYP2D6, CYP2C19, and CYP3A) and copy number variation in glutathione *S*-transferases (GSTM1 and GSTT1). Out of 496 individuals, 172 (34.7%) carried an allele for the K-variant or atypical BChE, while 58 (11.7%) presented the PON1 genotype for slow metabolism of OP pesticides. Null genotypes for GSTM1 and GSTT1 were found in 7.1% of the study population. When genetic polymorphisms in BCHE, PON1, GSTM1, and GSTT1 were considered together, the worst genotype combination was observed in one individual (0.2%). When CYP450 enzymes were assessed for the worst genotype combinations, only 2 indivduals carried risky genotypes, CYP3AP1-44GG and CYP2C19 681AA, which correspond to extensive and poor metabolizers, respectively. The individuals in the study had no toxic symptoms and no known exposure to OP. Future studies should correlate genotype, OP exposure, and signs of toxicity.

PON1 polymorphism has been studied for its association to pesticide toxicity.[76−78] Greenhouse workers had higher levels of PON1 activity than the control group[78] suggesting an adaptive response to pesticide exposure. Greenhouse workers also had higher levels of BChE activity but only when exposure levels were low.[79] Several esterases with phenyl valerate activity in chicken brain are inhibited by OP including neuropathy target esterase and BChE.[80,81]

The human studies of healthy young men summarized in section 3.1 document the fact that individuals respond differently to the same dose of nerve agent administered via the same route (iv or orally). At the time of the studies, methods for identifying genetic variants were not available. Polymorphism of BChE and OP-metabolizing genes is a likely explanation for why some people have toxic symptoms from low dose exposures that have little effect on others.

5.2. Animal Models for Testing Risk from OP Toxicity. The response of nonhuman primates to OP toxicants is closest to that of humans. Sequencing of the rhesus macaque exomes identified a family of monkeys with a naturally occurring mutation p.Gly180* in the BCHE gene in the heterozygous state. The nonsense mutation introduced a stop codon, resulting in a decrease of serum BChE activity.[82] Monkeys with this mutation could serve as a model for humans with BChE mutations in the heterozygous state to determine whether BChE deficiency is associated with increased sensitivity to OP toxicity. Directed breeding of heterozygous monkeys with this mutation could be used to produce homozygous animals for study of the effects of complete absence of BChE in a nonhuman primate.

The BChE knockout mouse with no BChE activity in any tissue[83] is not an appropriate model for the effect of soman in humans who are deficient in BChE because the mice have high levels of carboxylesterase activity in plasma. In contrast, humans have no carboxylesterase in blood.[84] Mouse plasma carboxylesterase, being an efficient bioscavenger of soman, obscures the contribution of BChE.

Mice with no plasma carboxylesterase, but with normal carboxylesterase in other organs, are a more suitable model for human response to nerve agents.[85,86] Their BChE activity per mL plasma is less than 50% of the BChE activity in human plasma. A low dose of the nerve agent analogue, soman coumarin, was lethal to ES1−/− mice but not to ES1+/+ mice, demonstrating that plasma carboxylesterase protects from a highly toxic soman analogue.[85] The mice can be purchased from The Jackson Laboratory, Bar Harbor, ME catalog # B6(Cg)-*Ces1c*$^{tm1.1Loc}$/J Stock No: 014096.

Studies in plasma carboxylesterase knockout mice demonstrated that carboxylesterase does not protect from OP pesticides.[86] The explanation for the failure of plasma carboxylesterase and BChE to protect from OP pesticides is as follows. OP pesticides react poorly with AChE and therefore cause toxicity only at high doses. A toxic dose of OP pesticide exceeds the concentration of bioscavenger in plasma. Even though the OP pesticide is effeciently scavenged in plasma, all of the scavenging capacity is used up before a significant amount of the OP is destroyed, thereby affording no protection.

Paraoxonase (PON1) in human plasma is a component of high density lipoprotein. PON1 hydrolyzes the active metabolites of OP pesticides including chlorpyrifos oxon and diazoxon, as well as the nerve agents sarin and soman.[76] Mice completely deficient in PON1 are highly sensitive to the toxicity of chlorpyrifos oxon and diazoxon. PON1 knockout mice[87] can be purchased from The Jackson Laboratory B6.129 × 1-*Pon1*^{tm1Lus}/J Stock No: 004160.

6. EFFECT OF BCHE GENETIC VARIANTS ON ANALYSIS OF EXPOSURE TO OP

6.1. Quantitative Variants. The atypical and K-variant have about 70% of the standard concentration of BChE molecules per mL plasma. A previously reported mass spectrometry method can detect as little as 3.2% nerve agent-labeled BChE in 0.5 mL of plasma.[88] This high level of sensitivity means that the atypical and K-variants of BChE are expected to yield detectable levels of BChE adducts.

Silent variants range from zero activity and no BChE protein, to 10% of normal activity and 50 nM BChE protein.[36] The silent BChE variants with no BChE protein will yield no BChE OP adducts. The missense mutation that changes the active site serine to glycine makes a BChE protein that is unable to make a covalent bond with OP. Plasma from individuals with these BChE mutations will give no information about OP exposure in the standard mass spectrometry assay.

An alternative assay could be implemented that analyzes adducts on AChE. The quantity of soluble AChE in plasma (8 ng/mL) is 500- to 1000-fold lower than that of BChE (4000 ng/mL).[89−91] RBC are a richer source of AChE than plasma,

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Chem. Res. Toxicol. 2016, 29, 1381−1392

but analysis of AChE on RBC requires additional sample preparation steps because AChE is membrane bound.

6.2. High BChE Activity. Higher than normal BChE activity, ranging from 30% to 400% of the normal, is found in the Cynthiana and C5+ genetic variants.[92,93] These variants have the wild-type BCHE nucleotide sequence in their exons and promoter region.[93] Heritable quantitative trait loci have been identified in intron 2 and the 3′-UTR of the BCHE gene and on chromosome 5q,[6] but it is unknown whether mutations at these loci increase or decrease BChE activity levels. Additional loci that affect BChE activity have been identified by the genome-wide association scan method.[94] The frequency of the C5+ variant is 10% in European and American populations. Plasma samples from these individuals are expected to give strong signals in previously reported mass spectrometry assays for confirming OP exposure.

7. SIGNIFICANCE OF ACHE AND BCHE GENETIC VARIANTS TO RISK OF TOXICITY FROM CHOLINESTERASE INHIBITORS

7.1. AChE Variants. AChE genetic variants have not been associated with the risk of toxicity from cholinesterase inhibitors. The most frequent variant His353Asn (H322N), the Yt blood group antigen, has normal AChE activity.[6,10] Missense mutations are rare, and when they do occur, they are paired with a normal ACHE allele. Some diseases are associated with very low AChE enzyme activity, but the cause is not a mutation in AChE. Congenital myasthenic syndrome with end-plate acetylcholinesterase deficiency is caused by mutations in the COLQ gene, resulting in failure to anchor AChE to the neuromuscular junction.[95,96] Patients with paroxysmal nocturnal hemoglobinuria have a deficiency in all glycolipid-anchored proteins including AChE, explained by mutations in enzymes that synthesize the glycosylphosphatidylinositol anchor.[97] It is predicted, but unproven, that patients with AChE deficiency have an increased risk of toxicity from cholinesterase inhibitors.

7.2. BChE Variants. It is commonly assumed that people with BChE deficiency are at increased risk of toxicity from cholinesterase inhibitors. In our opinion, this generalization is correct for nerve agents but fails for cholinesterase inhibitors that have a higher affinity for BChE than AChE. As pointed out by Maxwell[98] and confirmed by studies in carboxylesterase knockout mice,[86] poisons that preferentially react with plasma bioscavengers rather than with AChE produce no toxic signs until the bioscavenger capacity is exceeded. The binding affinity of OP pesticides for AChE is so poor that AChE is inhibited only by large doses. Toxic doses of OP pesticides are orders of magnitude higher than the BChE concentration in blood. The 50 nM BChE in human plasma scavenges 50 nM OP pesticides but is unable to scavenge 20,000 nM chlorpyrifos. Thus, BChE contributes little to no protection from the acute toxicity of OP pesticides. This means that risk from OP pesticide toxicity is indistinguishable in individuals with genetic variants from those with wild-type BChE.

Nerve agents inhibit AChE more readily than they inhibit BChE. Humans have moderate signs of toxicity after a single oral dose of 0.028 mg/kg sarin[42] or an intravenous dose of 0.0015 mg/kg VX.[43] The 50 nM BChE in plasma easily captures low doses of nerve agent, preventing the inhibition of AChE. Genetic variants with reduced levels or with inactive BChE are expected to be at increased risk of toxicity from nerve agents.

This prediction assumes that detoxication of nerve agents is accomplished primarily by BChE. It does not consider the role of other bioscavengers including the carboxylesterases in the liver and lung, acetylcholinesterase in RBC, albumin in plasma, metabolic destruction by cytochrome P450 and paraoxonase, glucuronide conjugation, and glutathione-mediated conjugation. Body fat also has a role because the uncharged cholinesterase inhibitors are sequestered in body fat. What is needed is a study that correlates the toxicity of nerve agents with BChE genotype. If archived blood samples exist from humans administered known doses of sarin and VX,[41] BChE genotype analysis would answer the question of whether BChE genetic variants explain the differences in toxic response noted in individual subjects.

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Notes

The findings and conclusions in this review are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement fy the Centers for Disease Control and Prevention, the Public Health Service, or the U.S. Department of Health and Human Services.

The authors declare no competing financial interest.

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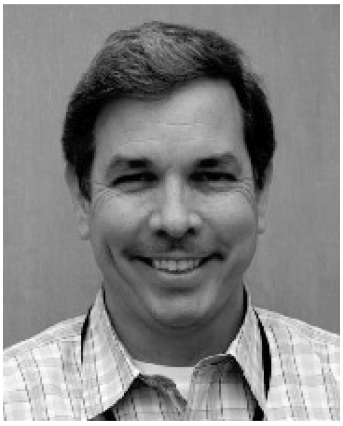
Chem. Res. Toxicol. 2016, 29, 1381−1392



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■ ABBREVIATIONS

ACHE, acetylcholinesterase gene; AChE, acetylcholinesterase enzyme; BCHE, butyrylcholinesterase gene; BChE, butyrylcholinesterase enzyme; CDC, Centers for Disease Control and Prevention; OP, organophosphorus toxicant; 2-PAM, 2-pyridine aldoxime methyl chloride; PON1, paraoxonase 1; RBC, red blood cell; VX, *O*-ethyl *S*-[2-(diisopropylamino)-ethyl] methylphosphonothioate)

■ REFERENCES

(1) Skinner, C., Thomas, J., Johnson, R., and Kobelski, R. (2009) Medical toxicology and public health–update on research and activities at the Centers for Disease Control and Prevention, and the Agency for Toxic Substances and Disease Registry: introduction to the Laboratory Response Network-Chemical (LRN-C). *J. Med. Toxicol. 5*, 46−49.

(2) Pantazides, B. G., Watson, C. M., Carter, M. D., Crow, B. S., Perez, J. W., Blake, T. A., Thomas, J. D., and Johnson, R. C. (2014) An enhanced butyrylcholinesterase method to measure organophosphorus nerve agent exposure in humans. *Anal. Bioanal. Chem. 406*, 5187−5194.

(3) Sporty, J. L., Lemire, S. W., Jakubowski, E. M., Renner, J. A., Evans, R. A., Williams, R. F., Schmidt, J. G., van der Schans, M. J., Noort, D., and Johnson, R. C. (2010) Immunomagnetic separation and quantification of butyrylcholinesterase nerve agent adducts in human serum. *Anal. Chem. 82*, 6593−6600.

(4) Lockridge, O. (2015) Review of human butyrylcholinesterase structure, function, genetic variants, history of use in the clinic, and potential therapeutic uses. *Pharmacol. Ther. 148*, 34−46.

(5) Grob, D. (1950) Uses and hazards of the organic phosphate anticholinesterase compounds. *Ann. Intern. Med. 32*, 1229−1234.

(6) Valle, A. M., Radic, Z., Rana, B. K., Mahboubi, V., Wessel, J., Shih, P. A., Rao, F., O'Connor, D. T., and Taylor, P. (2011) Naturally occurring variations in the human cholinesterase genes: heritability and association with cardiovascular and metabolic traits. *J. Pharmacol. Exp. Ther. 338*, 125−133.

(7) Eddleston, M., Buckley, N. A., Eyer, P., and Dawson, A. H. (2008) Management of acute organophosphorus pesticide poisoning. *Lancet 371*, 597−607.

(8) Fontoura-da-Silva, S. E., and Chautard-Freire-Maia, E. A. (1996) Butyrylcholinesterase variants (BCHE and CHE2 Loci) associated with erythrocyte acetylcholinesterase inhibition in farmers exposed to pesticides. *Hum. Hered. 46*, 142−147.

(9) Bartels, C. F., Zelinski, T., and Lockridge, O. (1993) Mutation at codon 322 in the human acetylcholinesterase (ACHE) gene accounts for YT blood group polymorphism. *Am. J. Hum. Genet. 52*, 928−936.

(10) Masson, P., Froment, M. T., Sorenson, R. C., Bartels, C. F., and Lockridge, O. (1994) Mutation His322Asn in human acetylcholinesterase does not alter electrophoretic and catalytic properties of the erythrocyte enzyme. *Blood 83*, 3003−3005.

(11) Sussman, J. L., Harel, M., Frolow, F., Oefner, C., Goldman, A., Toker, L., and Silman, I. (1991) Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein. *Science 253*, 872−879.

(12) Ordentlich, A., Barak, D., Kronman, C., Flashner, Y., Leitner, M., Segall, Y., Ariel, N., Cohen, S., Velan, B., and Shafferman, A. (1993) Dissection of the human acetylcholinesterase active center determinants of substrate specificity. Identification of residues constituting the anionic site, the hydrophobic site, and the acyl pocket. *J. Biol. Chem. 268*, 17083−17095.

(13) Szegletes, T., Mallender, W. D., Thomas, P. J., and Rosenberry, T. L. (1999) Substrate binding to the peripheral site of acetylcholinesterase initiates enzymatic catalysis. Substrate inhibition arises as a secondary effect. *Biochemistry 38*, 122−133.

(14) Taylor, P., and Radic, Z. (1994) The cholinesterases: from genes to proteins. *Annu. Rev. Pharmacol. Toxicol. 34*, 281−320.

(15) Roberts, W. L., Santikarn, S., Reinhold, V. N., and Rosenberry, T. L. (1988) Structural characterization of the glycoinositol phospholipid membrane anchor of human erythrocyte acetylcholinesterase by fast atom bombardment mass spectrometry. *J. Biol. Chem. 263*, 18776−18784.

(16) Massoulie, J., Anselmet, A., Bon, S., Krejci, E., Legay, C., Morel, N., and Simon, S. (1999) The polymorphism of acetylcholinesterase:

DOI: 10.1021/acs.chemrestox.6b00228
Chem. Res. Toxicol. 2016, 29, 1381−1392

post-translational processing, quaternary associations and localization. *Chem.-Biol. Interact. 119−120*, 29−42.
(17) Massoulie, J., and Bon, S. (2006) The C-terminal T peptide of cholinesterases: structure, interactions, and influence on protein folding and secretion. *J. Mol. Neurosci. 30*, 233−236.
(18) Exome-Aggregation-Consortium-Lek-et-al. (2015) Analysis of Protein-Coding Genetic Variation in 60,706 Humans, bioRxiv the preprint server for biology, DOI: http://dx.doi.org/10.1101/030338.
(19) Duysen, E. G., Stribley, J. A., Fry, D. L., Hinrichs, S. H., and Lockridge, O. (2002) Rescue of the acetylcholinesterase knockout mouse by feeding a liquid diet; phenotype of the adult acetylcholinesterase deficient mouse. *Dev. Brain Res. 137*, 43−54.
(20) McAlpine, P. J., Dixon, M., Allderdice, P. W., Lockridge, O., and La Du, B. N. (1991) The butyrylcholinesterase gene (BCHE) at 3q26.2 shows two RFLPs. *Nucleic Acids Res. 19*, 5088.
(21) Arpagaus, M., Kott, M., Vatsis, K. P., Bartels, C. F., La Du, B. N., and Lockridge, O. (1990) Structure of the gene for human butyrylcholinesterase. Evidence for a single copy. *Biochemistry 29*, 124−131.
(22) Manoharan, I., Boopathy, R., Darvesh, S., and Lockridge, O. (2007) A medical health report on individuals with silent butyrylcholinesterase in the Vysya community of India. *Clin. Chim. Acta 378*, 128−135.
(23) McGuire, M. C., Nogueira, C. P., Bartels, C. F., Lightstone, H., Hajra, A., Van der Spek, A. F., Lockridge, O., and La Du, B. N. (1989) Identification of the structural mutation responsible for the dibucaine-resistant (atypical) variant form of human serum cholinesterase. *Proc. Natl. Acad. Sci. U. S. A. 86*, 953−957.
(24) Kalow, W., and Staron, N. (1957) On distribution and inheritance of atypical forms of human serum cholinesterase, as indicated by dibucaine numbers. *Can. J. Biochem. Physiol. 35*, 1305−1320.
(25) Lushchekina, S., Nemukhin, A., Varfolomeev, S., and Masson, P. (2016) Understanding the non-catalytic behavior of human butyrylcholinesterase silent variants: Comparison of wild-type enzyme, catalytically active Ala328Cys mutant, and silent Ala328Asp variant. *Chem.-Biol. Interact.*, DOI: 10.1016/j.cbi.2016.04.007.
(26) Wichmann, S., Faerk, G., Bundgaard, J. R., and Gatke, M. R. (2016) Patients with prolonged effect of succinylcholine or mivacurium had novel mutations in the butyrylcholinesterase gene. *Pharmacogenet. Genomics 26*, 351−356.
(27) Bartels, C. F., James, K., and La Du, B. N. (1992) DNA mutations associated with the human butyrylcholinesterase J-variant. *Am. J. Hum. Genet. 50*, 1104−1114.
(28) Li, B., Duysen, E. G., Saunders, T. L., and Lockridge, O. (2006) Production of the butyrylcholinesterase knockout mouse. *J. Mol. Neurosci. 30*, 193−195.
(29) Bartels, C. F., Jensen, F. S., Lockridge, O., van der Spek, A. F., Rubinstein, H. M., Lubrano, T., and La Du, B. N. (1992) DNA mutation associated with the human butyrylcholinesterase K-variant and its linkage to the atypical variant mutation and other polymorphic sites. *Am. J. Hum. Genet. 50*, 1086−1103.
(30) Rubinstein, H. M., Dietz, A. A., and Lubrano, T. (1978) E1k, another quantitative variant at cholinesterase locus 1. *J. Med. Genet. 15*, 27−29.
(31) Altamirano, C. V., Bartels, C. F., and Lockridge, O. (2000) The butyrylcholinesterase K-variant shows similar cellular protein turnover and quaternary interaction to the wild-type enzyme. *J. Neurochem. 74*, 869−877.
(32) van der Schans, M. J., Fidder, A., van Oeveren, D., Hulst, A. G., and Noort, D. (2008) Verification of exposure to cholinesterase inhibitors: generic detection of OPCW Schedule 1 nerve agent adducts to human butyrylcholinesterase. *J. Anal. Toxicol. 32*, 125−130.
(33) Fidder, A., Hulst, A. G., Noort, D., de Ruiter, R., van der Schans, M. J., Benschop, H. P., and Langenberg, J. P. (2002) Retrospective detection of exposure to organophosphorus anti-cholinesterases: mass spectrometric analysis of phosphylated human butyrylcholinesterase. *Chem. Res. Toxicol. 15*, 582−590.
(34) Carter, M. D., Crow, B. S., Pantazides, B. G., Watson, C. M., Thomas, J. D., Blake, T. A., and Johnson, R. C. (2013) Direct Quantitation of Methyl Phosphonate Adducts to Human Serum Butyrylcholinesterase by Immunomagnetic-UHPLC-MS/MS. *Anal. Chem. 85*, 11106−11111.
(35) Sakamoto, N., Hidaka, K., Fujisawa, T., Maeda, M., and Iuchi, I. (1998) Identification of a point mutation associated with a silent phenotype of human serum butyrylcholinesterase–a case of familial cholinesterasemia. *Clin. Chim. Acta 274*, 159−166.
(36) Primo-Parmo, S. L., Bartels, C. F., Wiersema, B., van der Spek, A. F., Innis, J. W., and La Du, B. N. (1996) Characterization of 12 silent alleles of the human butyrylcholinesterase (BCHE) gene. *Am. J. Hum. Genet. 58*, 52−64.
(37) Hidaka, K., Watanabe, Y., Tomita, M., Ueda, N., Higashi, M., Minatogawa, Y., and Iuchi, I. (2001) Gene analysis of genomic DNA from stored serum by polymerase chain reaction: identification of three missense mutations in patients with cholinesterasemia and ABO genotyping. *Clin. Chim. Acta 303*, 61−67.
(38) Rao, P. R., and Gopalam, K. B. (1979) High incidence of the silent allele at cholinesterase locus 1 in Vysyas of Andrha Pradesh (S. India). *Hum. Genet. 52*, 139−141.
(39) Scott, E. M., and Wright, R. C. (1976) A third type of serum cholinesterase deficiency in Eskimos. *Am. J. Hum. Genet. 28*, 253−256.
(40) Kadar, T., Raveh, L., Cohen, G., Oz, N., Baranes, I., Balan, A., Ashani, Y., and Shapira, S. (1985) Distribution of 3H-soman in mice. *Arch. Toxicol. 58*, 45−49.
(41) Sidell, F. R. (2007) A History of Human Studies with Nerve Agents by the UK and USA, in *Chemical Warfare Agents Toxicology and Treatment*, 2nd ed., (Marrs, T. C., Maynard, R. L., and Sidell, F. R., Eds.) pp 223−239, John Wiley & Sons, New York.
(42) Grob, D., and Harvey, J. C. (1958) Effects in man of the anticholinesterase compound sarin (isopropyl methyl phosphonofluoridate). *J. Clin. Invest. 37*, 350−368.
(43) Sidell, F. R., and Groff, W. A. (1974) The reactivatibility of cholinesterase inhibited by VX and sarin in man. *Toxicol. Appl. Pharmacol. 27*, 241−252.
(44) McGuire, J. M., Taylor, J. T., Byers, C. E., Jakubowski, E. M., and Thomson, S. M. (2008) Determination of VX-G analogue in red blood cells via gas chromatography-tandem mass spectrometry following an accidental exposure to VX. *J. Anal. Toxicol. 32*, 73−77.
(45) Solano, M. I., Thomas, J. D., Taylor, J. T., McGuire, J. M., Jakubowski, E. M., Thomson, S. A., Maggio, V. L., Holland, K. E., Smith, J. R., Capacio, B., Woolfitt, A. R., Ashley, D. L., and Barr, J. R. (2008) Quantification of nerve agent VX-butyrylcholinesterase adduct biomarker from an accidental exposure. *J. Anal. Toxicol. 32*, 68−72.
(46) Suzuki, T., Nozaki, H., Aikawa, N., Shinozawa, Y., Hori, S., Fujishima, S., Takuma, K., and Sagoh, M. (1995) Sarin poisoning in Tokyo subway. *Lancet 345*, 980−981.
(47) Masuda, N., Takatsu, M., Morinari, H., and Ozawa, T. (1995) Sarin poisoning in Tokyo subway. *Lancet 345*, 1446.
(48) Suzuki, T., Morita, H., Ono, K., Maekawa, K., Nagai, R., and Yazaki, Y. (1995) Sarin poisoning in Tokyo subway. *Lancet 345*, 980.
(49) Sidell, F. R. (1974) Soman and sarin: clinical manifestations and treatment of accidental poisoning by organophosphates. *Clin. Toxicol. 7*, 1−17.
(50) Grob, D., and Harvey, A. M. (1953) The effects and treatment of nerve gas poisoning. *Am. J. Med. 14*, 52−63.
(51) Hatta, K., Miura, Y., Asukai, N., and Hamabe, Y. (1996) Amnesia from sarin poisoning. *Lancet 347*, 1343.
(52) Miyaki, K., Nishiwaki, Y., Maekawa, K., Ogawa, Y., Asukai, N., Yoshimura, K., Etoh, N., Matsumoto, Y., Kikuchi, Y., Kumagai, N., and Omae, K. (2005) Effects of sarin on the nervous system of subway workers seven years after the Tokyo subway sarin attack. *J. Occup. Health 47*, 299−304.
(53) Polhuijs, M., Langenberg, J. P., and Benschop, H. P. (1997) New method for retrospective detection of exposure to organophosphorus anticholinesterases: application to alleged sarin victims of Japanese terrorists. *Toxicol. Appl. Pharmacol. 146*, 156−161.

DOI: 10.1021/acs.chemrestox.6b00228
Chem. Res. Toxicol. 2016, 29, 1381−1392

(54) Minami, M., Hui, D. M., Katsumata, M., Inagaki, H., and Boulet, C. A. (1997) Method for the analysis of the methylphosphonic acid metabolites of sarin and its ethanol-substituted analogue in urine as applied to the victims of the Tokyo sarin disaster. *J. Chromatogr., Biomed. Appl. 695*, 237−244.
(55) Noort, D., Hulst, A. G., Platenburg, D. H., Polhuijs, M., and Benschop, H. P. (1998) Quantitative analysis of O-isopropyl methylphosphonic acid in serum samples of Japanese citizens allegedly exposed to sarin: estimation of internal dosage. *Arch. Toxicol. 72*, 671−675.
(56) Ostergaard, D., Viby-Mogensen, J., Rasmussen, S. N., Gatke, M. R., and Varin, F. (2005) Pharmacokinetics and pharmacodynamics of mivacurium in patients phenotypically homozygous for the atypical plasma cholinesterase variant: effect of injection of human cholinesterase. *Anesthesiology 102*, 1124−1132.
(57) Skrinjaric-Spoljar, M., and Simeon, V. (1993) Reactions of usual and atypical human serum cholinesterase phenotypes with progressive and reversible inhibitors. *J. Enzyme Inhib. 7*, 169−174.
(58) Kalow, W., and Davies, R. O. (1959) The activity of various esterase inhibitors towards atypical human serum cholinesterase. *Biochem. Pharmacol. 1*, 183−192.
(59) Loewenstein-Lichtenstein, Y., Schwarz, M., Glick, D., Norgaard-Pedersen, B., Zakut, H., and Soreq, H. (1995) Genetic predisposition to adverse consequences of anti-cholinesterases in 'atypical' BCHE carriers. *Nat. Med. 1*, 1082−1085.
(60) Masson, P., Froment, M. T., Bartels, C. F., and Lockridge, O. (1997) Importance of aspartate-70 in organophosphate inhibition, oxime re-activation and aging of human butyrylcholinesterase. *Biochem. J. 325*, 53−61.
(61) Li, H., Ricordel, I., Tong, L., Schopfer, L. M., Baud, F., Megarbane, B., Maury, E., Masson, P., and Lockridge, O. (2009) Carbofuran poisoning detected by mass spectrometry of butyrylcholinesterase adduct in human serum. *J. Appl. Toxicol. 29*, 149−155.
(62) Reiner, E. (1971) Spontaneous reactivation of phosphorylated and carbamylated cholinesterases. *Bull. W. H. O. 44*, 109−112.
(63) Saxena, A., Viragh, C., Frazier, D. S., Kovach, I. M., Maxwell, D. M., Lockridge, O., and Doctor, B. P. (1998) The pH dependence of dealkylation in soman-inhibited cholinesterases and their mutants: further evidence for a push-pull mechanism. *Biochemistry 37*, 15086−15096.
(64) Heilbronn, E. (1963) In vitro reactivation and ″ageing″ of Tabuninhibited blood cholinesterases; studies with N-methyl-pyridinium-2-aldoxime methane sulphonate and N,N′-trimethylene bis (pyridinium-4-aldoxime) dibromide. *Biochem. Pharmacol. 12*, 25−36.
(65) Dimov, D., Kanev, K., and Dimova, I. (2012) Correlation between butyrylcholinesterase variants and sensitivity to soman toxicity. *Acta Biochim. Polym. 59*, 313−316.
(66) Shafferman, A., Ordentlich, A., Barak, D., Stein, D., Ariel, N., and Velan, B. (1996) Aging of phosphylated human acetylcholinesterase: catalytic processes mediated by aromatic and polar residues of the active centre. *Biochem. J. 318*, 833−840.
(67) Eckerson, H. W., Oseroff, A., Lockridge, O., and La Du, B. N. (1983) Immunological comparison of the usual and atypical human serum cholinesterase phenotypes. *Biochem. Genet. 21*, 93−108.
(68) Brock, A. (1990) Immunoreactive plasma cholinesterase (EC 3.1.1.8) substance concentration, compared with cholinesterase activity concentration and albumin: inter- and intra-individual variations in a healthy population group. *J. Clin. Chem. Clin. Biochem. 28*, 851−856.
(69) Howard, T. D., Hsu, F. C., Grzywacz, J. G., Chen, H., Quandt, S. A., Vallejos, Q. M., Whalley, L. E., Cui, W., Padilla, S., and Arcury, T. A. (2010) Evaluation of candidate genes for cholinesterase activity in farmworkers exposed to organophosphorus pesticides: association of single nucleotide polymorphisms in BCHE. *Environ. Health Perspect. 118*, 1395−1399.
(70) Steele, L., Lockridge, O., Gerkovich, M. M., Cook, M. R., and Sastre, A. (2015) Butyrylcholinesterase genotype and enzyme activity in relation to Gulf War illness: preliminary evidence of gene-exposure interaction from a case-control study of 1991 Gulf War veterans. *Environ. Health 14*, 4.
(71) Nolan, R. J., Rick, D. L., Freshour, N. L., and Saunders, J. H. (1984) Chlorpyrifos: pharmacokinetics in human volunteers. *Toxicol. Appl. Pharmacol. 73*, 8−15.
(72) van Gemert, M., Dourson, M., Moretto, A., and Watson, M. (2001) Use of human data for the derivation of a reference dose for chlorpyrifos. *Regul. Toxicol. Pharmacol. 33*, 110−116.
(73) Mink, P. J., Kimmel, C. A., and Li, A. A. (2012) Potential effects of chlorpyrifos on fetal growth outcomes: implications for risk assessment. *J. Toxicol. Environ. Health, Part B 15*, 281−316.
(74) Amitai, G., Moorad, D., Adani, R., and Doctor, B. P. (1998) Inhibition of acetylcholinesterase and butyrylcholinesterase by chlorpyrifos-oxon. *Biochem. Pharmacol. 56*, 293−299.
(75) Gomez-Martin, A., Hernandez, A. F., Martinez-Gonzalez, L. J., Gonzalez-Alzaga, B., Rodriguez-Barranco, M., Lopez-Flores, I., Aguilar-Garduno, C., and Lacasana, M. (2015) Polymorphisms of pesticide-metabolizing genes in children living in intensive farming communities. *Chemosphere 139*, 534−540.
(76) Costa, L. G., Giordano, G., Cole, T. B., Marsillach, J., and Furlong, C. E. (2013) Paraoxonase 1 (PON1) as a genetic determinant of susceptibility to organophosphate toxicity. *Toxicology 307*, 115−122.
(77) Adad, L. M., de Andrade, H. H., Kvitko, K., Lehmann, M., Cavalcante, A. A., and Dihl, R. R. (2015) Occupational exposure of workers to pesticides: Toxicogenetics and susceptibility gene polymorphisms. *Genet. Mol. Biol. 38*, 308−315.
(78) Lozano-Paniagua, D., Gomez-Martin, A., Gil, F., Parron, T., Alarcon, R., Requena, M., Lacasana, M., and Hernandez, A. F. (2016) Activity and determinants of cholinesterases and paraoxonase-1 in blood of workers exposed to non-cholinesterase inhibiting pesticides. *Chem.-Biol. Interact.*, DOI: 10.1016/j.cbi.2016.04.008.
(79) Garcia-Garcia, C. R., Parron, T., Requena, M., Alarcon, R., Tsatsakis, A. M., and Hernandez, A. F. (2016) Occupational pesticide exposure and adverse health effects at the clinical, hematological and biochemical level. *Life Sci. 145*, 274−283.
(80) Mangas, I., Estevez, J., Vilanova, E., and Franca, T. C. (2016) New insights on molecular interactions of organophosphorus pesticides with esterases. *Toxicology*, DOI: 10.1016/j.tox.2016.06.006.
(81) Mangas, I., Estevez, J., and Vilanova, E. (2016) Esterases hydrolyze phenyl valerate activity as targets of organophosphorus compounds. *Chem.-Biol. Interact.*, DOI: 10.1016/j.cbi.2016.04.024.
(82) Cornish, A. S., Gibbs, R. M., and Norgren, R. B., Jr. (2016) Exome screening to identify loss-of-function mutations in the rhesus macaque for development of preclinical models of human disease. *BMC Genomics 17*, 170.
(83) Li, B., Duysen, E. G., Carlson, M., and Lockridge, O. (2008) The butyrylcholinesterase knockout mouse as a model for human butyrylcholinesterase deficiency. *J. Pharmacol. Exp. Ther. 324*, 1146−1154.
(84) Li, B., Sedlacek, M., Manoharan, I., Boopathy, R., Duysen, E. G., Masson, P., and Lockridge, O. (2005) Butyrylcholinesterase, paraoxonase, and albumin esterase, but not carboxylesterase, are present in human plasma. *Biochem. Pharmacol. 70*, 1673−1684.
(85) Duysen, E. G., Koentgen, F., Williams, G. R., Timperley, C. M., Schopfer, L. M., Cerasoli, D. M., and Lockridge, O. (2011) Production of ES1 Plasma Carboxylesterase Knockout Mice for Toxicity Studies. *Chem. Res. Toxicol. 24*, 1891−1898.
(86) Duysen, E. G., Cashman, J. R., Schopfer, L. M., Nachon, F., Masson, P., and Lockridge, O. (2012) Differential sensitivity of plasma carboxylesterase-null mice to parathion, chlorpyrifos and chlorpyrifos oxon, but not to diazinon, dichlorvos, diisopropylfluorophosphate, cresyl saligenin phosphate, cyclosarin thiocholine, tabun thiocholine, and carbofuran. *Chem.-Biol. Interact. 195*, 189−198.
(87) Shih, D. M., Gu, L., Xia, Y. R., Navab, M., Li, W. F., Hama, S., Castellani, L. W., Furlong, C. E., Costa, L. G., Fogelman, A. M., and Lusis, A. J. (1998) Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. *Nature 394*, 284−287.

DOI: 10.1021/acs.chemrestox.6b00228
Chem. Res. Toxicol. 2016, 29, 1381−1392

(88) Knaack, J. S., Zhou, Y., Abney, C. W., Jacob, J. T., Prezioso, S. M., Hardy, K., Lemire, S. W., Thomas, J., and Johnson, R. C. (2012) A high-throughput diagnostic method for measuring human exposure to organophosphorus nerve agents. *Anal. Chem. 84*, 9470−9477.
(89) Sorensen, K., Brodbeck, U., Rasmussen, A. G., and Norgaard-Pedersen, B. (1986) Normal human serum contains two forms of acetylcholinesterase. *Clin. Chim. Acta 158*, 1−6.
(90) St Clair, D. M., Brock, D. J., and Barron, L. (1986) A monoclonal antibody assay technique for plasma and red cell acetylcholinesterase activity in Alzheimer's disease. *J. Neurol. Sci. 73*, 169−176.
(91) Brimijoin, S., and Hammond, P. (1988) Butyrylcholinesterase in human brain and acetylcholinesterase in human plasma: trace enzymes measured by two-site immunoassay. *J. Neurochem. 51*, 1227−1231.
(92) Yoshida, A., and Motulsky, A. G. (1969) A pseudocholinesterase variant (E Cynthiana) associated with elevated plasma enzyme activity. *Am. J. Hum. Genet. 21*, 486−498.
(93) Akizuki, S., Ohnishi, A., Kotani, K., and Sudo, K. (2004) Genetic and immunological analyses of patients with increased serum butyrylcholinesterase activity and its C5 variant form. *Clin. Chem. Lab. Med. 42*, 991−996.
(94) Benyamin, B., Middelberg, R. P., Lind, P. A., Valle, A. M., Gordon, S., Nyholt, D. R., Medland, S. E., Henders, A. K., Heath, A. C., Madden, P. A., Visscher, P. M., O'Connor, D. T., Montgomery, G. W., Martin, N. G., and Whitfield, J. B. (2011) GWAS of butyrylcholinesterase activity identifies four novel loci, independent effects within BCHE and secondary associations with metabolic risk factors. *Hum. Mol. Genet. 20*, 4504−4514.
(95) Donger, C., Krejci, E., Serradell, A. P., Eymard, B., Bon, S., Nicole, S., Chateau, D., Gary, F., Fardeau, M., Massoulie, J., and Guicheney, P. (1998) Mutation in the human acetylcholinesterase-associated collagen gene, COLQ, is responsible for congenital myasthenic syndrome with end-plate acetylcholinesterase deficiency (Type Ic). *Am. J. Hum. Genet. 63*, 967−975.
(96) Ohno, K., Brengman, J., Tsujino, A., and Engel, A. G. (1998) Human endplate acetylcholinesterase deficiency caused by mutations in the collagen-like tail subunit (ColQ) of the asymmetric enzyme. *Proc. Natl. Acad. Sci. U. S. A. 95*, 9654−9659.
(97) Krawitz, P. M., Hochsmann, B., Murakami, Y., Teubner, B., Kruger, U., Klopocki, E., Neitzel, H., Hoellein, A., Schneider, C., Parkhomchuk, D., Hecht, J., Robinson, P. N., Mundlos, S., Kinoshita, T., and Schrezenmeier, H. (2013) A case of paroxysmal nocturnal hemoglobinuria caused by a germline mutation and a somatic mutation in PIGT. *Blood 122*, 1312−1315.
(98) Maxwell, D. M. (1992) The specificity of carboxylesterase protection against the toxicity of organophosphorus compounds. *Toxicol. Appl. Pharmacol. 114*, 306−312.

DOI: 10.1021/acs.chemrestox.6b00228
Chem. Res. Toxicol. 2016, 29, 1381−1392