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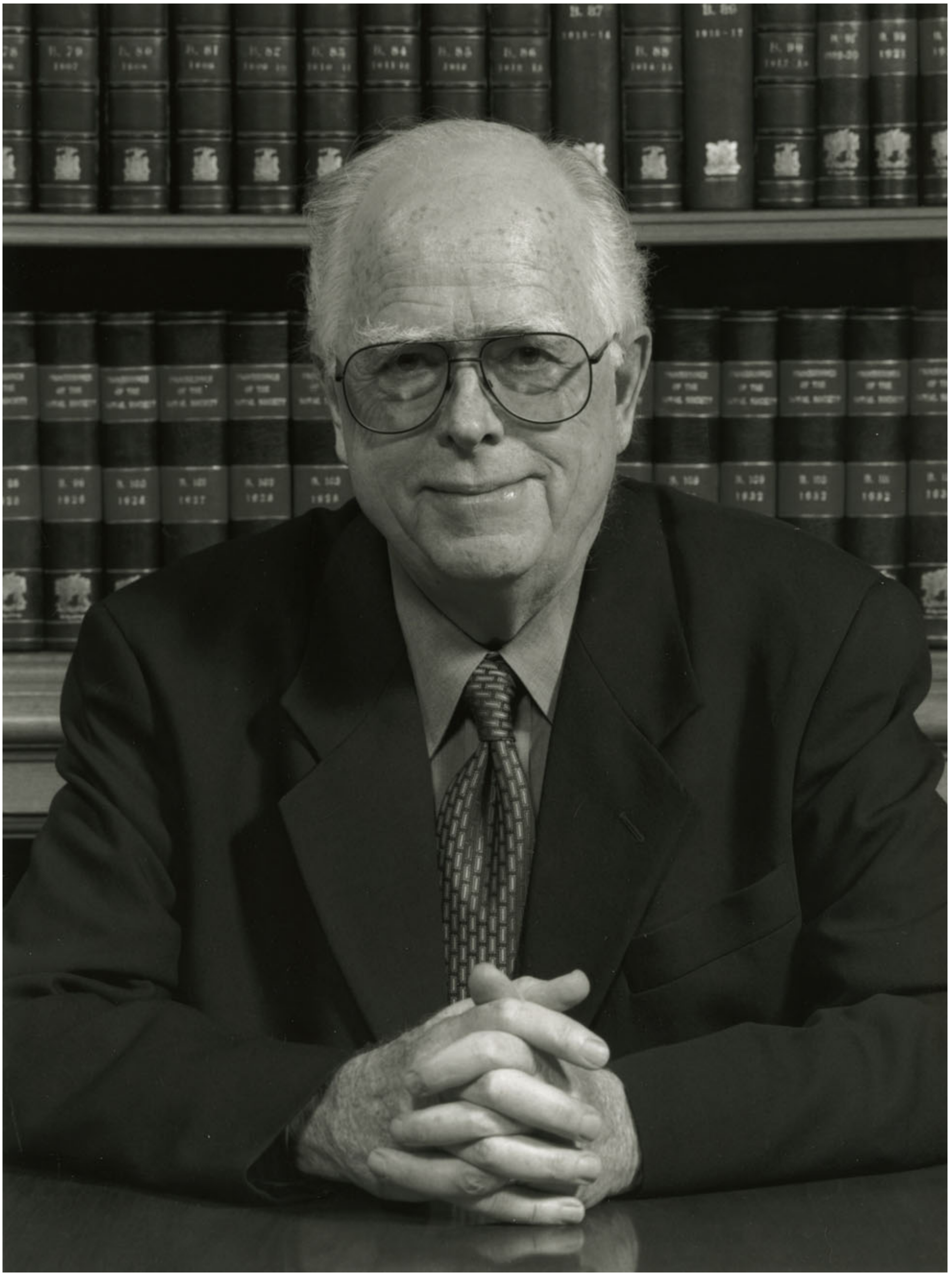

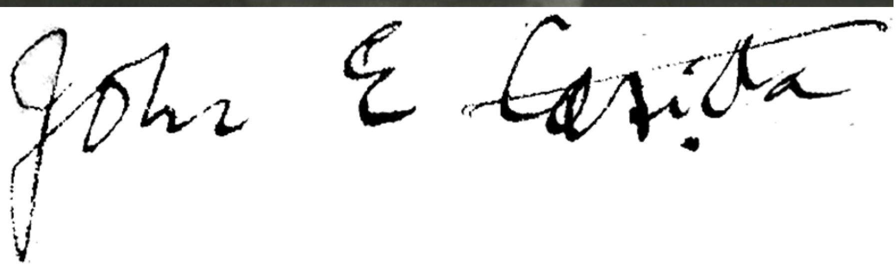

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Elected ForMemRS 1998

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John Edward Casida's research in pesticide toxicology led to more effective agricultural chemicals that are far safer for human and environmental health. He used pesticides as probes for his fundamental studies of metabolism and mode of action, resulting in great insight into biological chemistry and the underlying mechanisms of regulatory biology, ranging from voltage-gated sodium channels, through the ryanodine receptor and calcium regulation, the gamma-aminobutyric acid (GABA)-gated chloride channel, to the nicotinic acetylcholine receptors. These discoveries, among many others, have had a profound impact on pharmacology and toxicology. His research career started with the introduction of DDT into agricultural practice and continued to assist in the development of many pesticides that dominate the market today. John Casida trained multiple generations of toxicologists who obtained leading positions in government, industry and academics. He spent many of his formative years in Madison, Wisconsin, where he entered the University of Wisconsin, received his BS, MS and PhD and then joined the faculty to become a full professor six years later. He then moved to the Entomology Department at the University of California, Berkeley where he remained active in teaching and research until his death. He loved laboratory science and this, coupled with insatiable curiosity and a gift for finding the unexpected, led to papers from his laboratory sparkling with creativity. He similarly loved teaching at all levels and had just finished grading the final examination in his toxicology class at the time of his passing. John won numerous national and international awards and is widely viewed as the premiere pesticide toxicologist.

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Life

Early life

John's father, Lester Earl Casida, was a grade school teacher in a one-room country school when he decided to go to college. He was finishing his Master's degree in animal husbandry at the University of Missouri when the Great Depression hit. About this time, in 1927, he married Ruth Barnes and joined the Arizona State Teacher's College. It was here in Phoenix, Arizona, where John Casida was born on 22 December 1929. The senior Casida then received a one-year position at the Arkansas State Teacher's College in Conway, Arkansas (now University of Central Arkansas) and subsequently returned to Missouri to finish his PhD. John reported that his mother was very supportive of her husband's academic aspirations and tolerated the multiple moves with John, his sister Betty and brother Earl. In the mid 1930s L. E. Casida received a fellowship to the University of Wisconsin (UW) at Madison. He remained employed on sequential fellowships until his mid 30s. John reported that the family had a marginal income during this period, but that he never felt deprived. At that point his father became a tenure track faculty member at the university, where he spent his career working on the genetics and physiology of reproductive biology. L. E. Casida is well known in his own right for applying rigorous statistical methods to the genetics of cattle and was an inspiration for his children (Stormshak 2002).

University education

John reported this period as idyllic for a young biologist since he was given free access to the university arboretum and lake, and later the university entomology museum. It was here that he developed a deep love of biological science and collected insects and mites. During the Second World War (WWII), technical positions in agriculture opened up in the university owing to the draft for the war. Thus, John had ample opportunities as a teenager to perform field and laboratory research as well as pursue his hobby as an insect collector. John reported this was a period of increasing interest in field biology and science, encouraged by his parents and activity in the Boy Scouts. His first publication appeared when he was 22 in *Science* magazine (1)*.

John entered the UW Madison, where he received a BS in entomology in 1951 and developed an interest in agriculture and plant physiology. During this period he took up sabre fencing and was team captain (figure 1), but reported that most of his free time was spent in studies and independent research. John then obtained an MS degree in biochemistry in 1952 and received a joint PhD in entomology and biochemistry under the direction of M. A. Stahmann and T. C. Allen, with a minor in botany. John noted that hidden below the tremendous improvement in plant growth that came from control of insects with DDT (dichlorodiphenyltrichloroethane) was that DDT could both stimulate and retard plant growth directly. This dark side was later found to be due to a DDT metabolite acting as a plant hormone. With the success of DDT and the rise of organophosphates (OPs) as agricultural chemicals after WWII, John became increasingly interested in the interaction of exogenous chemicals with organisms and the impact of pesticides on agriculture and the environment.

His PhD work was interrupted by the Korean War and an increasingly active draft programme for the military. Since John was working on OPs, he was offered a direct commission by two military laboratories, Fort Detrick in Frederick, Maryland, on biological

* Numbers in this form refer to the bibliography at the end of the text.

Figure 1. Fencer John Casida. John demonstrates fencing technique for the camera on 13 December 1949. (Photograph from the University of Wisconsin at Madison Steenbock Library.)

warfare and at the Edgewood Arsenal of the Aberdeen Proving Ground, also in Maryland, working on OP chemicals. John went into the chemical branch with a direct commission as a second lieutenant, and continued with his own insect work at Fort Detrick. It was during this period when John was seriously poisoned with an OP, which certainly spurred an interest

in risks of pesticides to human health. By taking classes while serving in the military, and publishing his independent research, he was able to finish his PhD quickly when he returned to Madison (Rice 2019).

Academic positions

Only rarely do American research universities hire their own, but John was offered and accepted an assistant professor position when he received his PhD from UW Madison. Three years later he was offered the position opened by the retirement of William Hoskins as the toxicologist in the Department of Entomology at the University of California (UC), Berkeley. He was later awarded the Hoskins chair in chemical and molecular entomology, which he held from 1996 until 2018. Subsequent to his retirement, he was the Edward A. Dickson emeritus professor and professor of the Graduate School at UC Berkeley (Nomura 2018).

Throughout his UC career, John held court in an office at the north end of a long corridor in the lovely but old Wellman Hall (figure 2). The basement space in this historic building was ample but certainly illustrated that the quality of the space does not make the science. It was notable for chemistry laboratories without emergency showers and only one exit as well as for a room for new graduate students with such a low ceiling one could not stand up. It was here that John founded the Pesticide Chemistry and Toxicology Laboratory (PCTL), later renamed the Environmental Chemistry and Toxicology Laboratory (ECTL), which continued long after his retirement and was finally largely closed only two years before his death. Even then, John continued research in two smaller laboratories by his office, with some research published posthumously.

John was an avid teacher for his entire career (Garvey 2018). Generations of associates and visitors have commented that the most stimulating instruction was at the blackboard in his office (figure 3). For most of his career he also had a semiformal noon bag lunch termed ‘the gripe session’ with all laboratory personnel presenting their work on a rotating basis in a tiny windowless room under the slanted roof of Wellman Hall. John taught multiple courses, but a three-unit course on toxicology, started in Wisconsin and continued at UC Berkeley, was taught every year during his entire time at UC Berkeley. He in fact finished grading the final exam of 72 students just several weeks before his death.

Family life, interests, pastimes and values

John married Katherine Faustine Monson (a well-known artist), better known as Kati, in 1956. Kati was an American-born Norwegian descendant. John and Kati and, for a time, their two sons lived in the Berkeley Hills, north east of campus. John said the walk to and from work gave him time to think; however, whenever Kati drove up to his basement window of Wellman Hall, John never hesitated to rush out to join her. His advice to the students in his office was: ‘Never keep Kati waiting.’

John occasionally joked about his wife’s creative eccentricities, and complained about moving Kati’s often large and heavy art installations around the world. He quickly added that ‘she made me a whole human’. Kati and John were immersed in the Berkeley art scene and dedicated to culture and Greek dancing. He also developed an interest in archaeology, photography, Russian icons and pre-Columbian art. Kati was as fully engaged in John’s career and interests as he was in hers. Among other things, Kati sent unique Christmas letters about the Casida family and laboratory alumni (figure 4).

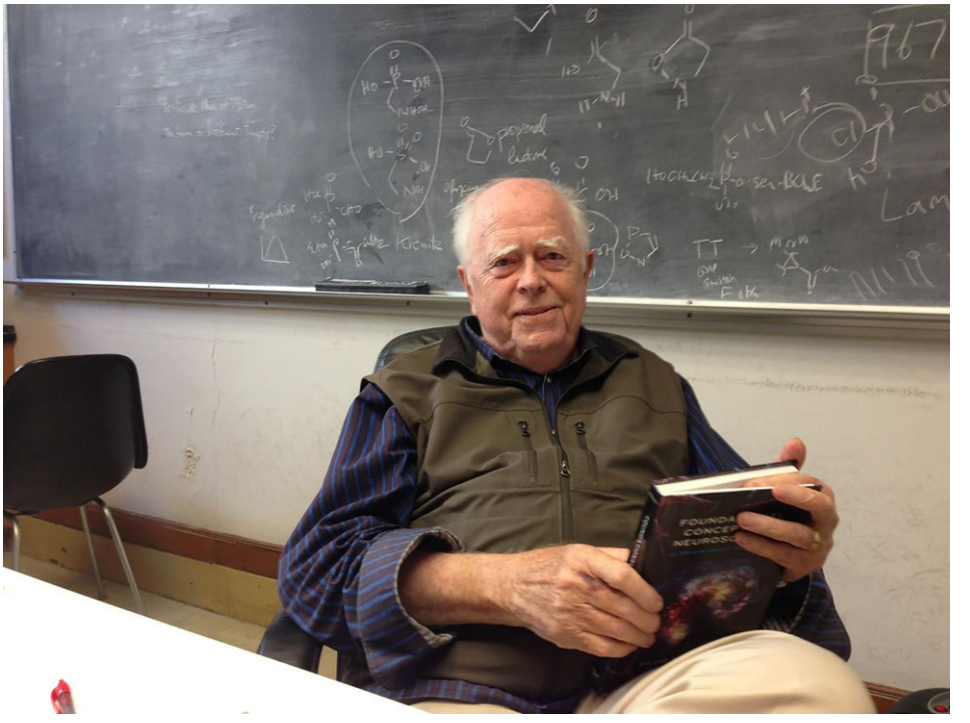

Figure 2. John in his office in Wellman Hall, UC Berkeley. (Photograph by David E. Presti.) (Online version in colour.)

Sadly, Kati died in 2021. They leave behind their two sons, Eric (BeRex Corp., Berkeley) and Mark (professor of theoretical chemistry, Grenoble-Alps University, Grenoble, France), Kim Collins Casida (Mark's wife) and their two grandchildren, Mariposa and Tenaya Casida (figure 5).

Works

The phrase 'long and productive career' is often used in remembrances, but this phrase is seldom more appropriately applied than when it describes J. E. Casida. John was productive until his last brief illness, and even during this period of hospitalization he was planning his next works. The editing on his final papers was completed by adoring alumni of his laboratory, leading to an exceptionally long career. Certainly, John was creative and productive by any measure, with over 30 patents and 800 peer-reviewed publications, and an exceptionally high citation index in diverse fields. Repeatedly he solved timely problems in agricultural chemistry and pesticide toxicology, and illuminated a path toward impactful and fruitful research in fundamental biology (Hammock & Casida 1998; Komives 2018; Wing 2019). His career spanned modern pesticide science and technology. Because of the breadth of his interests, some scientists have thought John a dilettante jumping from one exciting area to another. As described below, the broad swath that he cut through basic and applied science, while

Figure 3. John (second from left) in discussion with his visiting colleagues (names and date unknown). (Family collection.) (Online version in colour.)

pioneering many of the technologies employed, rather shows his methodical approach on how best to invest his multiple talents in research. This approach certainly worked for John and for the scientific endeavour.

Early research activities and achievements in agrochemicals and pesticide safety and metabolism

It is very appropriate that his first paper in 1951 was on DDT. At the dawning of modern insecticide toxicology, DDT was initially used in WWII to control malaria, typhus, body lice and bubonic plague. DDT is still used today in some countries for these purposes. DDT was also resulting in massive increases in crop yields and the profitability that ultimately fed the world. But, rather than praising the attributes of DDT, John cautioned in his first *Science* paper that pesticides could have unexpected and possibly deleterious consequences (1). This ability to see the attributes of pesticides in agriculture while laying the scientific framework for cautions regarding their use characterized his whole career. It is now well recognized that DDT in particular and pesticides in general can pose deleterious impacts on biodiversity. Certainly, John both contributed to and founded multiple fields in agricultural chemicals, pesticide toxicology and other areas, yet the evolution of research interests moved from one to another in a very methodical fashion. John, with his mentor T. C. Allen, explored in some depth pesticide-caused phytotoxicity. These studies led them to expand their interest

Figure 4. John with his wife Kati in Solvorn, Norway, May 2005. (Family collection.) (Online version in colour.)

into OP pesticides, first in plants and then with enzymes in a key paper by Casida, Allen and Stahmann (2). As mentioned earlier, this work with OPs attracted the attention of the US military, allowing John to enter service as an officer, continue his interests in OPs and other pesticides, and thus rapidly obtain his PhD. It also drove home the concept of selective toxicity in his work: the same basic chemistry at the site of action of OPs resulted in some of the most valuable and often safest agricultural chemicals as well as anti-personnel agents that remain a terror threat today. One also sees his evolving approach in science of building on technical and intellectual strengths in one field to facilitate exploration of new, exciting areas.

During this period we see strong publications in classical economic entomology journals, but also his realization that excellent biology is based on a firm understanding of chemistry. Thus, his publications emerged increasingly in both agricultural chemical and pure chemical journals. Although he later returned to the mechanism of action of DDT and the sodium channel, John increasingly worked on OP mode of action and mechanism of action. This coincided with a ‘swords to ploughshares’ transformation of the anti-personnel agents from WWII into some exceptionally important tools in agriculture and vector biology. Thus, in the first five years of his career, John was a key early worker on the first two important classes of modern insecticides. Over the next five years John became increasingly productive in the OP field, expanded his collaborators to some of the leading world figures and began to prepare reviews that were not merely compilations of references but critical evaluations of where the pesticide field was going and, more importantly, insightful comments on where it should be going.

Figure 5. John's family photo taken at the dedication of Kati's sculpture on 23 June 2011 in Skjolden, Norway. Back row, from left to right: Eric (son), Mark (son), Mark's wife Kim (daughter-in-law), Mariposa (granddaughter), Kati (wife) and John Casida. Front row, from left to right: Tenaya (grandson) and two local Norwegian children (names unknown). (Family collection.) (Online version in colour.)

It was with the OPs that John began to use his investigations into pesticide mechanism of action as a window into understanding fundamental processes in biology. This was also the time that he first ventured into pesticide metabolism. He quickly saw that understanding metabolism and environmental degradation was far more than a regulatory requirement, He illustrated that metabolic stability played a major role in efficacy and possible risk of deleterious environmental problems. Possibly of more importance, he illustrated that pesticides as structurally diverse and economically important xenobiotics provided tools and funding to study the enzymes involved in their metabolism. Often, the enzymes involved in xenobiotic degradation had more important endogenous functions in biology. This led to his later insights into esterases, cytochrome P450 (CYP) monooxygenases, amidases, epoxide hydrolases, lipases, chitin synthetase, serine hydrolases, arylformamidase, fatty acid amide hydrolase and so on.

Metabolism, mechanisms and mode of action of pesticides and creative use of radioisotopes

John's leadership role in advancing use of radioisotopes in biology, and particularly in metabolism of agricultural chemicals, soon led his laboratory to becoming the state of the art in the field. He set the standard of excellence for metabolism studies until good laboratory

practice requirements necessitated the field largely to move into industrial and governmental laboratories.

The pesticide metabolism field prospered under his leadership, with an initial focus on OPs and the new carbamate class of insecticides. It was in 1966, in a classical study, that he published in collaboration with Izuru Yamamoto on the radiosynthesis of the natural pyrethrins and early pyrethroids that initiated possibly his major contribution to the development of practical insect control (3). This returned John to the sodium channel as the site of action of both DDT and pyrethroids and the importance of xenobiotic metabolism, highlighting esterases and cytochrome P450 enzymes. This work led to a lifelong collaboration and friendship with Yamamoto and decades of collaboration with the Rothamsted Experimental Station and particularly Michael Elliott (FRS 1979) (Pickett 2016; Yamamoto 2018). His laboratory both hosted and created the leaders in the pyrethroid field, and probably his hosting of this cadre of scientists led in part to the expansion of the field to dominate insect pest control for decades. The metabolism studies of these complex molecules were exploited by innovative chemistry first at Rothamsted and then with multiple laboratories around the world, to make pyrethroids the world's dominant class of insecticides and dramatically increase the safety of insect control chemicals.

Radioligands creatively designed and well prepared in his laboratory led to many of his breakthroughs and in-depth investigations. These radioligands emerging from the Casida laboratory are either the insecticides themselves or analogues that bind at the same or coupled sites. Examples of these ligands and their targets, often in both insects and mammals, are trioxabicyclooctanes for the γ-aminobutyric acid (GABA) receptor, avermectin for the glutamate receptor, imidacloprid for the nicotinic acetylcholine receptor (nAChR), ryanodine and chlorantraniliprole for the ryanodine receptor, and rotenone or pyridaben for $NADH^{+}$ ubiquinone oxidoreductase (37). The radioligand binding assays are used to help define structure–activity relationships, target site modifications on selection of resistant pest strains, selectivity between insects and mammals, and interaction with antidotes and other chemicals at modulator sites. They were also the basis for the development of multiple pharmaceuticals. The binding assays serve for receptor isolation and photoaffinity labelling to characterize the interactions involved. For example, 9,21-dehydroryanodine was identified, leading to the synthesis of radioactive [9,21-^{3}H]ryanodine and showing that the new radioligand probe acts at the calcium release channel of muscle sarcoplasmic reticulum (14). The toxic principal of blister beetles and purported aphrodisiac cantharidin (the active ingredient of Spanish fly) was prepared as a radioligand, and the binding protein isolated and identified as protein phosphatase 2A (15), thereby explaining its pharmacological, toxicological and aphrodisiac properties and those of a related synthetic compound used as a herbicide. In his research related to nicotine action, the nitroguanidine insecticide imidacloprid, prepared as a radioligand, showed the unique sensitivity of nAChR of insects compared with that of mammals; related chemistry served to isolate the pure native receptor from *Drosophila melanogaster* by affinity chromatography (25).

A paper in 1971, asking how microsomes converted phosphonodithionate protoxins such as fonofos to proximate cholinesterase inhibitors, initiated a series of serendipitous studies that John said were among the most exciting events in his career (4). Ultimately, this led to several new classes of insecticides, discovery of the risks of a widely used class of compounds showing great danger to human health, development of some of the most commonly used tools in neurobiology, such as trioxabicyclooctane radioligand probes for

studying the GABA-gated chloride channel, and multiple new human and animal health therapeutics. What, of course, delighted John in retrospect was the unexpected discovery that some commercial fire retardants and the subsequent molecules derived from them were exquisitely toxic. In the conversion of P = S to P = O did active oxygen attack the phosphorus, attack the sulfur or carry out an as yet unknown chemical magic? The laboratory quickly modelled this microsomal reaction with chemical peroxides being used in the laboratory at that time for other things and isolated a putative intermediate in the P = S to P = O conversion termed a phosphorus-oxy-thionate. In one of the few blunders by Yamamoto and Casida, the structure was quickly shown to be wrong by M. Fahmy and John's lifelong friend and rival T. R. Fukuto. John described this as the very best blunder of his career. It initiated a series of studies to find the elusive phosphorus-oxy-thionate. One approach taken by a postgraduate fellow, Eugene Bellet (5), was to tie the R groups on phosphorus 'back in a bow' to be able to follow the reaction intermediates by ^{31}P nuclear magnetic resonance (^{31}P NMR). Based on a bet for a beer regarding the toxicity or lack of toxicity of these model compounds, one of these molecules was injected into a mouse, which instantly died. A survey of these molecules did show many of them to be exquisitely toxic. Several compounds were in wide-scale use as industrial fire retardants and certainly were very dangerous materials. These compounds and related natural products were found to bind tightly to the GABA-gated chloride channel, which inhibits the functioning of mammalian neurons (7). This serendipitous study led to some of John's most cited papers, great benefit to environmental and human health, and chemical probes that are used in most neurobiology laboratories worldwide. A related study that John particularly enjoyed was the determination of the mechanism of action of the drink absinthe (17). 'Because of this study,' quipped John, 'I had brief notoriety among the artistic community while before I had been viewed as just the eccentric companion of Kati Casida.'

Environmental chemistry and photochemistry

Repeatedly we see that John's work evolved from strength to strength. Investigations into first the OPs and the mechanistically related carbamates, extending into the metabolism of these agents and on into the far more complex pyrethroids, and then realizing that cytochrome P450 enzymes (CYPs) were key in this process and initiating projects on CYPs, each led to their own field. Shortly thereafter he began applying his technologies in metabolism chemistry to environmental degradation and the use of photosensitizers to speed degradation of pesticides (4). This also opened the door to fundamental studies in photochemistry of great relevance to environmental chemistry.

It was during this period that John saw the power of mass spectrometry in metabolism studies, and increasingly, from this period onwards, high resolution NMR and mass spectrometry played a massive role in his research (Johnston & Ruzo 2011). Identification of the metabolic basis for selective toxicity and environmental fate of OPs, methylcarbamates, pyrethroids and neonicotinoids was among major accomplishments of John's laboratory.

GABAergics and GABA-gated chloride channels

John was a keen observer and critical thinker. Two new classes of potential insecticides came from the chance observation described above of very simple and highly toxic bicyclophosphates and related bicycloorthocarboxylates with a totally unexpected mode of action, followed by fundamental research on neurobiology (12). Radioligands ([^{35}S]TBPS, [^{3}H]TBOB and [^{3}H]EBOB) and receptor binding studies

Figure 6. John (centre) with Lassie Hammock (left) and his former graduate students Sarjeet Gill (second left), now of UC Riverside, and Bruce Hammock (right), of UC Davis. This photograph was taken at UC Berkeley in 2016. (Bruce Hammock collection.) (Online version in colour.)

combined with physiological assays established that the new toxicants are non-competitive blockers of the GABA-gated chloride channel. The binding sites of tert-butylbicyclophosphorothionate (TBPS), tert-butylbicycloorthobenzoate (TBOB) and ethynylbicycloorthobenzoate (EBOB) were discovered to be the same as, or closely coupled to, that of the polychlorocycloalkane insecticides such as dieldrin, lindane and toxaphene, so finally establishing their mode of action after three billion pounds in weight had been used (29), as well as the rodenticide tetramethylenedisulfotetramine (TETS) (34). A rational approach to a totally new class of potential insecticides then led to non-halogenated and non-phosphorus compounds of unusually simple structure, some of which are more active than many commercial insecticides. His team extended the experimentally derived and computationally rationalized model of the GABA receptor–non-competitive binding site from the β3 homomer, which is ultrasensitive to insecticides, to α1β3 and other heteromers more relevant in normal brain function. With that of his alumni (figure 6), the work defined $GABA_AR$ sites for potential antidotes acting to prevent TETS binding or displace it from its binding site (34). Although now banned as a rodenticide, TETS is a feared chemical threat agent because of its high convulsant toxicity, ease of synthesis and availability.

We also see emerging from his environmental chemistry work ties back to the structures of other classical pesticides, such as toxaphene and cyclodienes, which act on the GABA-gated chloride channel, as well as new classes, including fipronil and many natural products. In looking back on his work on the GABA-gated chloride channel and many other major

discoveries from his laboratory, John credits a degree of serendipity (13). Although being in the right place at the right time is always a major driver in science, John seemed to realize when he was at the right time and place. John described one of his colleagues once by saying: 'He has yet another paper in *Science*. That guy has been so lucky for so long, one begins to think he may be good.' John's statement seems to apply equally to his own career.

Calcium-activated calcium channel and ryanodine receptor

John's interests were always wide-ranging, but why did he have such a knack for finding seemingly minor questions overlooked by others that led to important fundamental discoveries in both basic science and agriculture? His wife, Kati Casida, may have provided part of the answer when she said that he needed little sleep. 'He reads incessantly and widely, but is constantly reading broadly in science.'

While reading Donald Crosby's book on natural insecticides, he rediscovered how very toxic the natural insecticide ryanodine was. He was long an advocate of the value of high specific activity tritium in probing basic biology. Thus he pulled together a superb chemist (10) and skilled neurobiologist (11), added in his tritium technology, and the resulting synergism led to the discovery of a fundamental receptor regulating calcium metabolism. As with the discovery of ligands for the GABA-gated chloride channel, the impact of having tools for the ryanodine receptor did far more than provide a mechanism of action for a minor natural insecticide: it has revolutionized our understanding of calcium regulation and led to a number of therapies for maladies ranging from heart disease to convulsions. It has also led to several new classes of insecticides binding to the ryanodine receptor.

Organophosphate toxicology and safety

John carried his studies into extraordinary depth and precise understanding, which is well demonstrated with his remarkable and prolific research on OP toxicants for his entire career. OP toxicants are the principal chemical warfare and terrorism agents. OP esters are one of the most important classes of insecticides, with approximately 150 compounds used for protecting crops, livestock and human health in the past 70+ years. They remain major insecticides. The selective toxicity of OP insecticides is based on specificity differences in the acetylcholinesterase (AChE) targets, more rapid detoxification in mammals than insects and the use of proinsecticides undergoing preferential activation in insects as compared with mammals. AChE is the primary target of OP insecticides and chemical warfare agents. OPs cause more cases of human poisoning than any other class of pesticides. Many attempts to replace or ban them have been unsuccessful because they are cheap and effective, which in some instances take priority over safety. John spent years on uncovering non-AChE systems, on the contribution of alternative targets to the acute lethal action and on secondary effects of short- or long-term exposure (23). He envisioned the importance of OP-sensitive serine hydrolases, OP insecticides and chemical threat agents that phosphorylate serine in the catalytic triad of AChE. Other serine hydrolases can be secondary OP targets, depending on the OP structure, and include neuropathy target esterase (NTE), lipases and endocannabinoid hydrolases (36).

His research took careful consideration of several OP-sensitive serine hydrolases and receptors as secondary targets. His OP research ties together OP pesticides, Gulf War syndrome (GWS) and attention deficit/hyperactivity disorder (ADHD). GWS is a chronic and multi-symptomatic disorder affecting military veterans of the 1990–1991 Persian Gulf

War, while ADHD is a common neurodevelopmental disorder of childhood. The first, seminal mouse model developed in his laboratory provided a much-needed system for studies of potential clinical syndromes caused by OP exposure (22). NTE is involved in neural development and is the target for neurodegeneration induced by selected OP pesticides and chemical warfare agents. His studies showed that genetic or chemical reduction of NTE activity results in a neurological phenotype of hyperactivity in mammals and indicated that the NTE-inhibiting compound ethyl octylphosphonofluoridate toxicity occurs directly through inhibition of NTE without the requirement for NTE gain of function or aging. His studies further identified mouse brain NTE as a lysophospholipase (LysoPLA) (19) and showed that delayed toxicity from the OPs is more closely associated with *in vivo* inhibition of brain NTE-LysoPLA than with that of the cannabinoid CB1 receptor or AChE (20). LysoPLAs are a large family of enzyme for removing lysophospholipids from cell membranes.

His studies with tri-*o*-cresyl phosphate identified a saligenin cyclic phosphate as the highly potent activated metabolite and established that the biochemical lesion probably involved phosphorylation and aging of NTE. Arylformamidase (Afmid, also known as kynurenine formamidase) was shown to be the target for OP and methylcarbamate insecticides which are extremely potent in producing embryonic abnormalities (teratogenic effects) in avian embryos. In his paper published in 2005, he speculated that there must be enzymes other than Afmid (possibly in the kidney) capable of metabolizing formyl-kynurenine into kynurenine. His findings let him propose that the most significant function of the kynurenine pathway and Afmid in mice may be in eliminating toxic metabolites and to a lesser extent in providing intermediates for other processes (24). Afmid is the second enzyme of the kynurenine pathway metabolizing tryptophan to nicotinic acid and nicotinamide dinucleotide cofactors.

His work on the cannabinoid CB1 receptor demonstrated his curiosity about and ability to extend his research to unknowns and into depth. Three principal components of the cannabinoid system are the endogenous ligand *N*-(2-hydroxyethyl)arachidoamide (anandamide), the cannabinoid CB1 receptor and fatty acid amide hydrolase (FAAH). His team discovered that the CB1 receptor is sensitive to inhibition *in vitro* and *in vivo* by OP pesticides and analogues (18). Use of arachidonylsulfonyl fluoride derivatives synthesized in his laboratory as potent inhibitors of mouse brain FAAH and CB1 defined the toxicological and structural features of organophosphorus and organosulfur cannabinoid CB1 receptor ligands acting at the proposed nucleophilic site (20). His work further defined that monoacylglycerol (MAG) lipase inhibition by OPs leads to elevation of brain 2-arachidonoylglycerol (2-AG) and the associated hypomotility in mice (30). MAG lipase is a key enzyme in the hydrolysis of the major endogenous agonist 2-AG.

Serine hydrolase KIAA1363 is among many OP insecticide secondary targets identified in the Casida laboratory. His studies showed that chlorpyrifos oxon (CPO) and other OP toxicants potently inhibit CPO binding protein (CPO-BP) *in vivo* and *in vitro*. Mouse brain CPO-BP is identified as serine hydrolase KIAA1363 of unknown function. KIAA1363 is the principal enzyme for metabolizing CPO in brain and may play a general role in detoxification of OP neurotoxicants (26). Acylpeptide hydrolase (APH) is another sensitive target for OPs. It is a major serine hydrolase and unblocks *N*-acetyl peptides. His creative OP research team paid close attention to selectivity and found that blood APH activity is a sensitive marker for exposure to some but not all OP pesticides and chemical warfare agents (27). However, platelet-activating factor acetylhydrolase (PAF-AH) is not a major secondary target of OP

pesticide poisoning (28). PAF is an endogenous phospholipid modulator of diverse biological activities including inflammation and shock. PAF levels are primarily regulated by PAF-AHs.

Neonicotinoid metabolism and toxicology

A series of his 60+ papers have encompassed neonicotinoid metabolism, agonist action, selective toxicity, photoaffinity probes and structure–activity relationships. Neonicotinoid insecticide toxicology was an interest for much of his career, but evolved as a significant part of his research. Neonicotinoids have been important insecticides to control insect pests on crops since the 1990s, although there are anxieties about the impact of neonicotinoids on bees, birds and pollinators. They are also critically important for parasitic insect control on domestic animals such as dogs and cats. John's studies clearly defined the neonicotinoids' target site, identified its many toxic metabolites and enzymes responsible for the metabolism, and elucidated its special structural features and the basis for neonicotinoid safety. His work defined major enzymes metabolizing neonicotinoids as CYP isozymes and aldehyde oxidase reduction at the nitro substituent of neonicotinoids. His work indicated that neonicotinoids activate the extracellular signal-regulated kinase (ERK) cascade triggered by primary action at the $\alpha 4\beta 2$ nAChR with an involvement of intracellular Ca^{2+} mobilization possibly mediated by inositol 1,4,5-triphosphate (IP3). His study using radioligand assays also provided direct pharmacological evidence for distinct imidacloprid- and α-bungarotoxin (α-BGT)-sensitive sites or subtypes in the fruit fly brain. His studies well defined the low affinity of neonicotinoids for vertebrate relative to insect nAChRs as a major factor in their favourable toxicological profiles as insecticides (21,25). His neonicotinoid work has been highly impactful, as indicated by high citation numbers, for example, approximately 1000 times for the paper of Tomizawa & Casida (21) as of 2021.

His studies provided essential knowledge for safe use of neonicotinoid insecticides, and he gave cautions for their non-target effects. John's emphasis on the difference between insect and mammalian nAChRs associated with the action of selective agonists and the safe use of neonicotinoid insecticides illustrates the theme of selective toxicity that dominated his career. His team characterized the nicotinoid receptor–neonicotinoid binding site interactions using photoaffinity labelling and other techniques in the preparation of a binding site model describing insecticide ligand–target site disruptions. His research elucidated the pathways and enzymes involved in neonicotinoid metabolism with special attention to nitrosamines and other reactive intermediates. His group established the relevance of neonicotinoid metabolites with iminium-type structures to their acute and chronic toxicity to mammals and interpretation of the long-term safety of this class of insecticides. His team probed insect nAChR interactions *in vivo* with neonicotinoid, organophosphorus and methylcarbamate insecticides, which bettered understanding of the action of these three principal insecticide chemotypes (33). Interestingly, his work uncovered the links between the neonicotinoid insecticides and salicylate-associated plant defence responses which effect a similar global transcriptional response to that of salicylic acid, including genes involved in (a)biotic stress responses (31). Those findings have laid a solid foundation for risk assessment of the studied pesticides, particularly when molecular action mechanism and 'the risk cup' are considered. The analogy of a 'risk cup' is used to describe aggregate exposure estimates. The US Environmental Protection Agency uses the risk cup as a conceptual approach to determine the 'cumulative risk' posed by the groups of pesticides with common mechanisms of action.

Insect growth regulators and chitin synthesis inhibitors

In addition to neuron toxicants, John contributed greatly to hormonal insect growth regulators and chitin synthesis inhibitors. In 1978, investigations of the potent benzoylphenyl urea class of chitin synthesis inhibitors took place in his laboratory, describing the quick and direct action of diflubenzuron analogues within the insect integument to block the terminal polymerization step in chitin formation. This discovery paved the path to develop fungal and insecticidal chitin synthesis inhibitors (8,9).

Herbicide safeners

A paper published in 1975 from the Casida laboratory initiated the field of herbicide chemical safeners. The study described the amazing ability of dichloroacetamides in preventing the injury of maize (*Zea mays*) by the major thiocarbamate and chloroacetanilide herbicides without affecting the sensitivity of weeds (6). This discovery led to practical applications of chemical safeners to selectively control weeds. The dichloroacetamide safeners, such as benoxacor and dichlormid, used with the herbicides metolachlor and EPTC (*S*-ethyl-*N*,*N*-dipropylthiocarbamate), respectively, induce the crop-specific synthesis of glutathione and glutathione *S*-transferases which rapidly detoxify the metabolically-activated herbicide. Later, John's team used a radioligand synthesized in his laboratory and verified that the dichloroacetamide safeners competitively bind to the same site as the thiocarbamate and chloroacetanilide herbicides (16). In general, detoxification plays a major role in pesticide action and resistance. Genetically modified and chemical-safener-modified crops often involve herbicide detoxification by design to achieve the required crop tolerance (38).

Pesticides and public health

In addition to protection of crops and domestic animals, John's research has significant implications for West Nile virus and malaria through the development of pesticides suitable for mosquito control. His decades of effort on pyrethrum and pyrethroids are of critical importance, since these agents are the most effective tools for control of vectors of West Nile virus and malaria, with approximately 300 million cases and one million deaths per year of children in Sub-Saharan Africa. His fundamental research on the chemistry, stereochemistry, structure–activity relationship, metabolism, photolysis and mode of action of pyrethrum and pyrethroids ultimately has had a major influence on pyrethroid discovery, development and safety evaluations. Related investigations demonstrated that important insecticide synergists act as both substrates and inhibitors for microsomal cytochrome P450-dependent mixed-function oxidases, thereby blocking detoxification of pyrethroids and other insecticides, prolonging their persistence and increasing their potency and cost effectiveness.

His research has also significant implications for Parkinson's disease (PD). He proposed a catecholaldehyde hypothesis that the autotoxic dopamine metabolite DOPAL plays a pathogenic role in PD (35). His team defined the mechanism by which dithiocarbamate fungicides such as benomyl and their metabolites induce a PD-type neurodegeneration in mice and the possible relevance of these changes to human PD (32,35). Benomyl exposure in primary mesencephalic neurons inhibits aldehyde dehydrogenase (ALDH) and alters dopamine homeostasis. It induces selective dopaminergic neuronal damage *in vitro* in primary mesencephalic cultures and *in vivo* in animal models. The epidemiology study supports the association of higher exposure to benomyl with increased PD risk. This ALDH model for

PD aetiology helps to explain the selective vulnerability of dopaminergic neurons in PD and provides a potential mechanism through which environmental toxicants contribute to PD pathogenesis.

John's research was dynamic, well extended and in-depth. It spanned from practical agriculture, classical entomology and fundamental toxicology, to basic biology and biochemistry. His research addressed significant scientific questions in a timely manner, which was in accord with the organizational changes and the change of his laboratory name from PCTL to ECTL in 1994 (Quistad 2000). The Department of Entomology where he worked in Berkeley also evolved and was merged with several departments into the Department of Environmental Science, Policy and Management in 1993.

John Edward Casida: his legacy

As John's career advanced there was always the sparkle of new concepts, ranging from how absinthe works as a liquor (17) to lipases in neurotoxicology (19). His diving into new fields might have been because there were always deeper and more interesting aspects to investigate in the fields that his laboratory opened. In the 2010s, as he was going deeper and deeper into the fields he pioneered, it was a time of the university withdrawing space and resources from his programme, making it harder to run a major research laboratory and compete as one of the longest-ever continuously funded NIH programmes for more than 50 years. Thus, during this period John turned even more attention to his teaching and to writing reviews and perspectives of the field. Between 2015 and 2018 he published over a dozen reviews and perspectives that light up a path toward fruitful and impactful research in the fields. Those reviews and perspectives covered pesticide toxicology, GABA receptors, lipases, novel metabolic reactions and pesticide secondary targets, prodrugs and propesticides, OP toxicology, pesticide detoxification, radioligands and neonicotinoids and other insect nicotinic receptor competitive modulators. At the time he passed away, he was outlining several subsequent reviews in pesticide toxicology. John was the pesticide toxicologist of his generation, based on his research, his leadership and the impact of the many alumni from his laboratory. With pesticide toxicology as his base, his insight and brilliance illuminated multiple adjunct fields and inspired generations of scientists.

In this remembrance we have touched on but a few of the many contributions of the Casida laboratory and John's collaborators. These were selected in large part to illustrate our perception of John's approach to research. Some of these areas remained lifelong but minor interests of his, but never had the recognition to attract funding. Other areas probably are evidence of John 'tasting' a new field for possible future interests. Many of the fields he tasted went on to become major research endeavours, often led by the alumni of his laboratory. The authors apologize for the many topics and scientists not mentioned here. Perhaps it is a consolation that the authors themselves during their time in the Casida laboratory were confined to what he referred to as 'low priority projects' not covered in this remembrance; yet, these low priority projects shaped both of our subsequent careers. John was well known for his ability to match scientific talents with scientific questions. There is no space to discuss the cross-fertilization of ideas among fields, and our attempt has been just to exemplify John's extraordinary ability to focus on the most important and exciting science with enthusiasm, originality and success.

John always gave great credit to Berkeley, the city, and UC Berkeley, the campus, for his wonderful personal and professional life. He once said Berkeley is not so much a place as a spirit and a time. John was fortunate to be in a lovely old building, Wellman Hall, of great historical significance. Many visitors to the mecca of John's laboratory were aghast at the facilities; however, the basement of Wellman Hall buzzed with creativity and excitement. Productivity clearly was not constrained by facilities in John's case. Without overtly encouraging laboratory personnel to initiate collaborations, John somehow facilitated an intellectual ferment that extended far beyond his own laboratory and continued in the careers of many of his alumni.

John's legacy is his science, and this knowledge fostered subsequent science. A second legacy is the scientists he mentored in his career and the next generation who grew up with tales of 'when we were in John's laboratory'. At scientific meetings there is always a period of informal 'Casida tales' ranging from practical jokes that extend for decades to stories of John and the charming eccentrics in his laboratory. There is also a uniform awe and respect among his alumni. John set a high standard of ethics as well as work ethic in the field (the latter tempered by the caution to 'never keep Kati waiting'). What drives any of us, and particularly John Casida? Clearly wealth and fame were not important drivers, but there was a competitive spirit. The success of his many alumni brought him pleasure. We are sure John appreciated the tremendous contribution his career made to pesticide toxicology, the environment, human health and agriculture. However, in talking to numerous Casida alumni, the conclusion was that he did it for the fun of science (Hammock & Casida 1998).

Education, appointments, honours and awards

Education

1951	BS, entomology, University of Wisconsin, Madison
1952	MS, biochemistry, University of Wisconsin, Madison
1954	PhD, joint major in entomology and biochemistry with botany minor, University of Wisconsin, Madison

Appointments

1954–1963	Assistant, associate and full professor of entomology, University of Wisconsin, Madison
1958–1959	Visiting researcher, University of Stockholm and several European laboratories
1964–2018	Director of the Environmental Chemistry and Toxicology Laboratory, University of California, Berkeley
1964–2007	Professor of entomology and toxicology, University of California, Berkeley
1970–1971	Visiting researcher, University of Cambridge, University of Stockholm and Kyoto University
1986–1987	Visiting researcher, University of Crete (Greece) and Centre National de Recherche Scientifique (Gif-sur-Yvette, France)
1996–2018	William Muriece Hoskins chair in chemical and molecular entomology, University of California, Berkeley
2008–2018	Professor of the Graduate School, University of California, Berkeley

Fellowships

1958 Haight fellow
1970 Guggenheim fellow
1989 Fellow, Entomological Society of America
1991 Member, United States National Academy of Sciences
1997 Honorary Member, Society of Toxicology
1998 Foreign Member, Royal Society
2004 Member, European Academy of Sciences
2005 Honorary Member, Pesticide Science Society of Japan

Awards

1969 International Award for Research in Pesticide Chemistry, American Chemical Society
1978 Spencer Award for Research in Agricultural and Food Chemistry, American Chemical Society
1988 Distinguished Service Award for Research, USDA
1989 J. E. Bussart Award, Entomological Society of America
1992 Sterling B. Hendricks Memorial Lectureship, Agricultural Research Service, USDA and American Chemical Society
1993 Wolf Foundation Prize in Agriculture
1994 Founders Award, Society of Environmental Toxicology and Chemistry
1995 Koro-Sho Prize, Pesticide Science Society of Japan
2009 Career Achievement Award, College of Natural Resources, University of California, Berkeley
2009 Distinguished Service Award, American College of Toxicology

Lectureships

1983 Jeffery Lectureship, University of New South Wales, Australia
1985 Messenger Lectureship, Cornell University at Ithaca
1987 Yabuta Lectureship, Fukuoka University, Japan
1987 Botyu-Kagaku Lectureship, Kyoto University, Japan
1997 Honorary Doctor Degree and Third World Academy of Sciences Lectureship, University of Buenos Aires, Argentina
1998 Faculty Research Lecturer, University of California, Berkeley

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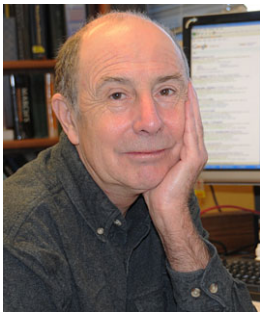
The portrait photograph was taken in 1998 by Prudence Cuming Associates and is © the Royal Society.

Author profiles

Bruce D. Hammock



Bruce D. Hammock is a distinguished professor in the Department of Entomology and Nematology and the Comprehensive Cancer Center at UC Davis, USA. After receiving his BS at Louisiana State University in 1969, he moved to UC Berkeley, where he did a PhD with John E. Casida. His work focused on synthesis, evaluation and metabolism chemistry of mimics of insect juvenile hormones as green pesticides. JEC, as he was often known, remained a lifelong mentor, inspiration and friend.

After graduation in entomology and toxicology in 1973, Bruce entered the US Army as a medical officer and then was a Rockefeller Foundation postdoctoral scientist with Larry Gilbert in biochemistry at Northwestern University, studying insect developmental biology. In 1975 he moved to UC at Riverside as a toxicologist. There, he continued studying insect developmental biology and pesticide chemistry, and pioneered making immunoassays for the analysis of pesticides in the environment. Based on his PhD work, and in collaboration with Sarjeet Gill, he continued exploring an epoxide hydrolase enzyme in mammals they found in the Berkeley laboratory of JEC. The enzyme degraded inflammation- and pain-resolving natural lipids.

In 1980 he moved to UC Davis, where he initiated a programme on the production of natural recombinant baculoviruses for insect control. This work continued during a year at the University of Oxford. While at UC Davis he published a joint biography of JEC with John's wife, Kati. Increasingly, Bruce began to emphasize the role of the arachidonate cascade in human physiology. This work led to a pain-relieving pharmaceutical now in human trials based on inspiration from JEC almost 50 years earlier. Professor Hammock has won the Distinguished Teaching and the Distinguished Research awards from his university and is a member of the National Academy of Sciences and National Academy of Inventors.

Qing X. Li

Qing X. Li is a professor in the Department of Molecular Biosciences and Bioengineering, University of Hawaii (UH) at Manoa, Honolulu, USA. He received his BS in agriculture from Shandong Agricultural University, China. In 1986, he went to UC Davis to pursue his PhD in agricultural and environmental chemistry under the guidance of Bruce D. Hammock and James N. Seiber. After his doctoral degree, he joined the Casida laboratory in UC Berkeley in 1991, where he synthesized 1,3-dithiane inhibitors and chemical probes for the characterization of the non-competitive blocker site of the GABA-gated chloride channel. Not long before John's passing, Li had a long phone conversation with John about his health, family, lab closing processes, teaching, review manuscripts and research as well as mentoring and advice.

Li joined the UH Manoa in 1995. He was director of the pesticide residue chemistry laboratory at the UH Manoa from 1995 to 2013. Since 2011, he has served as director of

the UH Manoa Proteomics core facility. His research has been centred on agrochemicals, with emphasis on immunoassays, functional proteomics, pesticide chemistry and transformation and environmental fate of agrochemicals. He has remained interested in the mechanism of action of bioactive compounds and the GABA receptor, which he started investigating in the Casida laboratory. Recently, he began to explore potential values and mechanism of action of natural products against Alzheimer's disease and obesity. He has served as an associate editor for the *Journal of Agricultural and Food Chemistry* since 2015. He has received the University of Hawaii research medal, and is a member of the National Academy of Inventors.

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