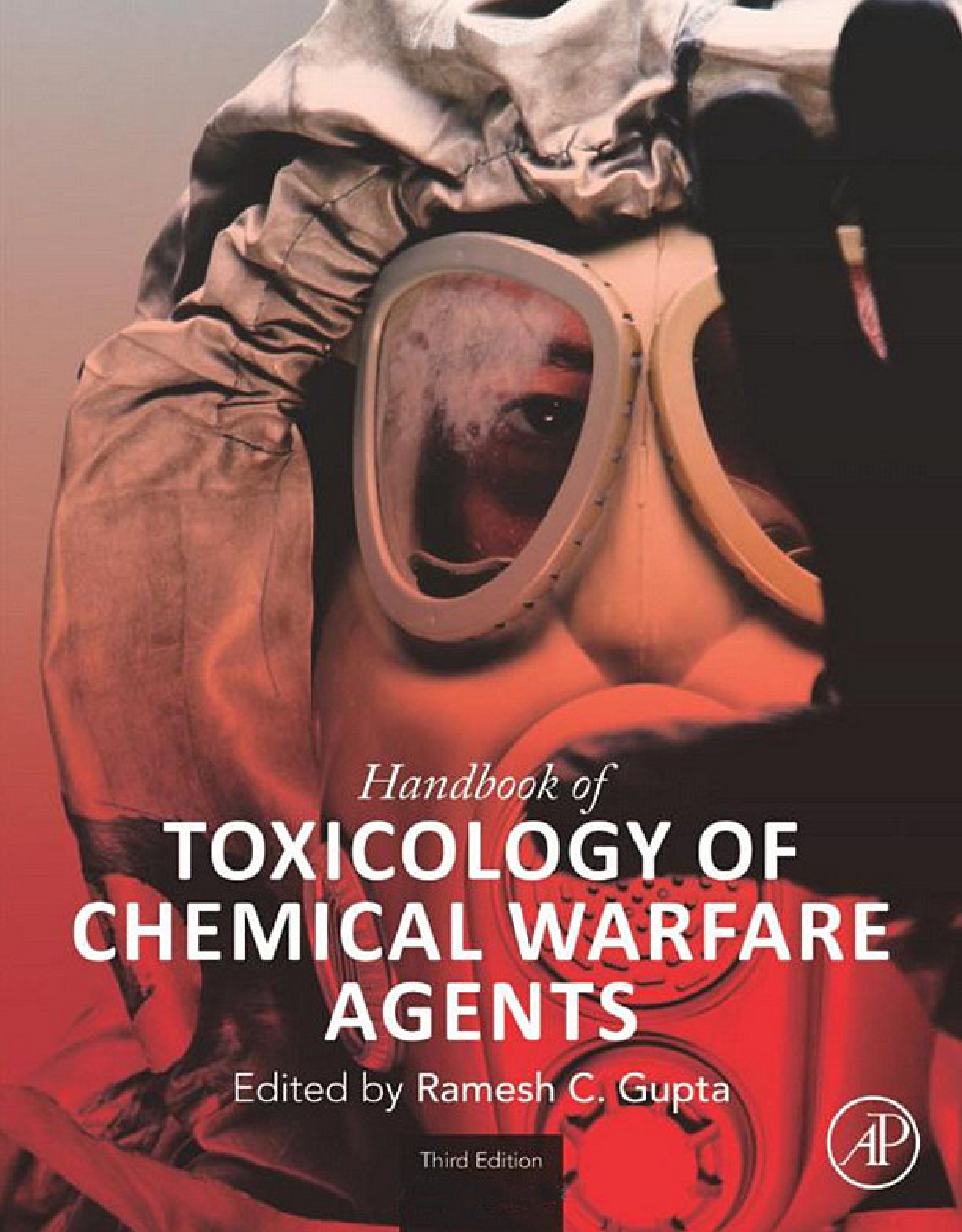

Handbook of

TOXICOLOGY OF CHEMICAL WARFARE AGENTS

Edited by Ramesh C. Gupta

Third Edition

Handbook of Toxicology of Chemical Warfare Agents

THE UNCERTAINTY OF THE DANGER BELONGS TO THE ESSENCE OF TERRORISM

Jurgen Habermas (1929—Present)

Handbook of Toxicology of Chemical Warfare Agents

Third Edition

Edited by

Ramesh C. Gupta, DVM, MVSc, PHD, DABT, FACT, FACN, FATS
Professor and Head, Toxicology Department, Breathitt Veterinary Center,
Murray State University, Hopkinsville, KY, United States

ACADEMIC PRESS
An imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-819090-6

For Information on all Academic Press publications
visit our website at https://www.elsevier.com/books-and-journals

Publisher: Andre Gerhard Wolff
Acquisitions Editor: Kattie Washington
Senior Editorial Project Manager: Kristi Anderson
Senior Production Project Manager: Kiruthika Govindaraju
Cover Designer: Christian Bilbow

Typeset by MPS Limited, Chennai, India

Dedication

This book is dedicated to my beloved wife Denise, daughter Rekha, and parents, the late Chandra and Triveni Gupta.

Contents

List of contributors

Arturo Anadón Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain

Rudolf Andrys University of Hradec Kralove, Faculty of Science, Department of Chemistry, Rokitanskeho 62, 500 03 Hradec Kralove, Czech Republic

Pavel Avdonin Koltzov Institute of Developmental Biology, Russian Academy of Sciences, Moscow, Russia

Jiri Bajgar Department of Chemistry, Faculty of Science, University of Hradec Kralove, Hradec Kralove, Czech Republic; Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic; University Hospital Hradec Kralove, Sokolska, Czech Republic

Mahdi Balali-Mood Medical Toxicology Research Centre; Department of Clinical Toxicology; Imam Reza Hospital, Faculty of Medicine Mashhad University of Medical Sciences, Mashhad, IR, Iran

Frank Balszuweit Bundeswehr Medical Service Headquarters, Koblenz, Germany

Atrayee Banerjee Senior Toxicologist, Reckitt Benckiser, United States

Cheryl B. Bast Keller and Heckman LLP, Washington, DC, United States

Daria Belinskaia Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Saint Petersburg, Russia

Vijay K. Bharti Defence Institute of High Altitude Research (DIHAR), DRDO, Ministry of Defence, Leh-Ladakh, India

Claire E. Bollinger College of Health & Rehabilitative Sciences, The Ohio State University, Columbus, OH, United States

Robert P. Casillas Latham BioPharm Group, Cambridge, MA, United States

Ryan Clark *Office for Educational Outreach and Health Careers*, Morehouse School of Medicine, Atlanta, GA, United States

Edward D. Clarkson Medical Toxicology Branch, Analytical Toxicology Division, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Grounds - Edgewood Area, MD, United States

Toby B. Cole Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, United States; Center on Human Development and Disability, University of Washington, Seattle, WA, United States

Rhian B. Cope Australian Pesticides and Veterinary Medicines Authority, Canberra, Australia

Robert W. Coppock Toxicologist and Associates, Ltd, Vegreville, AB, Canada

Lucio G. Costa Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, United States; Department of Medicine and Surgery, University of Parma, Parma, Italy

Wolf-D. Dettbarn Vanderbilt University, Nashville, TN, United States

Alzbeta Dlabkova Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic

Robin B. Doss Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, United States

Margitta M. Dziwenka Health Sciences Laboratory Animal Services (HSLAS), Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada; ToxAlta Consulting, Vegreville, AB, Canada

Jorge Estevez University Miguel Hernandez of Elche, Elche, Spain

Tim J. Evans Veterinary Medical Diagnostic Laboratory, Department of Veterinary Pathobiology, University of Missouri, Columbia, MO, United States

John K. Fink Department of Neurology, University of Michigan, Ann Arbor, MI, United States

Swaran J.S. Flora National Institute of Pharmaceutical Education and Research-Raebareli, Transit Campus, Lucknow, India

Clement E. Furlong Department of Medicine (Division of Medical Genetics), University of Washington, Seattle, WA, United States; Department of Genome Sciences, University of Washington, Seattle, WA, United States

Josef Fusek Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic; Faculty of Health Studies, University of Pardubice, Pardubice, Czech Republic

Jacqueline Garrick Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, WA, United States

Jeffery M. Gearhart The Henry M. Jackson Foundation for Military Medicine, 711th HPW, Airman Readiness Optimization Branch, Wright-Patterson AFB, Dayton, OH, United States

Donald R. Gerecke Department of Pharmacology and Toxicology, Rutgers University, Piscataway, NJ, United States

Dana F. Glass-Mattie Oak Ridge National Laboratory, Oak Ridge, TN, United States

Saryu Goel Expert Consultants, Leesburg, VA, United States

Nikolay Goncharov Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint Petersburg, Russia; Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Saint Petersburg, Russia

Richard K. Gordon US Army Medical Research and Material Command, Ft. Detrick, MD, United States

Joshua P. Gray Department of Science. US Coast Guard Academy, New London, CT, United States

Zoran Grubic Laboratory for Molecular Neurobiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Kavita Gulati Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, New Delhi, India

Ramesh C. Gupta Toxicology Department, Breathitt Veterinary Center, Murray State University, Hopkinsville, KY, United States; Division of Pharmacology & Toxicology, IVRI, Bareilly, Uttar Pradesh, India

Sharon M. Gwaltney-Brant Veterinary Information Network, Davis, CA, United States

Tracey A. Hamilton Department of Toxicology, US Army Medical Research Institute of Chemical Defense, MD, United States

Kenneth J. Harris Department of Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN, United States

Darryl B. Hood Division of Environmental Health Sciences, College of Public Health, The Ohio State University, Columbus, OH, United States

Edward M. Jakubowski Toxicology and Obscurants Division, Combat Capabilities Development Command-Chemical Biological Center, Aberdeen Proving Ground, MD, United States

Richard Jenkins School of Allied Health Sciences, De Montfort University, Leicester, United Kingdom

David A. Jett National Institutes of Health/National Institute of Neurological Disorders and Stroke, Rockville, MD, United States

Yuqin Jiao Department of Neuroscience, Colorado State University, Fort Collins, CO, United States

Harald John Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany

Nathan H. Johnson Department of Laboratory Sciences, University of Arkansas for Medical Sciences, AR, United States

Milan Jokanović Experta Consulting, Belgrade, Serbia

Daniel Jun Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic; Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defense in Brno, Trebesska, Czech Republic; University Hospital Hradec Kralove, Sokolska, Czech Republic

Georgy Karakashev Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint Petersburg, Russia

Jiri Kassa Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic; Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defense in Brno, Trebesska, Czech Republic

Maja Katalinic Institute for Medical Research and Occupational Health, Zagreb, Croatia

Kai Kehe Bundeswehr Medical Service Academy, Division F, Medical CBRN Defense, Munich, Germany

Natalia Khlebnikova Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint Petersburg, Russia

Urmila P. Kodavanti Public Health and Integrated Toxicology Division, Center for Public Health and Environmental Assessment, US Environmental Protection Agency, Research Triangle Park, NC, United States

Ekaterina Korf Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Saint Petersburg, Russia

Nadezhda Koryagina Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint Petersburg, Russia

Bojan Kovač Military Medical Academy, Belgrade, Serbia

Gopala Krishna Expert Consultants, Ellicott City, MD, United States

Mayur Krishna Expert Consultants, Ellicott City, MD, United States

Kamil Kuca Philosophical Faculty, University of Hradec Kralove, Hradec Kralove, Czech Republic; Department of Chemistry, Faculty of Science, University of Hradec Kralove, Hradec Kralove, Czech Republic

Dinesh Kumar FJD-R, IVRI, Rajdhani Enclave, Dehradun, Uttarakhand, India

Yukio Kuroiwa Japan Poison Information Centre, Tsukuba, Japan

Sergey Kuznetsov Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Saint Petersburg, Russia

Kamil Kuča University Hospital Hradec Kralove, Sokolska, Czech Republic; Department of Chemistry, Faculty of Science, University of Hradec Kralove, Rokitanskeho, Czech Republic; Faculty of Science and Philosophical, University of Hradec Kralove, Hradec Kralove, Czech Republic

Joseph C. Larsen Strategic Portfolio Development, VenatoRx Pharmaceuticals, PA, United States

Mikhail Leninskiy Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint Petersburg, Russia

Jing Liu Preclinical Safety Assessment, Charles River Laboratories, Inc. Reno, NV, United States; Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, United State

Heather Lochotzki Division of Environmental Health Sciences, College of Public Health, The Ohio State University, Columbus, OH, United States

Oksana Lockridge University of Nebraska Medical Center, Eppley Institute, Omaha, NE, United States

Jordana Lockwich Department of Physical Therapy, University of Evansville, IN, United States

Bommanna G. Loganathan Department of Chemistry and Watershed Studies Institute, Murray State University, Murray, KY, United States

Dragana Lončar-Stojiljković Department of Anesthesiology and Intensive Care, Institute for Cardiovascular Diseases, Dedinje, Belgrade, Serbia

Sofya V. Lushchekina Emanuel Institute of Biochemical Physics of Russian Academy of Sciences, Moscow, Russian Federation

Megan E. Lyman Department of Toxicology, US Army Medical Research Institute of Chemical Defense, MD, United States

Mark Maguire Department of Neuroscience and Pharmacology, Meharry Medical School, Nashville, TN, United States

Galina F. Makhaeva Institute of Physiologically Active Compounds, Russian Academy of Sciences, Chernogolovka, Russia

Jitendra K. Malik FJD-R, IVRI, Rajdhani Enclave, Dehradun, Uttarakhand, India

David Malinak University of Hradec Kralove, Faculty of Science, Department of Chemistry, Rokitanskeho 62, 500 03 Hradec Kralove, Czech Republic

Tomaz Mars Laboratory for Molecular Neurobiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Judit Marsillach Department of Medicine (Division of Medical Genetics), University of Washington, Seattle, WA, United States

María-Rosa Martínez-Larrañaga Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain

Patrick Masson Neuropharmacology Laboratory, Kazan Federal University, Kazan, Russian Federation

Shigeki Masunaga Yokohama National University, Yokohama, Japan

Monique McCallister Cooperative Extension Program, Tennessee State University, Nashville, TN, United States

Linda A. McCauley Nell Hodgson Woodruff School of Nursing, Emory University, Atlanta, GA, United States

Roger O. McClellan Toxicology and Human Health Risk Analysis, Albuquerque, NM, United States

Patrick M. McNutt Department of Toxicology, US Army Medical Research Institute of Chemical Defense, MD, United States

Edward C. Meek Center for Environmental Health Sciences, College of Veterinary Medicine, Mississippi State University, MS, United States

Elaine Merrill Air Force Research Laboratory, 711/HPW RHBBF, Wright-Patterson AFB, OH, United States

Sylvia Milanez Oak Ridge National Laboratory, Oak Ridge, TN, United States

Igor Mindukshev Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Saint Petersburg, Russia

Katarina Mis Laboratory for Molecular Neurobiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Jan Misik Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic; Biomedical Research Centre, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic

Michael J. Murphy University of Minnesota, St. Croix Trail N, Stillwater, MN, United States

Kamil Musilek University of Hradec Kralove, Faculty of Science, Department of Chemistry, Rokitanskeho 62, 500 03 Hradec Kralove, Czech Republic

Tamie Nakajima Department of Life and Health Sciences, Chubu University, Kasugai, Japan

Marian R. Nelson Department of Toxicology, US Army Medical Research Institute of Chemical Defense, MD, United States

Eugenie Nepovimova Department of Chemistry, Faculty of Science, University of Hradec Kralove, Hradec Kralove, Czech Republic

Tetsu Okumura Japan Poison Information Centre, Tsukuba, Japan

Jiri Patocka Department of Radiology and Toxicology, Faculty of Health and Social Studies, University of South Bohemia Ceske Budejovice, Hradec Kralove, Czech Republic

Katarina Pegan Laboratory for Molecular Neurobiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Jaroslav Pejchal Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

Sergej Pirkmajer Laboratory for Molecular Neurobiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

René Pita Chemical Defense Department, Army CBRN Defense School, Madrid, Spain

Jason Pitt Department of Physical Therapy, University of Evansville, Evansville, IN, United States

Yiuka Pitt Department of Anesthesia, Methodist Health, KY, United States

Gennady E. Platoff, Jr. National Institutes of Health/ National Institute of Allergy and Infectious Diseases, Rockville, MD, United States

Carey N. Pope Department of Physiological Sciences, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, United State

Andrey Radilov Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint Petersburg, Russia

Anu Rahal Division of Animal Health, ICAR-Central Institute for Research on Goats, Mathura, India

Aramandla Ramesh Department of Biochemistry and Cancer Biology, Meharry Medical College, Nashville, TN, United States

Arunabha Ray Department of Pharmacology, Hamdard Institute of Medical Sciences and Research (HIMSR), Hamdard University, New Delhi, India

Vladimir Rembovskiy Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint Petersburg, Russia

Raina Rhoades Department of Neuroscience and Pharmacology, Meharry Medical College, Nashville, TN, United States

Rudy J. Richardson Department of Environmental Health Sciences, Department of Neurology, Center of Computational Medicine and Bioinformatics, University of Michigan, Ann Arbor, MI, United States

Dragana Ristić Military Medical Academy, Belgrade, Serbia

Peter J. Robinson The Henry M. Jackson Foundation for Military Medicine, Air Force Research Laboratory, 711/HPW RHBBF, Wright-Patterson AFB, Dayton, OH, United States

Alejandro Romero Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain

Chris Ruark The Procter and Gamble Company, Mason, OH, United States

Harry Salem Columbus, OH, United States

Natalia Samchenko Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint Petersburg, Russia

Tetsuo Satoh Japan Poison Information Centre, Tsukuba, Japan

Russell E. Savage Toxicology Education Foundation, Cincinnati, OH, United States

Elena Savelieva Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint Petersburg, Russia

Lawrence M. Schopfer University of Nebraska Medical Center, Eppley Institute, Omaha, NE, United States

Alfred M. Sciuto (Retired), Biochemical and Physiology Branch, Analytical Toxicology Research Division, US Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, United States

Yasuo Seto RIKEN SPring-8 Center, Hyogo, Japan

Michael P. Shakarjian Environmental Health Science, New York Medical College, School of Health, Sciences and Practice, Valhalla, NY, United States

Vladimir Shmurak Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint Petersburg, Russia; Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, Saint Petersburg, Russia

Adam Skarka University of Hradec Kralove, Faculty of Science, Department of Chemistry, Rokitanskeho 62, 500 03 Hradec Kralove, Czech Republic

Ranko Škrbić Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

Miguel Sogorb University Miguel Hernandez of Elche, Elche, Spain

Shardell M. Spriggs National Institutes of Health/ National Institute of Neurological Disorders and Stroke, Rockville, MD, United States

Sakshi Srivastava National Institute of Pharmaceutical Education and Research-Raebareli, Transit Campus, Lucknow, India

Dirk Steinritz Bundeswehr Medical Service Academy, Division F, Medical CBRN Defense, Munich, Germany

Miloš P. Stojiljković Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

Neera Tewari-Singh Department of Pharmacology & Toxicology, Michigan State University, East Lansing, MI, United States

Horst Thiermann Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany

Suresh Kumar Thokchom Department of Pharmacology, Vallabhbhai Patel Chest Institute, University of Delhi, New Delhi, India

Larry J. Thompson Thompson Tox, Edwardsville, IL, United States

Anton Ukolov Research Institute of Hygiene, Occupational Pathology and Human Ecology, Saint Petersburg, Russia

Luis G. Valerio Toxicology Expert, Ellicott City, MD, United States

M.J. van der Schans TNO, Rijswijk, The Netherlands

Daya R. Varma Montreal, Canada

Eugenio Vilanova University Miguel Hernandez of Elche, Elche, Spain

Annetta Watson Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, United States

Sanjeeva J. Wijeyesakere Department of Environmental Health Sciences, Department of Microbiology and Immunology, University of Michigan, Ann Arbor, MI, United States

Tina Wismer ASPCA Animal Poison Control Center, Urbana, IL, United States

R. Mark Worden Department of Biomedical Engineering, Department of Chemical Engineering and Materials Science, Michigan State University, East Lansing, MI, United States

Franz Worek Bundeswehr Institute of Pharmacology and Toxicology, Munich, Germany

Linzzi K. Wright CCDC Chemical Biological Center, Aberdeen Proving Ground, MD, United States

Hidenori Yamasue Department of Psychiatry, Hamamatsu University School of Medicine, Hamamatsu city, Japan

Takemi Yoshida Japan Poison Information Centre, Tsukuba, Japan

Toshiharu Yoshioka Japan Poison Information Centre, Tsukuba, Japan

Robert A. Young Senior Research Staff (Retired), Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, United States

Snjezana Zaja-Milatovic PAREXEL International, Alexandria, VA, United States; PAREXEL International, Billerica, MA, United States

Valeriy Zinchenko Institute of Cell Biophysics, Russian Academy of Sciences, Pushchino, Russia

Csaba K. Zoltani Emeritus, ARL, Aberdeen Proving Ground, MD, United States

Introduction

Extremely toxic chemicals have been used for thousands of years in wars, conflicts, and extremist activities by terrorists and dictators in malicious poisonings and executions. One of the earliest forms of chemical warfare agents (CWAs) was natural toxins from plants or animals, which were used to coat arrowheads, commonly referred to as "arrow poisons." Ancient use of some CWAs and riot control agents (RCAs) dates back to the 5th century BCE during the Peloponnesian War, when the Spartans used smoke from burning coal, sulfur, and pitch to temporarily incapacitate and confuse occupants of Athenian strongholds. The Spartans also used bombs made of sulfur and pitch to overcome the enemy. The Romans used irritant clouds to drive out adversaries from hidden dwellings. In the 15th century, Leonardo da Vinci proposed the use of a powder of arsenic sulfide as a chemical weapon.

Modern use of CWAs and RCAs or incapacitating agents dates back to World War I (WWI). With advancements in science and chemistry in the 19th century, the possibility of chemical warfare increased tremendously. The first full-scale use of CWAs began in April 1915 when German troops launched a poison gas attack at Ypres, Belgium, using 168 tons of chlorine gas, killing about 5000 Allied (British, French, and Canadian) soldiers. During WWI, the deployment of CWAs, including toxic gases (chlorine, phosgene, cyanide, and mustard), irritants, and vesicants in massive quantities (about 125,000 tons), resulted in about 90,000 fatalities and 1.3 million nonfatal casualties. The majority of deaths in WWI were a result of exposure to chlorine and phosgene gases. During the Holocaust, the Nazis used carbon monoxide and the insecticide Zyklon-B, containing hydrogen cyanide, to kill several million people in extermination camps. Poison gases were also used during the Warsaw Ghetto Uprising in 1943. Again, in November 1978, religious cult leader Jim Jones murdered over 900 men, women, and children with cyanide.

Prior to, during, and after World War II, anticholinesterase organophosphate (OP) nerve agents/gases were developed in Germany, the United States, the United Kingdom, and Russia, and were produced in large volumes in many other countries. They were maximally produced and stockpiled during the "Cold War" period. These nerve agents have been used in wars and by dictators, extremists, cult leaders, and terrorist groups as chemical weapons of mass destruction (CWMD) on many occasions. In 1980, Iraq attacked Iran, employing mustard and OP nerve gases. During the period of the Iraq and Iran conflict (1980–88), Iran sustained 387 attacks and more than 100,000 troops were victims along with a large number of civilians. Thousands of these victims still suffer from long-term health effects. Shortly after the end of the Iraq–Iran war in 1988, the brutal dictator of the Iraqi regime, Saddam Hussein, used multiple CWAs against the Kurdish minorities in Halabja, killing more than 10% of the town's 50,000 residents. To date, mustards have been used in more than a dozen conflicts, killing and inflicting severe injuries in millions of military personnel and civilians.

During the Persian Gulf War, exposure to OP nerve agents occurred from the destruction of munitions containing 8.5 metric tons of sarin/cyclosarin housed in Bunker 73 at Khamisyah on March 4, 1991, and additional destruction of these nerve agents contained in rockets in a pit at Khamisyah on March 10, 1991. Although exposure levels to nerve agents were too low to produce signs of acute toxicity, military personnel serving in and around the Khamisyah area still suffer from long-term adverse health effects, most notably "Gulf War syndrome." In 1996, about 60,000 veterans of the Persian Gulf War claimed to suffer from "Gulf War syndrome" or "Gulf veterans' illnesses," possibly due to low-level exposure of nerve agents, mustard, pyridostigmine bromide, and pesticides. Exposed veterans had an increased incidence of chronic myelocytic leukemia and increased risk of brain cancer deaths compared to unexposed personnel.

In the mid-1990s, two terrorist attacks by a fanatic religious cult, Aum Shinrikyo (Supreme Truth), known as Aleph since 2000, took place in Japan (Matsumoto, 1994 and Tokyo subway, 1995). In both incidents, the OP nerve agent sarin was used as a CWA. Aum Shinrikyo in Kamikuishiki, Japan, manufactured an estimated 70 tons of sarin. Although the total fatality count involved not more than 20 civilians, injuries were observed in more than 6000 and millions were terrified. These acts of chemical terrorism were unprecedented and the impact propagated throughout not only Japan, but the entire world. In the past few decades, many incidents have also occurred with biotoxins such as ricin and anthrax. Publicity surrounding frequent recent use due to easy access and copycat crimes increase the possibility of future use of these chemicals and biotoxins, which warrants advancement in emergency preparedness planning at the federal, state, and local government levels.

It is interesting to note that toxic chemicals have been used by governmental authorities against rebels, or civilians. In the 1920s, Britain used chemical weapons in Iraq "as an experiment" against Kurdish rebels seeking independence. Winston Churchill strongly justified the use of "poisoned gas against uncivilized tribes." The Russian OSNAZ forces used an aerosol containing fentanyl anesthetic during the Moscow theater hostage crisis in 2002. RCAs were frequently used in the US in the 1960s to disperse crowds in riot control.

Intoxications or deaths by poisoning of emperors, heads of countries, and other significant individuals have been recorded for a long time. The French Emperor Napoleon Bonaparte was poisoned with a mixture of heavy metals including arsenic and mercury. Napoleon Bonaparte died on May 5, 1821, while he was in exile on the island of St. Helena. In December 2004, during the presidential campaign, the former President of Ukraine, Viktor Yuschenko, was poisoned by a very high dose of 2,3,7,8-tetrachlorodibenzodioxin (TCDD). Ex-lieutenant of the Russian Federal Service, Alexander Litvinenko (1962–2006) died on November 23, 2006, from intoxication with polonium 210. Kim Jong-Nam, a half-brother of North Korean dictator Kim Jong-Un was poisoned with VX nerve agent at Kuala Lumpur airport in Malaysia. He died within 20 min of exposure. On March 4, 2018, the former officer of the Russian Main Intelligence Directorate, Sergey Skripal, and his daughter, Yuliya Skripal, were poisoned with Novichoks in Salisbury, United Kingdom. Following an aggressive antidotal therapy, fortunately both survived.

At present, more than 25 countries and possibly many terrorist groups possess CWAs, while many other countries and terrorist groups are seeking to obtain them, due to their easy access. Some of these agents are stockpiled in enormous quantities and their destruction and remediation are not only expensive but also associated with significant health risks. There is also the possibility of accidental release of CWAs or CWMD at their production sites, as well as during transportation, dissemination, and deployment. The intentional or accidental release of highly toxic chemicals, such as the nerve agent VX (Dugway Proving Ground, Utah, 1968), Agent Orange (Vietnam, 1961–72), PBB (Michigan, USA, 1973), dioxin (Seveso, Italy, 1976), and methyl isocyanate (Bhopal, India, 1984), has caused injuries to more than a million people, and deaths in several thousands. A 1968 accident with VX nerve gas killed more than 6000 sheep in the Skull Valley area of Utah.

After September 11, 2001, the chances are greater than ever before of the use of CWMD by extremist and terrorist groups like Al Qaeda, which presents great risks to humans, domestic animals, and wildlife in many parts of the world. On November 26, 2008, Pakistani Islamic terrorists attacked Mumbai city in India at 10 different sites, including two luxury hotels, a Jewish center, a train station, and hospitals and cafes. Approximately 200 innocent people died and about 300 people were injured by bullets and smoke. It is more likely that these terrorist groups may use toxic industrial chemicals (agents of opportunity) either as such or as a precursor for more deadly CWMD. At present, many countries have established Defense Research Institutes with two major missions: (1) to understand the toxicity profile of CWAs/CWMDs and (2) to develop strategic plans for prophylactic and therapeutic countermeasures. By the turn of the 21st century, the US established the Department of Homeland Security. Many other countries also developed similar governing branches and agencies at the state and national levels to protect people and property from terrorist attacks. Among chemical, biological, and radiological weapons, the possibility of CWMD is more likely because of their easy access and delivery system. It is important to mention that understanding the toxicity profile of CWAs/CWMD is very complex, as these chemical compounds are of a diverse nature, and, as a result, treatment becomes very difficult or in some cases impossible.

In the past, many accords, agreements, declarations, documents, protocols, and treaties have been signed at the international level to prohibit the development, production, stockpiling, deployment, and use of CWAs, yet dictators and terrorists produce and/or procure these chemicals to harm or kill enemies, create havoc, and draw national and international attention. In 1907, The Hague Convention outlawed the use of chemical weapons, yet during WWI, many countries used these chemicals. The first international accord on the banning of chemical warfare was agreed upon in Geneva in 1925. Despite the General Protocol, the Japanese used chemical warfare against China in 1930. In 1933, the Chemical Weapon Convention banned the development, possession, and use of CWAs. The document was signed and implemented by more than 100 countries. Yet, during WWI many chemicals of warfare were developed, produced, and used by several countries. In 1993, another global convention banning the production and stockpiling of CWAs was signed by over 100 countries.

The delayed health effects from CWAs used in the Iraq–Iran conflict of the 1980s, sarin subway attacks in Japan, and the First Gulf War in the 1990s are still not well understood. Recently, the Syrian government stockpiled over 1300 metric tons of chemical agents, including sarin, VX, and sulfur mustard. In August 2013, the Syrian military repeatedly attacked civilians with chemical weapons, including sarin and chlorine. More than 1300 people died and thousands were injured. Again, on April 11−13, 2014, Syrian military forces attacked civilians in Hama province with chlorine gas, killing and injuring an unaccounted number of people. Despite warnings from many countries, the Syrian army continues to use CWAs against civilians. In the present world situation, it is highly likely that these agents will be used in wars, conflicts, terrorist attacks, and with malicious intent. In such scenarios, these extremely toxic agents continuously pose serious threats to humans, animals, and wildlife.

The first edition of this *Handbook of Toxicology of Chemical Warfare Agents* was prepared in 2009 in order to offer the most comprehensive coverage of every aspect of the deadly toxic chemicals that can be used as CWAs/CWMD. Since the publication of the first edition of this *Handbook*, concerns over the use and misuse of CWAs and BWAs have become greater than ever before. The second edition of the *Handbook of Toxicology of Chemical Warfare Agents* was published in 2015. This third edition of this *Handbook* is prepared to meet the current challenges facing academicians and lay persons alike. The format employed is user friendly and easy to understand. Standalone chapters on individual chemical and a few biological agents, target organ toxicity, biosensors and biomarkers, risks to man, animals, and wildlife, and prophylactic and therapeutic countermeasures are just a few of the many novel topics covered in this volume. The chapters are enriched with historical background as well as the latest information and up-to-date references. With 73 chapters, this book will serve as a reference source for biologists, toxicologists, pharmacologists, forensic scientists, analytical chemists, local/state/federal officials in the Department of Homeland Security, Department of Defense, Defense Research Establishments, Department of Veterans Affairs, physicians at medical and veterinary emergency care units of hospitals, poison control centers, medical and veterinary diagnostic labs, environmentalists and wildlife interest groups, researchers in the area of nuclear, chemical, and biological warfare agents, and college and university libraries.

Contributors of the chapters in this book are the most qualified scientists in their particular areas of chemical and biological warfare agents and radiation. These scientists are from around the globe and are regarded as authorities in the fields of pharmacology, toxicology, and military medicine. The editor sincerely appreciates each author for his/her dedicated hard work and invaluable contributions to this volume. The editor gratefully acknowledges Robin B. Doss and Denise M. Gupta for their technical assistance. Finally, the editor remains indebted to the editors at Elsevier (Kristi Anderson, Kattie Washington, and Kiruthika Govindaraju) for their immense contributions to this book.

Ramesh C. Gupta

Toxicology Department, Breathitt Veterinary Center,
Murray State University, Hopkinsville, KY, United States

Chapter 1

History of toxicology: from killers to healers

Eugenie Nepovimova[1] and Kamil Kuca[2]

[1]Department of Chemistry, Faculty of Science, University of Hradec Kralove, Hradec Kralove, Czech Republic, [2]Philosophical Faculty, University of Hradec Kralove, Hradec Kralove, Czech Republic

1.1 Introduction

The word *toxicology* comes from the Greek words *toxicon*, meaning a poison, and *logos*, meaning a scientific study. Today, the term *toxicology* refers to a scientific discipline dealing with the physical-chemical properties of toxic substances, their mechanisms of action on the body, clinical symptoms of intoxications, and the prevention and treatment of various poisonings (Klaassen, 2018). It would not be an exaggeration to claim that toxicology is almost as old as mankind. The earliest mentions of toxic substances and intoxications can be found not only in ancient scientific literature, but also in Greek myths. For example, Homer describes how Odysseus sent a warrior to Egypt to bring back traditional Egyptian poisons used for arms ammunition. In another legend, Hercules soaked his weapons in poison of the sacred Lernean hydra. Last, but not least, the myth of Helen of Troy tells how her captor died because of a wound caused by a poisoned arrow.

1.2 Ancient times

Among the oldest literary sources focused on toxicology is the Ebers Papyrus (1550 BCE) (Fig. 1.1), found in 1872 in Thebes. This 20.5 m long scroll, glued from 108 smaller sheets of papyrus, is also called "The book of preparation of remedies for all parts of the body." The oldest pharmacopoeia of the ancient Egyptians contains more than 900 prescriptions for drugs for the treatment of diseases associated with the gastrointestinal tract, respiratory tract, ear, throat, nose, eye, and skin. Such prescriptions involved substances like opium, arsenic trioxide, aconitine, cyanogenic glycosides, or a herb called Dia-Dia, currently known as mandrake (*Mandragora officinalis*, *Solanaceae*) (Hallmann-Mikołajczak, 2004). Ancient Egyptian surgeons used the juice of mandrake root for anesthesia and analgesia. Later, the art of the preparation of hypnotic and painkilling remedies isolated from the mandrake root transferred from Egypt to ancient Greece. During surgical operations, the Greeks used a sponge soaked in mandrake hot juice for anesthesia. Inhalation of the vapors of this juice resulted in a deep sleep of the patient. In the works of the Roman physician Galen, we can find passages telling about large quantities of mandrake tincture that were delivered daily to Rome. Apart from the medicinal use of mandrake, one of the Roman writers mentions mandrake wine used for war purposes, thanks to which the Carthaginians defeated the enemy. The soldiers of ancient Carthage left their camp with the mandrake wine in a conspicuous place. After returning back to the camp, they thereafter easily overpowered their sleeping enemies (Emboden, 1989; Mion, 2017).

Probably the most famous poisoning of the Hellenistic period was the execution of the Greek philosopher Socrates (470−399 BCE), who was condemned to drink the extract from hemlock (*Conium maculatum*, *Apiaceae*). His death is depicted in detail in Plato's tract *Phaedo* (Hotti and Rischer, 2017; Nepovimova and Kuca, 2019). The description of poisoning corresponds exactly to the present knowledge of coniine, the main component of hemlock.

The period of ancient Greece in world history is known for the flourishing of various scientific disciplines, including medicine. The founder of the most famous school of medicine, that was located in the Greek town of Kos, was the so-called "Father of Medicine" Hippocrates

Handbook of Toxicology of Chemical Warfare Agents. DOI: https://doi.org/10.1016/B978-0-12-819090-6.00001-5

FIGURE 1.1 Ebers Papyrus found in 1872 in Thebes (https://commons.wikimedia.org/wiki/File:A_page_from_the_Ebers_Papyrus,_written_circa_1500_B.C._Wellcome_M0008455.jpg).

(460–370 BCE) (Fig. 1.2). Hippocrates rejected using poisons for removing unwanted persons. Therefore in his works, toxic substances are rarely mentioned. Some of his disciples, such as Pliny or Galen, followed the same principle, describing in their works only the antidotes. Such an informal rule has been preserved until the modern era, when young doctors, by taking the Hippocratic Oath, promise neither to administer a poison to anybody when asked to do so, nor to suggest such a course (Emery, 2013).

The ancient scientists abounded in a deep knowledge of various poisons. Usually they gained such knowledge from the observation of accidental poisonings, as well as from intentional exposure to poisons. In contrast to Eastern countries, in ancient Greece and subsequently Rome, toxic substances were quite often used as a means of killing convicts. Thus the ancient Greek poet and physician Nikander of Kolophon in his poem *Theriaca* describes clinical symptoms of intoxications by various animal toxins. In Nikander's further work *Alexipharmaca* that has survived to the present time, we find descriptions of the characteristics of plant poisons as well as methods for their treatment. As very effective therapeutic approaches he recommended invoking vomiting by drinking warm flaxseed oil or irritating the throat by simple devices made from paper or bird feathers. The majority of the knowledge reported by Nikander was based on his own experiments on convicted criminals. In addition,

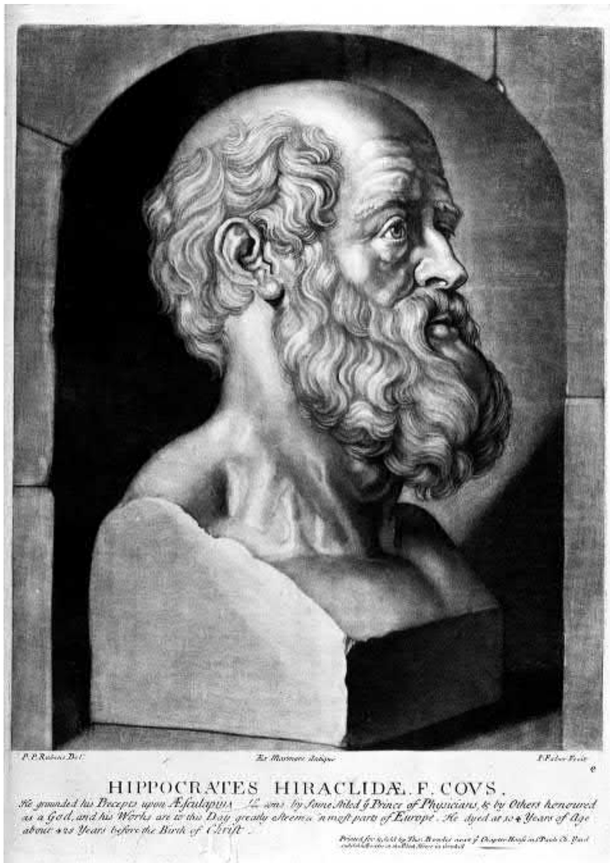

FIGURE 1.2 Father of Medicine, Hippocrates (https://commons.wikimedia.org/wiki/File:A_marble_bust_said_to_represent_Hippocrates_by_J._Faber._Wellcome_M0017663.jpg).

Nikander was by all accounts the first to describe the signs of lead poisoning (Clauss, 2012). In ancient Rome, lead was widely used in everyday life. For example, lead plates were added to wine to improve its quality. At that time, lead was very expensive and only rich people could afford it. Therefore it is not surprising that chronic lead poisoning became a scourge of the ancient Roman aristocracy (Hernberg, 2000).

From the point of view of the history of toxicology as a medical discipline, not only were poisoners and crimes committed by means of poisons important, but also searching for potent antidotes. Especially in ancient times, there was an obsession to discover a universal antidote, able to protect against most, if not all, poisons. Quite instructive is the story of King Mithridates VI of Pontus (132–63 BCE) (Fig. 1.3). Mithridates was terribly afraid of poisons, therefore, he began to study toxicology in depth—he observed the effects of various poisons on people (mostly convicts or slaves), designed antidotes, and subsequently tested their efficacy on the same groups of people. Finally, he managed to prepare a universal antidote consisting of 36 components. Such

FIGURE 1.3 King Mithridates VI of Pontus (https://commons.wikimedia.org/wiki/File:Mithridates_VI_Louvre_white_background.jpg).

an antidote even received a special designation in the Roman Pharmacopoeia—*mithridaticum.* The reputation of this antidote was excellent. It was even considered the best antidote of those times, capable of preventing the actions of aconitine, snake, scorpion, or spider toxins, etc. King Mithridates believed in his recipe so much that he decided to take this remedy daily. Acquired resistance, however, played a crucial role in his life. In old age, Mithridates attempted to commit suicide by taking a large dose of poison, but survived. Therefore he was forced to use other means (a sword) to finish this act. Based on this legend, the term *mithridatism* has been adopted into the modern toxicology indicating the increased resistance of an individual to poisons (Griffin, 1995; Valle et al., 2012, 2009).

The last wife of the Roman Emperor Claudius (10 BCE−CE 54) was his niece Agrippina the Younger (CE 15−59) (Fig. 1.4). Soon after their marriage, she decided to get rid of her husband as well as his first-born son Britannicus to make her own son, Nero, the emperor (Aveline, 2004). First, she poisoned Claudius using the toxin muscarine present in toxic mushrooms, fly agaric (*Amanita muscaria*, *Amanitaceae*), in one of his meals. The Emperor's physician, Aesculapius, tried to evoke vomiting in Claudius. However, Agrippina foresaw such a turn of events and had prepared in advance a poisoned feather. This feather was in all probability the product of the famous ancient poisoner—Locusta (Marmion and Wiedemann, 2002). After Claudius' death, Nero (CE 37−68) (Fig. 1.4) became the Emperor of Rome. Despite this, Nero's stepbrother Britannicus still constituted a threat to him (Shotter, 2008). Similarly to his mother Agrippina, Nero also asked for the help of the poisoner Locusta. In this case, she gave him a poison that was added to Britannicus' wine. After removal of his competitor, 17-year-old Nero became the only possible Emperor of Rome. Thus he decided to reward Locusta by an extraordinary right—to educate her own students. This story was one of many examples in world history where poisons were used for criminal purposes. Therefore in 81 BCE, the Roman dictator Sulla was forced to pass a special law ordering punishment, including the death penalty, for those who used poisonous substances with criminal intent (Telford, 2014).

Dioscorides (CE 40−90), the physician of the Roman Emperor Nero, in his tract *De Materia Medica* (Fig. 1.5) classified poisons based on their origin to plant, animal, and mineral. Additionally, in *De Materia Medica* we may find the methods of identification of several poisons. Such identification occurred in the scientific literature for the first time. For the next 15 centuries, Dioscorides' work was considered the "Holy Bible" of toxicology (Staub et al., 2016).

1.3 The Middle Ages

In medieval Europe, poisons were freely available in pharmacies. The first attempt to stop such a trade in poisons was made in Italy. In 1365 in Siena, apothecaries were forbidden from selling arsenic and mercury to people unknown to them. In France, a ban on toxic substances was issued in 1662, whereas in Russia this took place only in 1773 (Nepovimova and Kuca, 2019). Despite these restrictions, the question of searching for novel more potent poisons and corresponding antidotes remained relevant.

In Europe, the search for novel antidotes as well as their use in the prevention and treatment of poisoning continued until the beginning of the 18th century, and in Turkey until the beginning of the 20th century. The ancient works of Galen *De Theriaca, ad Pisonem*, *De Usu Thericae, ad Pamphilianum*, and *De Antidotis*, mainly inspired by the achievements of King Mithridates, were within the period of the Renaissance and the Middle Ages enriched with the knowledge of the Jewish physician and philosopher Moses Maimonides (1135−1204) (Fig. 1.6). His tract focused on poisons and antidotes and was published in Arabic in Cordoba (Spain) in 1198 (Rosner, 2000). This literary work constitutes a noticeable milestone in the history of toxicology, outlining the 1000-year

FIGURE 1.4 Agrippina the Younger and her son Nero (https://commons.wikimedia.org/wiki/File:Ner%C3%B3n_y_Agripina.jpg).

experience of treating various poisonings and also described the clinical picture of intoxication by poisons that were previously unknown. The first part of the tract describes intoxications as well as poisons of animal origin (bites by enraged dogs, wasps, snakes, spiders, scorpions, and other animals). As historically the first attempt, Maimonides distinguished the neurotoxic and hematotoxic symptoms of intoxication. In the second part, he focused on mineral and plant poisons. For instance, in the case of *Atropa belladonna* (*Solanaceae*) intoxication, Maimonides reported skin redness and some kind of "excitement" of the patient. As a therapeutic tool he recommended vomiting evoked by warm milk, vegetable oil, etc. (Rosner, 1968).

None of the noble families left such a significant imprint in the history of Italy and the whole world as the Spanish "holy family" of Borgia that was sadly famous for numerous murders committed by means of poisons. These Spaniards twice occupied the throne of Saint Peter—firstly as Pope Callixtus III and subsequently as Pope Alexander VI (Hibbert, 2009). "Cantarella" was the name of the poison used by the Borgias. Allegedly, Cesare Borgia (1474–1507) (Fig. 1.7), the son of the Pope Alexander VI (1431–1503) (Fig. 1.7), received the recipe for this poison from his mother. Apparently, the mystic poison contained arsenic, salts of copper, and phosphorus. According to the literary sources, the papal alchemists prepared such toxic mixtures that a drop was enough to kill a bull. Not only the poison, but also the tools containing such poison, were unique. Cesare Borgia was the owner of a ring with a huge ruby that bore the name the "Flame of Borgia." Several times he pronounced that this ring had repeatedly saved his life. Presumably, under the gemstone there was a skillfully made secret container with a poison. Cesare poured this poison into the drink of those who dared to encroach on

FIGURE 1.5 A page from Dioscorides' work *De Materia Medica* (https://en.wikipedia.org/wiki/De_Materia_Medica#/media/File:NaplesDioscuridesMandrake.jpg).

his life. The Pope himself also had a gold ring with a secret. In the process of shaking hands, a small thorn appeared on the inner side of the ring which slightly scratched the skin of the sentenced person and released a deadly drop of poison (Poole, 2010). Finally, destiny punished Pope Alexander VI, who accidentally drank poisoned wine that was intended for his victim (Hibbert, 2009).

Despite the wide use of poisons within the struggle for power, the development of toxicology in European countries in the Middle Ages was significantly hampered by the influence of religious ideologies. Medieval monks followed the principle "Like is cured by like" (*Similia similibus curantur*) (Zebroski, 2015). The exception was Swiss physician, alchemist, botanist, astrologer, and occultist of the era of the German Renaissance, Philippus Aureolus Theophrastus Bombastus von Hohenheim (1492–1541), also known as Paracelsus (Fig. 1.8). He chose this pseudonym for himself and it means "more than Celsus." Aulus Cornelius Celsus was a Roman naturalist, living more than one and half thousand years before Paracelsus (Grell, 1998). Paracelsus' groundbreaking contribution to life sciences consisted mainly in the interconnection between chemistry and medicine. Therefore it is not surprising that his life credo was: "The real purpose of chemistry is not to make gold, but to make remedies!" Paracelsus has been also considered the Father of Toxicology, since in one of his books he stated: "Dose makes the poison" (*Dosis facit venenum*). Thus substances that are taken to be toxic could be harmless in small doses, whereas normally harmless substances could be fatal if consumed excessively. This postulate still belongs among the basic pillars of modern toxicology. He was also known for his revolutionary views on the observation of nature and man, created by himself instead of simply quoting ancient texts. Last but not least, he gave the designation to the chemical element zinc and noted that certain diseases stem from the mind of the patient (Paracelsus, 1999).

With regard to poisonings, medieval Italy and later France were considered the most powerful countries in the world. The French Queen, Catherine de' Medici (1519−89) (Fig. 1.9), also known as the Queen-Poisoner, perfectly mastered the Italian technique of poisoning to achieve her intended political goals (Kruse, 2003). Alexandre Dumas, in his historical novel "Queen Margot," wrote that Queen Catherine was involved in the death of her political rival Jeanne d'Albret by giving her an insidious present—poisoned gloves (Dumas, 1994). Within the same novel, Dumas also describes the fatal mistake of the Queen, who at the end of her life decided to remove the son of poisoned Jeanne d'Albret—Henry. She commanded he be given a poisoned book dealing with the art of hunting. Unfortunately, this book got into the wrong hands, to her own son King Charles IX. Apart from removing the competitors within the battle for the royal throne, Queen Catherine was also known for experiments with various toxic mixtures that she conducted on poor and sick people. Catherine de' Medici carefully reported each experiment, recording the velocity of the toxic response (onset of the

FIGURE 1.6 Jewish physician and philosopher Moses Maimonides (https://he.wikipedia.org/wiki/%D7%A7%D7%95%D7%91%D7%A5:Maimonides-2.jpg).

FIGURE 1.8 Philippus Aureolus Theophrastus Bombastus von Hohenheim, also known as Paracelsus (https://commons.wikimedia.org/wiki/File:Aureolus_Theophrastus_Bombastus_von_Hohenheim_(Paracelsus)._Wellcome_V0004452.jpg).

FIGURE 1.7 The Pope Alexander VI (right) and his son Cesare Borgia (left) (https://www.flickr.com/photos/hinkelstone/38111166521; https://www.flickr.com/photos/eriktorner/32671151065).

FIGURE 1.9 French Queen Catherine de' Medici (https://commons.wikimedia.org/wiki/File:Catherine-de-medici.jpg).

FIGURE 1.10 French poisoner Catherine Monvoisin (https://fr.wikipedia.org/wiki/Fichier:Catherine_Deshayes_(Monvoisin,_dite_%C2%ABLa_Voisin%C2%BB)_1680.jpg).

toxic effect), the efficacy of the toxic mixture, the strength of the toxic effect in various parts of the body (organ specificity, site of action), and the clinical picture of intoxication. Thus despite the poor reputation of the Queen-Poisoner, she can be considered the first experimental toxicologist in history (Whyte, 2001).

The development of industry in the 16th century gave rise to several highly specialized works dealing with occupational diseases. In 1556 Georgius Agricola (1494–1555), a German doctor and metallurgist, in his work "On Mining and Metallurgy" described severe occupational diseases of miners (Weber, 2002). The first real systematic contribution to occupational toxicology was made by Italian physician Bernardino Ramazzini (1633–1714). In his work "Diseases of the workers," published in 1700, where he for the first time described the diseases of workers in almost 70 professions, such as miners, gilders, chemists, plasterers, blacksmiths, etc. (Dhungat, 2017).

The Golden Age of King Louis XIV of France was not associated just with the development of the country, but also with several famous cases of poisoners—Marquis de Brinvilliers, Catherine Monvoisin, and others. Catherine Monvoisin (1640–80) (Fig. 1.10) was among the most popular poison suppliers of that time. A frequent client of Madame Monvoisin was also a hot favorite of the Sun King—Marquis de Montespan. Due to the fact that poisons were perceived as the simplest means of solving problems among the aristocratic families, King Louis XIV issued a special law, where the definition of poison was given as: "Everything that can cause a rapid death or slowly destroy human health, regardless of the fact whether it is a simple or complex substance, must be considered as a poison." To complete the story of French poisoners, Marquis de Brinvilliers, Catherine Monvoisin, and their associates were executed. Marquis de Montespan, a mother of eight illegitimate children of Louis XIV, was sent to exile in the Netherlands (Somerset, 2004).

In the late 17th–early 18th centuries, Neapolitan poisoner Teophania, more commonly known as Tophana, operated in Europe. Apparently, this Italian was responsible for the deaths of more than 600 people. Tophana was an inventor of an original product called *Aqua Tophana*. Aqua Tophana had a water-like, odorless, and colorless consistency. Allegedly, five or six drops of this magical water was enough to kill a man. The onset of the toxic effect was gradual—painless, without any sign of fever or inflammation. Death occurred due to weakness, loss of appetite, and incessant thirst. Among the most frequent customers of Aqua Tophana were women who desired to get rid of their husbands. The exact content of Aqua

Tophana remains unknown. According to one source, it was made of arsenic acid with an addition of *Herba cymbalariae* (*Scrophulariaceae*). Other sources claim that the main component of Aqua Tophana was lead acetate solution. Seniora Tophana was eventually sentenced to death and in 1709 burnt to death (Nepovimova and Kuca, 2019; Wexler, 2017).

FIGURE 1.11 The founder of toxicology Mathieu Orfila (https://commons.wikimedia.org/wiki/File:Pierre_Matthieu_Joseph_Bonaventure_Orfila._Lithograph_by_Z._Wellcome_V0004368.jpg).

At the beginning of the 19th century, the most prominent figure in toxicology was considered to be the Spanish physician Mathieu Orfila (1787–1853) (Fig. 1.11). He was the first to separate toxicology from pharmacology, clinical and forensic medicine, giving toxicology the status of an independent scientific discipline. At the age of 27, Orfila wrote a book "Treatise on poisonings," that was later published in five editions. Several years later, another work by Orfila's "A treatise on the remedies to be employed in cases of poisoning and apparent death: including the means of detecting poisons, of distinguishing real from apparent death, and of ascertaining the adulteration of wines" met with great interest from the scientific community. In his writings, the Spanish physician classified all known toxic substances, described the clinical picture of intoxications typical for various classes of poisons, and also recommended chemical methods for poison identification in biological matrices (Myers, 1961). Based on his works, it became obligatory to conduct a forensic chemical analysis for official confirmation of poisoning as the cause of the death. In addition, Mathieu Orfila gave the most general definition of poison that remains widely used "Poison is a substance, that by coming in contact with a living organism in a small amount, destroys its health and subsequently life" (Hadengue, 1987).

The 1850s could be characterized as the time of the formation of modern toxicology. The decisive influence belonged to the successes achieved in analytical chemistry and experimental analysis that won its place in theoretical medicine (Oser, 1987). The fundamental works of French scientists Francois Magendie (1783–1855) (Fig. 1.12) and his student Claude Bernard (1813–78) (Fig. 1.12) dealing with the mechanism of action of strychnine, cyanide, curare, carbon monoxide, and other poisons, served to strengthen the role of toxicology among the other scientific disciplines (Bloch, 1989). Numerous methods of

FIGURE 1.12 Francois Magendie (left) and his student Claude Bernard (right) (https://commons.wikimedia.org/wiki/File:Fran%C3%A7ois_Magendie._Lithograph_by_N._E._Maurin._Wellcome_V0003781.jpg; https://commons.wikimedia.org/wiki/File:Portrait_of_Claude_Bernard_(1813-1878),_French_physiologist_Wellcome_M0000114.jpg).

particular physiological function evaluation, such as respiration and neuromuscular conduction, proposed by Claude Bernard were preserved in experimental practice for more than 100 years. Claude Bernard was also an author of the brilliant idea that toxic substances can serve as an excellent tool in physiology research. He said: "These substances could be considered as real life reagents that are carried by the blood stream to all points of the body, act on some tissues and finally lead to the death. The mechanism of death points to the physiological role of particular tissues on which they act." This finding became a significant milestone in general physiology (Breathnach, 2014). Additionally, within his experiments with curare, Claude Bernard revealed that this poison paralyzes voluntary muscles with no effect on impulse conduction in the motor nerves as well as on contractility of the muscles. This observation led to discovery of the special sensitivity of the neuromuscular junction to curare. Several years later, these investigations served as a strong argument for the development of a theory of the neurochemical basis of excitatory transmission within the nervous system (Gomes et al., 2014).

For almost two centuries after the death of the French Emperor Napoleon Bonaparte (1769−1821) (Fig. 1.13), his demise has remained a hot topic, and the scientists continue to investigate this case. After he was sent into exile in 1820, the health status of the Corsician sharply deteriorated. Throughout his stay on the island of St. Helen, he complained about severe stomachache, weakness, and frequent attacks of nausea. Finally, on May 5, 1821, he died. According to the findings of an international group of scientists, Napoleon Bonaparte passed away due to progressive stomach cancer with metastases in the lymph nodes (Leys, 2015). However, according to conspirologists, the symptoms of the ex-Emperor's death more resemble arsenic poisoning. Moreover, recent analysis of his hair has shown an almost 40-fold increase in arsenic concentration in comparison to normal people of that time. Several theories have been formulated, explaining how Napoleon Bonaparte could have been poisoned. Among the most curious being poisonous wallpaper, or the theory claiming that Napoleon was poisoned by a mixture of heavy metals (Hindmarsh and Corso, 1998). The first hypothesis builds on the fact that adding green arsenic-based pigment into wallpaper was quite common at that time. From a chemical point of view this pigment, called Paris Green, was copper(II) acetoarsenite. The humid weather of the island promoted the proliferation of microscopic fungi which could convert inorganic arsenic in Paris Green to an organic form. It is widely recognized that organic forms of heavy metals more easily cross the biological barriers compared to their corresponding inorganic salts. Therefore this theory assumes that Napoleon was intoxicated in particular by these organic forms of

FIGURE 1.13 French Emperor Napoleon Bonaparte (https://thorvaldsensmuseum.dk/en/collections/work/E876/zoom).

arsenic. The latter theory already assumes foreign blame. According to this hypothesis, low doses of arsenic were added to Napoleon's food and drinks. The clinical picture of chronic intoxication with arsenic usually manifests as severe pain in the stomach. To relieve vomiting in Napoleon, the doctors administered him potassium tartarate of antimony. In addition to this remedy, his physicians prescribed him calomel and orgeat to combat constipation and thirst, which represent other characteristic symptoms of arsenic intoxication. The main component of orgeat is bitter almond oil, whereas calomel is a trivial name for mercury dichloride (Hg_2Cl_2). Hydrolyses of orgeat to hydrocyanic acid in the acidic gastric environment, together with calomel, gives rise to mercury cyanide. Therefore either arsenic, hydrocyanic acid, antimony, mercury cyanide, or a mixture of some or all of them could be one of the causes of Napoleon's death (Mari et al., 2004).

1.4 The modern era

The Industrial Revolution in the middle of the 19th century allowed the synthesis of natural toxins in unlimited quantity. Moreover, novel entities derived from natural compounds were prepared. Due to all the above-mentioned events, poisons were gradually losing their mystery (Nepovimova and Kuca, 2019). At the beginning of the 20th century, the development of toxicology was strongly influenced by progress in the chemical industry. From the perspective of chemical production, Germany was among the most developed countries. Within several branches of the chemical industry, German chemists even maintained a monopoly position, for example, in dye production. One of the most famous German chemists of the time was Fritz Haber (1868–1934) (Fig. 1.14), who discovered a method of ammonia synthesis from atmospheric nitrogen. Such an invention was of high importance for the large-scale synthesis of fertilizers and explosives. Therefore in 1918, Fritz Haber was awarded the Nobel Prize (Manchester, 2002). In the history of toxicology, F. Haber is better known for another reason, he is called the "Father of Chemical Weapons" due to his longlasting reasearch in the field of weaponization of chlorine and other toxic gases in World War I (WWI). In addition, it was Haber's suggestion to use chlorine in the first chemical attack by Germans against British/French troops on April 22, 1915, near the town of Ypres (Belgium) (Charles, 2005). Subsequently, the Allies (France, Great Britain, United States, and Russia) also started to use chemicals for military purposes. During the 4 years of WWI (1914–18), about 1.3 million people were affected by chemical weapons on both sides of the conflict, of which more than 100,000 died (Tucker, 2006).

FIGURE 1.14 The "Father of Chemical Weapons" Fritz Haber (https://commons.wikimedia.org/wiki/File:Fritz_haber_1929_PI_29-C-0097.jpg).

On September 7, 1978, Bulgarian dissident Georgi Markov (1929–78), after an evening broadcast on the BBC, went around a crowded bus stop on the Waterloo Bridge in London and suddenly felt a slight sting in his leg. Looking around, the Bulgarian noticed a man picking up an umbrella from the ground. The stranger spoke with a strong accent, apologizing, and then caught a taxi and left. Due to a high fever, acute stomachache, and severe diarrhea, Markov was hospitalized that night, and a few days later he died. Fortunately, he managed to talk about the incident with the umbrella. Doctors, who performed an autopsy, found a small iridium-platinum capsule in the leg of the dissident. According to the findings of further investigations, this capsule with a diameter of less than 2 mm was filled with ricin (Crompton and Gall, 1980). Ricin is a plant toxin obtainable from the castor bean (*Ricinus communis*, *Euphorbiaceae*). By all accounts, Georgi Markov was shot by the Bulgarian special services because of his active criticism of the communist regime of Todor Zhivkov (Papaloucas et al., 2008). The killing device was an umbrella endowed with a hidden sting that shot small capsules filled with toxic ricin.

On March 20, 1995, the nerve agent sarin was used at several subway stations in Tokyo (Japan). About 10,000 people were affected, with 5000 seriously intoxicated and 12 people died. Sarin was used by the terrorists of the sect Aum Shinrikyo. They stored the nerve agent in plastic bags, which were subsequently punctured by an umbrella with a sharpened tip. The terrorists managed to puncture 10 of 11 bags. Fortunately, due to the low purity of the sarin (approximetely 30%) and the subway ventilation system, the loss of life was not as high as it might have been (Okumura et al., 2005). The question that still needs to be answered is: "Why did the sect select sarin?" There are plenty of possibilities why: (1) inspiration from the Gulf War; (2) simple synthesis; (3) starting compounds availability; and (4) low production costs (Nozaki and Aikawa, 1995). Many sect members who participated in the sarin production process claimed that they were unaware of its toxic effects. However, the handbook "Magic song of sarin," found in one of the buildings used by Aum Shinrikyo, apart from the instructions of how to synthesize the nerve agent, gave a description of its lethal effects. Therefore it was obvious that the members of the sect had lied (Kimura, 2002). The terrorist use of sarin in the Tokyo subway pointed out the serious risk of misuse of chemical warfare agents for nonmilitary purposes and highlighted the need for the development of appropriate protection including antidotal therapy.

The former President of Ukraine, Viktor Yushchenko (born 1954) (Fig. 1.15), was intoxicated by a very high dose of 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in