ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

Research of Pesticide Metabolites in Human Brain Tumor Tissues by Chemometrics-Based Gas Chromatography−Mass Spectrometry Analysis for a Hypothetical Correlation between Pesticide Exposure and Risk Factor of Central Nervous System Tumors

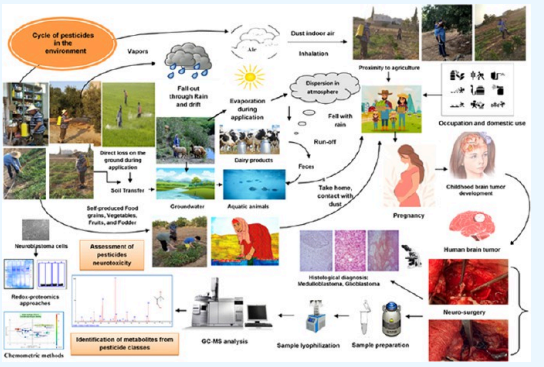
Kaouthar Louati,* Fatma Kolsi, Rim Kallel, Yassine Gdoura, Mahdi Borni, Leila Sellami Hakim, Rania Zribi, Sirine Choura, Amina Maalej, Sami Sayadi, Mohamed Chamkha, Basma Mnif, Zouheir Khemakhem, Tahya Sellami Boudawara, Mohamed Zaher Boudawara, and Fathi Safta

Cite This: *ACS Omega* 2023, 8, 29812−29835

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Pesticides are widely used, resulting in continuing human exposure with potential health impacts. Some exposures related to agricultural works have been associated with neurological disorders. Since the 2000s, the hypothesis of the role of pesticides in the occurrence of central nervous system (CNS) tumors has been better documented in the literature. However, the etiology of childhood brain cancers still remains largely unknown. The major objective of this work was to assess the potential role of pesticide exposure as a risk factor for CNS tumors based on questionnaires and statistical analysis of information collected from patients hospitalized in the Neurosurgery Department of the Habib Bourguiba Hospital Medium in Sfax, Tunisia, during the period from January 1, 2022, to May 31, 2023. It also aimed to develop a simple and rapid analytical method by the gas chromatography−mass spectrometry technique for the research traces of pesticide metabolites in some collected human brain tumor tissues in order to more emphasize our hypothesis for such a correlation between pesticide exposure and brain tumor development. Patients with a history of high-risk exposure were selected to conduct further analysis. Chemometric methods were adapted to discern intrinsic variation between pathological and control groups and ascertain effective separation with the identification of differentially expressed metabolites accountable for such variations. Three samples revealed traces of pesticide metabolites that were mostly detected at an early age. The histopathological diagnosis was medulloblastoma for a 10-year-old child and high-grade gliomas for 27- and 35-year-old adults. The bivariate analyses (odds ratio >1 and *P* value <5%) confirmed the great probability of developing cancer by an exposure case. The Cox proportional hazards model revealed the risk of carcinogenicity beyond the age of 50 as a long-term effect of pesticide toxicity. Our study supports the correlation between pesticide exposure and the risk of development of human brain tumors, suggesting that preconception pesticide exposure, and possibly exposure during pregnancy, is associated with an increased childhood brain tumor risk. This hypothesis was enhanced in identifying traces of metabolites from the carbamate insecticide class known for their neurotoxicity and others from pyridazinone, organochlorines (OCs), triazole fungicide, and N-nitroso compounds known for their carcinogenicity. The 2D-OXYBLOT analysis confirmed the neurotoxicity effect of insecticides to induce oxidative damage in CNS cells. Aldicarb was implicated in brain carcinogenicity confirmed by the identification of oxime metabolites in a stress degradation study. Revealing "aziridine" metabolites from the OC class may better emphasize the theory of detecting traces of pesticide metabolites at an early age. Overall, our findings lead to the recommendation of limiting the residential use of pesticides and the support of public health policies serving this objective that we need to be vigilant in the postmarketing surveillance of human health impacts.



1. INTRODUCTION

Pesticides are toxic agrochemicals used for crop protection against insects, fungi, and pests. Their widespread use in various agricultural practices not only resulted in environmental contamination but also entailed a direct exposure to a population from a variety of sources, including residues in water, air, dust, and foodstuff,[1−4] which represent routes of long-term exposures that have caused potential health problems, such

Received: June 27, 2023
Accepted: July 25, 2023
Published: August 3, 2023

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

as respiratory diseases, neurological dysfunctions, cancers, and reproductive disorders.[5−11]

Pesticides' hazardous effects are seriously perceived by many international organizations, like the U.S. Environmental Protection Agency (US-EPA), the World Health Organization (WHO), and the Food and Agriculture Organization (FAO) of the United Nations.[12,13] However, the insecurity problem may persist due to either the use of some banned toxic products that may escape from control or the low level of education particularly in low-development countries, where some farmers do not respect the recommendations indicated by the supplier and multiply the doses and frequencies for better production, which poses a great danger for human and environmental health.

Pesticides are classified into a number of types (defined in the Supporting Information). They display a variety of biological modes of action in both target and nontarget organisms.[14] The central nervous system (CNS) is the main target of acute toxic action by diverse insecticides including organophosphates (OPs), carbamates (CARs), organochlorines (OCs), fungicides, and fumigants.[5,15,16] Various epidemiologic studies have been investigated to assess pesticide's human health risk with a special focus on agricultural settings.[17] Farmers were revealed to be at a greater risk for brain cancer as well as psychiatric and neurological disorders compared to other professions.[18−21] Parental occupation and home proximity to treated fields have also been used to assess pesticide exposures among children since pesticides may affect neurodevelopment.[22]

Mass spectrometry (MS) and nuclear magnetic resonance (NMR) have emerged as the most common analytical tools in metabolomics studies.[23−25] Despite an improvement in the sensitivity and reproducibility of NMR spectroscopy, it remains less superior and nonselective to the targeted analysis, compared with MS. Indeed, MS is a high-throughput technology that has been steadily emerging in "omics" studies to provide chemical information, isotope distribution patterns, and structural elucidation through the characteristics of parent and fragment ions. In addition, due to the development of different ionization techniques and mass analyzers, it is now possible to conduct metabolomics analysis with high sensitivity and accuracy in mixed samples with a large number of detected metabolites at picomole and femtomole levels.[23−25]

Mass spectrometers are typically hyphenated to either gas chromatography (GC) or liquid chromatography (LC) to facilitate the separation of complex biological mixtures and achieve comprehensive biochemical assessment.[26,27] The main advantages of using GC-MS include high separation efficiency, high resolution, and reproducible chromatographic separations due to modern capillary GC. Although LC-MS is more widely used in metabolic studies, GC-MS is well suited for the qualitative and quantitative analyses of volatile compounds or compounds that can be derivatives to become volatile.

Thus, the motivation behind this study is to assess the potential role of pesticide exposure as a risk factor for human brain tumors based on questionnaires and geographic information collected from patients hospitalized in the neurosurgery department of the Habib Bourguiba university hospital in Sfax, Tunisia, during the period from January 1, 2022, to June 30, 2022. Then, the research traces pesticide metabolites in some collected brain tissues by using the GC-MS technique as a powerful tool since many pesticides are volatile, and the inhalation route is the most common route through which farmers get exposed to pesticides.

2. MATERIALS AND METHODS

2.1. Ethical Considerations. The procedure carried out in this work was in accordance with the Helsinki Declaration. It was approved under the number CPP SUD-0443/2022 by our medical human research committee (Ethics committee) related to the Habib Bourguiba university hospital where the work was conducted. Zouhir et al., specialized in legal and toxicological medicine, facilitated the use of human brain tumor tissues in the experimental part of my paper by confirming that the study is well in accordance with the Code of Ethics of the World Medical Association.

The selected participants gave their agreement to such research. A written informed consent was obtained from each patient before undergoing any neurosurgery for possible risk of paralysis or mortality. Information collected through a questionnaire was kept confidential.

2.2. Population Study Design. The study population corresponds to patients attending the neurological surgery center of the Habib Bourguiba Hospital Medium for CNS tumor surgery during the period from January 1, 2022, to May 31, 2023.

2.2.1. Questionnaire. In order to collect data from patients, face-to-face interviews were conducted and a structured questionnaire was developed based on some epidemiological studies in the literature.[17,28−31] The questionnaire included four different sections: (1) socio-demographics, (2) residence, (3) occupation, and (4) exposure and agricultural practice. The socio-demographic section involves age, sex, family situation, and educational level. The residence section includes the localization level (urban, suburban, or rural). In the occupational section, the history of crops on farms and detailed information on the origin of agricultural activities were reported as well as details on chronic exposure among farmers and farmworkers who are not farm owners or operators and do not often know what pesticides have been used on crops but can accurately report the crops worked on. Patients who reported at least one year of agricultural occupation were asked to determine pesticide exposure and if possible to provide the names of the products used, the number of applications per year, application methods, and personal protective equipment (PPE) usage (such as waterproof gloves, disposable suits, masks with a filter cartridge).

Parental occupation and home proximity to treated fields have been used to assess pesticide exposures among children since pesticides may affect neurodevelopment. Additional information, such as the type of crop, pest, and agricultural activities (farm manager, worker, or rancher), duration of farming, the area of farming surface, accuracy of the pesticide label, eating habits during pesticide application, and showering after spraying, was also collected.

From all of these collected information, patients could be classified into two subgroups with an agricultural activity: high and low intensity of risk exposure based on the duration of exposure since acute and chronic exposure may exhibit different mechanisms, frequency of works, exposure doses (e.g., dose/hectare in kg), educational levels for security measurement, absence of appropriate PPE protection, etc. Afterward, to avoid challenges with brain cancer etiologies and to best define the subgroup population of "exposure cases" on whom our further analyses were conducted, some inclusion and exclusion criteria were established.

2.2.2. Inclusion Criteria. The selected population of our study corresponds to patients operated for brain tumors with the

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

history of working within agricultural sectors as their main or secondary occupation and/or living in rural regions that can be exposed via distinct routes. For further GC-MS analysis, since the research of trace metabolites whether in urine, blood, or tissues entails a high cost and cannot be used for large-scale studies, only patients who reported a history of high-risk exposure to pesticides were taken into consideration.

2.2.3. Exclusion Criteria. Since smoking and alcohol are known as risk factors for carcinogenicity, all of the selected patients were nonsmokers and nonalcoholic in order to eliminate their causative effects and avoid challenges. Also, they did not receive hormonal therapy, chemotherapy, or radiotherapy before the surgery. Patients who had never worked within agricultural sectors as their main or secondary occupation and had lived in urban and suburban regions were not considered. In addition, those who had either a positive family history of human brain cancer or a previous surgery and came for recurrence or metastasis were also excluded.

2.3. Chemicals and Reagents. All solvents and reagents used in this study were of analytical grade. Phosphate-buffered saline (PBS) (D8537), Tween-20 (9005-64-5), sodium dodecyl sulfate (SDS) (436143), hydrogen peroxide 30% (7722-84-1), dichloromethane (75-09-2), and pyridine (110-86-1) (all with purity >99.0%) were purchased from Sigma-Aldrich laboratories. The chromatographic-grade chemical *N*,*O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) was from Macherey-Nagel laboratories (Düren, Germany).

Aldicrab, ($C_7H_{14}N_2O_2S$), a methyl-2-(methylthio)-2-propionaldehyde-*O*-methyl-carbamoyl-oxime, (CAS#116-06-3), with purity 99.0−100%, was from Chem-Service, Greyhound laboratories, Birkenhead, U.K.

2.4. Brain Tumor Tissue Collection. During surgery, immediately after brain tumor resection, samples were collected rapidly (average processing time ≤60 s minimizing any potential changes), washed with cold PBS solution, placed in a microtube soaked in serum 0.9%, and immediately frozen in liquid nitrogen to be stored at −80 °C until subsequent analysis. In the same way, we collected specimens of brain tissues derived from temporal lobe epilepsy surgeries, which are generally used as experimental controls in research studies.[32]

2.5. Sample Preparation and Extraction. For sample analysis, tissues were thawed, vortexed for 30 s, homogenized in ice in 10 volumes of water for injection using a T10 basic Ultra-Turrax homogenizer (IKAm Group, Staufen, Germany), and then centrifugated at 2500 rpm, 4 °C, 5 min to remove debris. After this, tissue homogenates were collected, lyophilized using a freeze-dryer (CHRIST), and then ground. Each sample was accurately weighed (15.3 mg) and dissolved into 0.5 mL of dichloromethane and then centrifuged for 5 min at 2500 rpm for extraction. The supernatant was collected for derivatization. For 100 μL of supernatant, 100 μL of pyridine and 200 μL of BSTFA were added, and the sample was heated at 70 °C for 1 h before injection. 1 μL of the extract was injected into GC-MS for qualitative analysis.

2.6. GC-MS Analysis. *2.6.1. Analytical Procedure.* The GC-MS analysis was equipped with an Agilent 7000 GC/MS Triple Quad, GC system 7890A, and an autosampler 7693. Helium was used as a carrier gas at a flow rate of 1.0 mL/min. Nitrogen was used as a collision gas. Separations were conducted using an HP 5-MS 19091S-433 (30 m × 0.25 mm; 0.25 μm) column. The ion source was operated by electron impact ionization. The injection volume was 1 μL, and the injector temperature was held at 280 °C. Using an automatic injection system, samples were analyzed as follows: the temperature program was set for an initial temperature of 70 °C (held for 2 min), increased to 150 °C at 25 °C/min (held for 1 min), raised to 200 °C at 3 °C/min (held for 1 min) and, finally, increased to 280 °C at 8 °C/min (held for 15 min). The mass spectrometer was operated in the scan mode, between m/z 45 and m/z 520 Da. Injection occurred in the splitless mode. Matching of chromatographic peaks (by >80%) using the National Institute of Standards and Technology (NIST) MS database Library (2014) was a qualitative criterion for metabolites. Metabolite data (compound name, retention time, and peak area) were obtained.

2.6.2. Raw Data Processing. Data from GC/MS analysis were converted to the NetCDF format via the data analysis interface of the Agilent Instrument (Agilent Technologies), and pretreatment procedures were carried out using custom scripts in MAT-LAB 7.0 (The MathWorks, Natick) such as baseline correction, peak deconvolution and alignment, exclusion of solvent peaks, and normalization to a total chromatogram. Three replicates for each sample were used for calibration.

2.7. Proteomic Study. *2.7.1. Culture of SH-SY5Y Cells.* Human neuroblastoma cells (*SH-SY5Y*) were obtained from the European Collection of authenticated Cell Culture (ECACC, U.K.), seeded at a density of 1×10^6 cells/mL into T75 flasks and cultured in DMEM (D5796, Sigma) supplemented with Ham's F-12 nutrient (51651C, Sigma), 1% nonessential amino acid solution (M7145), 10% fetal bovine serum (FBS) (F2442, Sigma), 100 U/mL penicillin, 100 μg/mL streptomycin (P4333), and 2 mM L-glutamine (G7513) in a humidified incubator (Thermo-Fisher Scientific Inc., 3110, Waltham, MA) at 5% CO_2/37 °C. Media was changed every 2 days. Cells were allowed to reach 80% confluence before exposure to pesticides.

2.7.2. Study of Neurotoxicity Effects. Pesticides were prepared in ethanol (99.5%) as 50 mM stocks. Experimental concentrations were prepared by serial dilution in 10% FBS media. Cells were seeded into three separate 96-well plates; then, the culture media was removed and 100 μL of fresh medium containing pesticides (at increasing concentrations from 10 to 50 μM) was added to each well and incubated for 24 h with the aim of calculating the IC_{50} value (inhibitory concentration for 50% cell death) by the respective dose−response curve. Cells without treatment served as background controls. Thereafter, cell viability was assessed using the MTT assay kit (Abcam, U.K.).

2.7.3. Protein Extraction. At the end of pesticide treatments at IC_{50}, *SH-SY5Y* cells were washed several times in cold PBS and then scraped off the plate and lysed in a modified RIPA lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% triton X-100, 5 mM EDTA pH 8.0) containing 1× protease inhibitor cocktail (HALT, Thermo Scientific), and phosphatase inhibitor cocktail (1 mM $NaVO_4$, 50 nM NaF), for 30 min on ice. Cell lysates were centrifuged at 10 000 rpm, 15 min at 4 °C to produce a crude cytosolic preparation; then, protein concentrations were assayed by the modification of the Lowry assay (Bio-Rad, Hertfordshire, U.K.).

2.7.4. Solubilization and Denaturation of Proteins. Proteins were diluted with 10 mM Tris-HCl buffer pH 7.5, Novex 10% reducing agent (Invitrogen, U.K.) and 4× LDS sample buffer (Invitrogen, U.K.), then heated at 70 °C for 10 min, centrifuged at 5000*g* for 5 s, and denatured with an equal volume of 12% SDS buffer.

2.7.5. Protein Derivatization. Protein carbonyls were derivatized with DNPH (2,4-dinitrophenylhydrazine) immedi-

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

ately before electrophoresis using 1% DNPH in HCl 2N for 15 min at room temperature in the dark. Next, we added the neutralization solution supplied with the assay kit (Abcam, U.K.) as well as 2-mercaptoethanol at 5% v/v.

2.7.6. SDS-PAGE and Western Blot Analysis (2D-OXYBLOT). Protein extracts (20 μg proteins/lane) were separated by SDS-PAGE using 10% Bis-Tris NuPAGE Novex Gel (Fisher Scientific, Loughborough, U.K.) according to the protocol described in the Supporting Information. After running, gels were separated and transferred to PVDF (poly(vinylidene difluoride)) membranes using a Trans-Blot Turbo Transfer System (Bio-Rad Laboratories, U.K.) for 100 min at 125 V. Afterward, PVDF membranes were removed and blocked in 5% milk in PBST (PBS, pH 7.2−7.5, 0.05% Tween-20) overnight at 4 °C. Membranes were washed in PBST and then incubated overnight at 4 °C with primary antibody mouse monoclonal anti-DNP (dinitro-phenyl-hydrazone) (Sigma-Aldrich Co., St. Louis, Cat# A2831, RRID: AB_258033) at 1:1000 dilution. The following day, membranes were washed with PBST and incubated at room temperature with corresponding horseradish goat antimouse IgG/peroxidase (HRP)-conjugated secondary antibody (polyclonal goat antimouse IgG; HRP, Millipore Cat# AP340P-50 ML, RRID: AB_1587164) diluted at 1:1000. After 1 h of incubation, membranes were washed in PBST and soaked with Western Enhanced Chemiluminescence substrates supplied by the kit (Bio-Rad, Hertfordshire, U.K.). The emitted chemiluminescent signals were detected by a ChemiDoc MP digital imaging system (Bio-Rad, Hertfordshire, U.K.), and protein visualization was captured by Image Lab 5.0 software (Bio-Rad).

2.8. Statistical Analysis. For the population design analysis, a bivariate analysis model was used with the significance threshold set at 5% (P values ≤0.05). The odds ratio (OR) was calculated by the MedCalc Software Ltd. Odds ratio calculator (https://www.medcalc.org/calc/odds_ratio.php (Version 20.113; accessed August 7, 2022)). On multivariate analyses adjusted for the status of exposure (exposure to pesticides versus nonexposure) and gender as predictable variables with age as the time axis, instantaneous hazard risk ratios were estimated by a Cox proportional hazards model. The statistical results and regression curves were performed with SPSS Statistic software (Version 20).

For chemometric techniques, data sets generated from GC-MS analysis were mean-centered and pareto-scaled before the performance of the multivariate statistical analysis by SIMCA-P Version 14.0 software for principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA). These analyses were carried out on the metabolomics of each tissue divided into two groups: pathological samples with positive results versus control samples. PCA is an unsupervised pattern recognition method used to grasp data in general, whereas PLS shows effective separations between groups by relating the design matrix X (e.g., different extracted metabolites) with matrix Y (e.g., peak area of each resolved GC-MS peak). PLS-DA, a derived method of PLS in which the Y matrix is usually set as a descriptor (e.g., 0/1/2), was employed to maximize biological variations and identify differentially expressed metabolites accountable for such variations by calculating the variable influence in the projection (VIP). In parallel, univariate data analysis was set using the Student t-test implemented in GraphPad Prism 8.0 software (San Diego, CA). Thus, differentially expressed metabolites were screened based on P (<0.01) and VIP (>1) values.

Default cross-validation in the SIMCA-P software package was used with one-sixth of the sample being pre-eluted from the PLS-DA model in each round to confirm the reliability of the regression model. Then, to ascertain the validity of the PLS-DA model, a permutation test (20 times) was undertaken. The intercepts of R^2 and Q^2 on the Y-axis in the permutation test reflect a measure of overfit.

Experiments by GC-MS and SDS-PAGE were carried out at least in triplicate. Data were expressed as mean ± SD (standard deviation). Statistical comparisons on Western blot analysis were performed using one-way analysis of variance (ANOVA) by GraphPad Prism 8.0 Software. Differences were considered statistically significant when $P < 0.05$.

3. RESULTS

3.1. Characteristics of the Study Population and Descriptive Analysis. Questionnaires have been used successfully. Our first step consisted in listing the adjustment variables classically used in the recent literature for brain tumor risk assessment in agriculture. The collected information is described in Table 1 for population characteristics, including sex, age, residence, and pesticide exposure with high and low exposure risk. Overall, during the period of our study, there were 114 cases of brain tumor surgery and 1607 controls (exhibiting other neurological diseases) for the entire study population (N =

Table 1. Summary of the Population Study Characteristics with Statistical Analysis[a]

study design				
characteristics of patients with brain tumors	age (years)	0−10	7	n_1 = 114 (6.62%)
		11−20	11	
		21−30	13	
		31−40	15	
		41−50	21	
		51−60	17	
		61−70	19	
		>70	11	
	sex	F	39	
		M	75	
	exposed cases	low-risk exposure	45	
		high-risk exposure	20	
	unexposed cases		49	
control group (patients with other neurological diseases)	exposed non-cases		369	n_2 = 1607 (93.38%)
	unexposed non-cases		1238	
the entire study population				N = 1721 (100%)
statistical analysis				
odds ratio				4.4505
95% of CI[b]				3.0175−6.5642
z statistic				7.530
significance[c] level				$P < 0.0001$
RR[d]	exposure status (exposure to pesticides versus non-exposure)			2.4831

[a]F, female; M, male. P, statistical significance. [b]CI, confidence interval. [c]The significance threshold for the bilateral statistical tests was set at 5% (P = 0.05). [d]RR: instantaneous hazard risk ratio (or relative risk) estimated in a Cox model adjusted for the status of exposure (exposure to pesticides versus non-exposure), with age as the time axis.

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

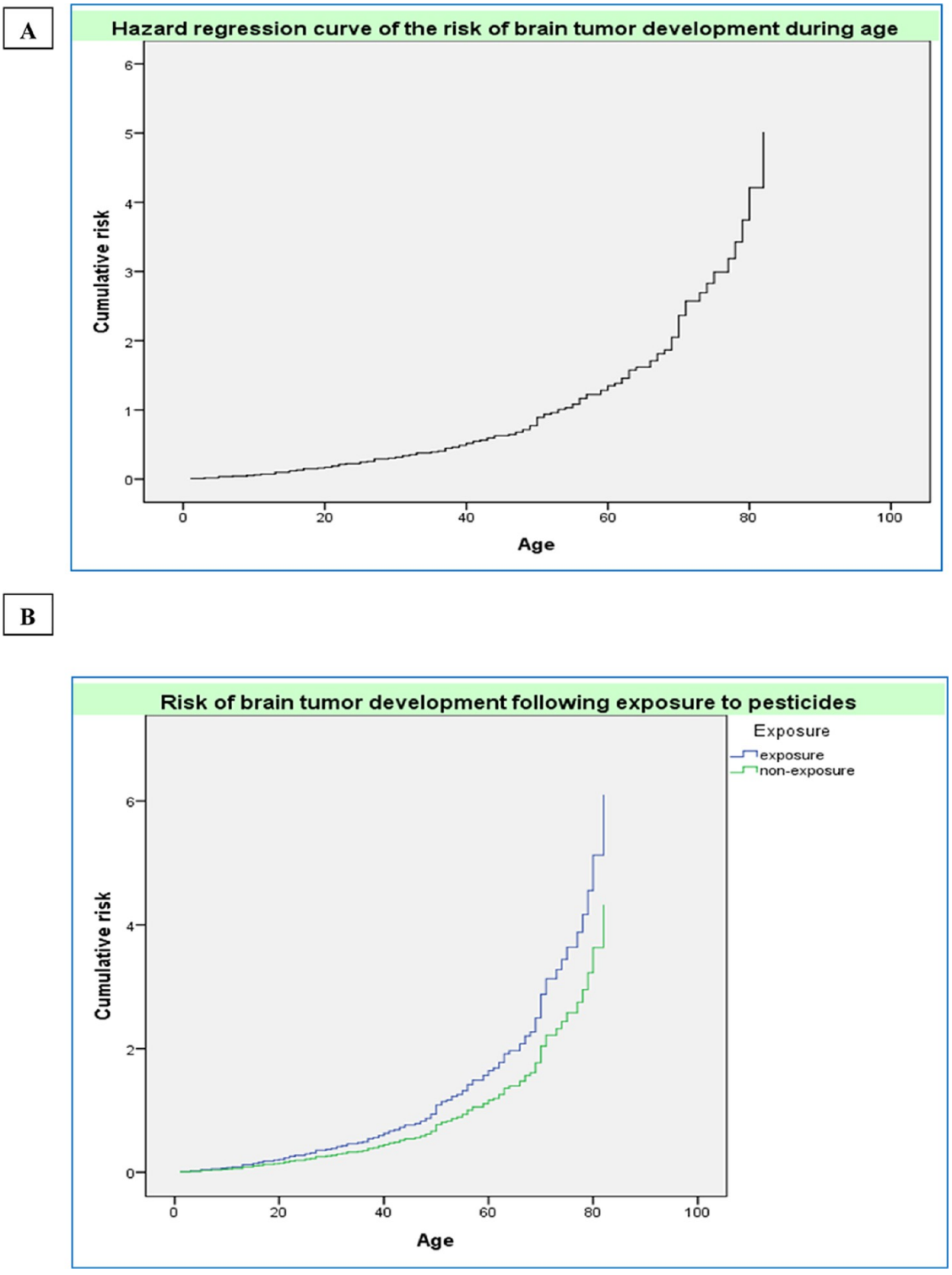


Figure 1. Hazard regression curve by the Cox model. (A) Brain tumor development following the age of the population study: the risk of brain tumor development beyond the age of 50 proves that carcinogenicity is a major risk factor for long-term effects of pesticide toxicity. (B) Brain tumor development following exposure to pesticides: the risk of brain tumor development is higher in exposure cases.

1721) classified according to pesticide exposure into four subgroups, namely, (a) exposed cases (whether at low- or high-risk exposure), (b) unexposed cases, (c) exposed non-cases, and (d) unexposed non-cases, with the aim of calculating OR, which represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure.

Statistical analyses are displayed in Table 1. The calculated OR was greater than 1.0, which means that the odds of exposure among case-patients are greater than the odds of exposure among controls. Thus, exposure might be a risk factor for the disease. *P* value (<0.0001) confirmed the lowest probability of association as a result of chance and the greatest probability of the disease related to exposure.

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

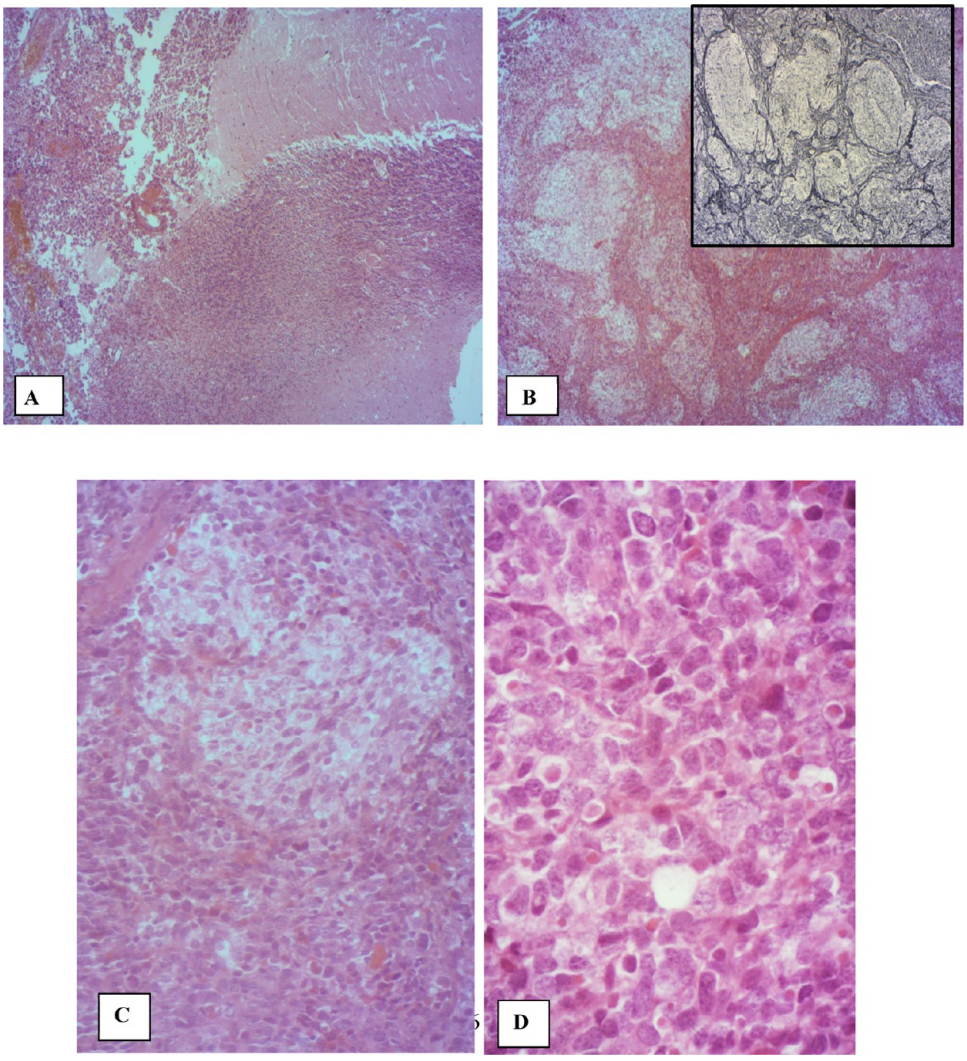


Figure 2. Representative photomicrographs of the histopathological identification of medulloblastoma obtained from brain tumor tissue sections for the positive case study of a 10-year-old child. (A) Cerebellum invasion by small-cell proliferation (magnification ×50). (B) Desmoplastic medulloblastoma: pale nodular areas surrounded by densely packed hyperchromatic cells (magnification ×100). Inset: Reticulin stain showing reticulin-free pale islands. (C) Pale nodular areas with neuronal differentiation (magnification ×200). (D) Anaplastic areas characterized by increased nuclear size and pleomorphism (magnification ×400).

Based on the COX proportional hazards model, statistical tests were significant (<5%), proving that the Cox regression model provides a better estimate of the cumulative hazard function than the baseline model. The estimated instantaneous hazard risk ratio (RR) adjusted for the "exposure" variable was positive and significant. Thus, the "exposure" variable can well predict the risk of brain tumor development by the model.

Graphs issued from the statistical analysis by the Cox proportional hazards model are presented in Figure 1A,B. The detailed demographic and clinical information of the selected patients with high exposure risks are reported in Table SI.

3.2. Histopathological Diagnosis of Brain Tumors for Positive Case Studies. The histopathological diagnosis identified by Kallel et al. for our patients' case studies whose samples yielded positive results were posterior fossa medulloblastoma for a 10-year-old child and high-grade gliomas (IDH1-mutant glioblastomas) for a 27- and a 35-year-old adult. Some representative photomicrophotographs of these identified brain tumors after formalin fixation and paraffin embedding techniques are presented, respectively, in Figures 2 and 3. Some neurosurgical photos are shown in Figure S1. Multiple sections from the temporal neocortex lobe epilepsy tissues used as control were analyzed both morphologically and immunohistochemically. The obtained results did not show any abnormalities by light microscopy. Furthermore, IHS with antibodies directed against phosphorylated neurofilament and synaptophysin proteins did not reveal any abnormal neurons in the cortex.

3.3. GC-MS Analysis for the Screening of Pesticide Metabolites in Human Brain Tumor Tissues. From all of the analyzed sets, three samples were positively identified for metabolites that strictly correlate with pesticide molecules and which could be issued by different catalytic pathways. It is the case of two agriculture farmers aged between 35 and 45 years and a 10-year-old child whose parents have well-reported agricultural occupations with high use of pesticides during many years in rural areas.

We succeeded in separating all of the traces of pesticide metabolites in the single and short run by the developed GC-MS analytical method. The typical chromatograms are shown in Figure 4. Overall, 10 compounds have been identified as

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

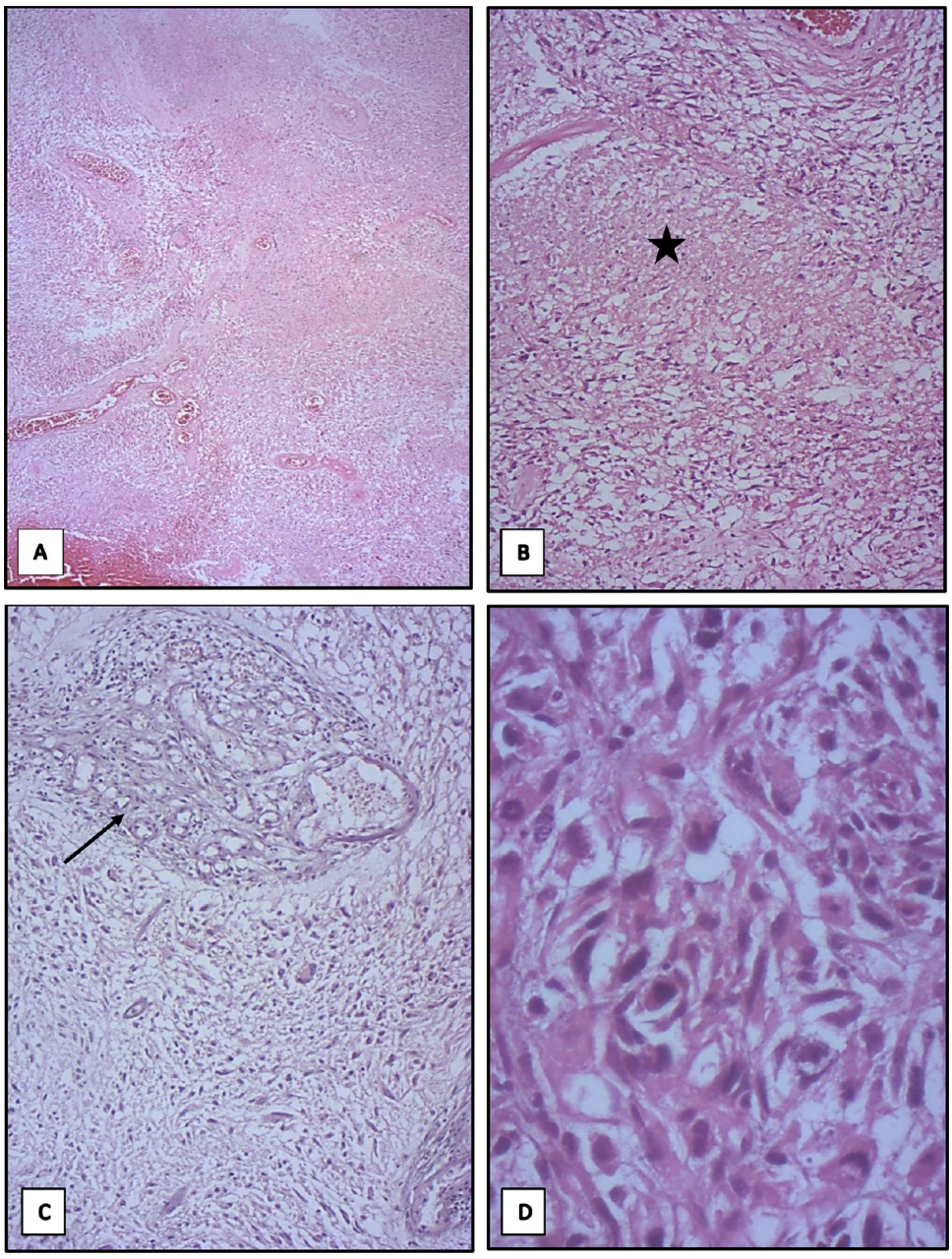


Figure 3. Representative photomicrographs of the histopathological identification of high-grade gliomas (WHO Grade IV) obtained from brain tumor tissue sections for the two positive case studies of a 27-year-old and a 35-year-old adult. (A) Cerebral infiltration by tumor diffuse proliferation (×25). (B) Tumor proliferation is focally necrotic (★) (×100). (C) Proliferated vessels (→) in the diffuse astrocytomas (Grade IV) with glomeruloid neovascular proliferation (×100). (D) Tumor cells are large with significant atypia (×400).

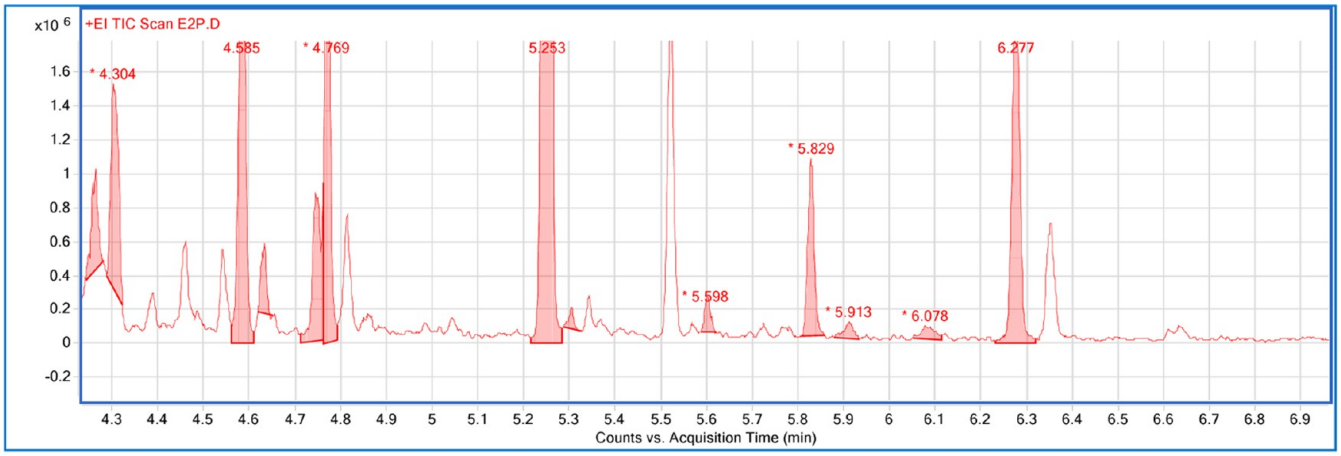
pesticides' metabolites by the use of the NIST database library with the criteria >80%.

In the first positive sample of a 10-year-child, we detected five metabolites from the CAR insecticide class, wherein the most major peak was eluted at RT 5.249 min. A metabolite extracted from N-nitroso compounds (pyrrolidine 1-nitroso) was identified at RT 5.604 min. Aziridine metabolites at RT 4.300 and 6.081 min were identified with high matching percentages. A triazol fungicide metabolite (1,2,4 triazolol) was identified at RT 4.624 min. A metabolite from the pyridazinone class (6-chloro-pyridazinone) was identified at RT 4.261 min. The two main peaks at RT 4.152 and 4.188 min, identified, respectively, as phenol-2,4-difluo-6-nitro and pentanol formate, were not considered in our investigations. The other minor peaks were identified as traces of organic acids, amino acids, sugars, and lipids.

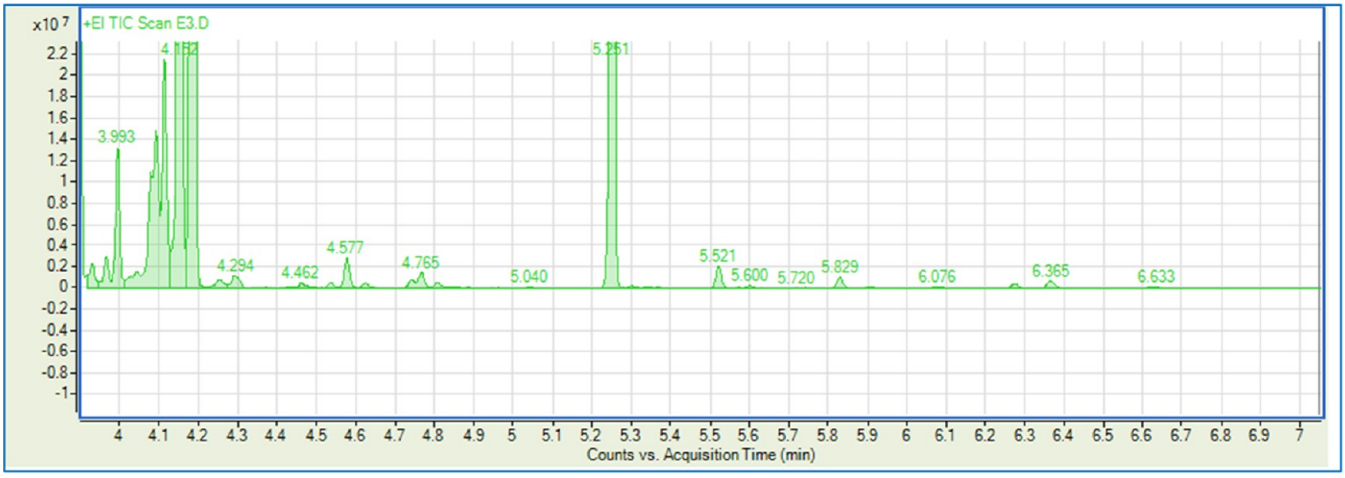
In the second positive sample from a 27-year-old adult, we detected two major peaks eluted at RT of 3.993 min and 5.251 min, identified for oxime and CAR metabolites, respectively. In the third sample extracted from a 35-year-old farmer, one major peak was detected at RT 5.257 min and another minor peak was obtained at RT 4.098 min, identified for CAR and oxime metabolites, respectively. The other peaks were revealed at trace levels, and they were not confirmed for pesticide metabolites.

The summaries of all identified metabolites with their corresponding pesticide classes are presented in Table 2. Their MS spectra and chemical structures are shown in the Supporting Information as follows: Figures S2−S10 for the

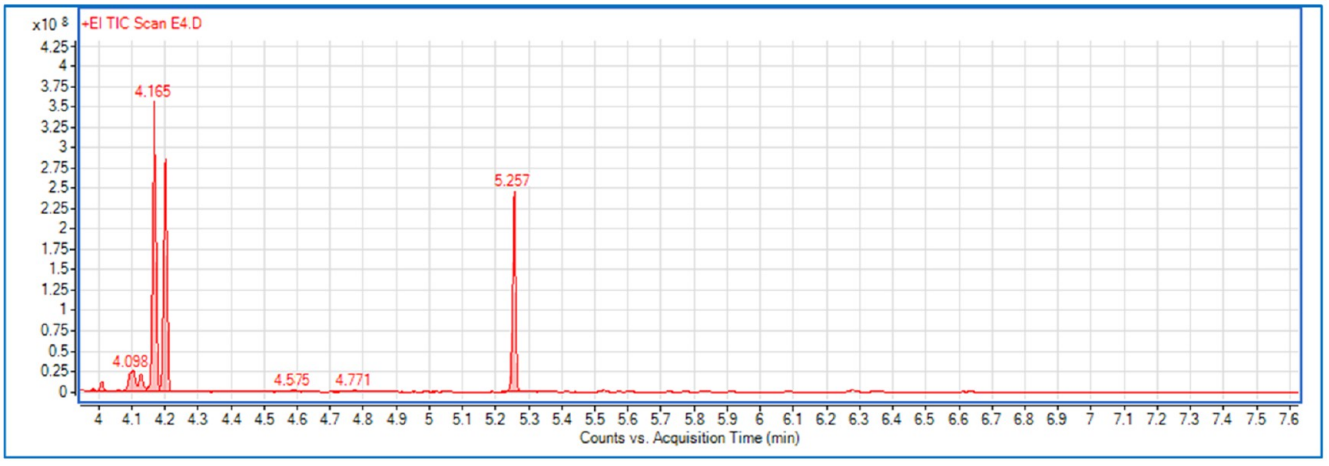
https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835



Pathological Sample 1



Pathological Sample 2



Pathological Sample 3

Figure 4. Typical chromatograms for the detection and separation of pesticide metabolites by GC-MS analysis of human brain tumor tissues.

identified metabolites in sample 1, Figure S11 for the identified metabolites in sample 2, and Figure S12 for the identified metabolites in sample 3.

3.4. Chemometric Analysis. Concerning multivariate statistical analysis, a two-component PCA score plot of GC/MS data was used to examine the intrinsic variation of metabolomics composition between two groups. R^2X, the measure of fit, is close to 1, proving a good stable model, and Q^2Y, the cumulative predicted variation, is superior to 0.5, suggesting a reliable predictive ability (Figure 5).

As shown in Figures 6 and 7, the clear separation between the two groups is therefore a strong indication of the real differences

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

Table 2. Summary of the Identified Pesticides' Metabolites Extracted from the Positive Case Samples

sample no.		name of compound	chemical structure	MW[a]	RT[b]	peak area (mean ± SD[c])	major m/z ions	pesticide class
sample 1	1	butanol-*O*-methyl oxime	$C_5H_{11}NO_2$	101	4.109	17 496 825 ± 0.001	41, 59, 86, 73	carbamates
	2	6-chloro pyridazinone	$C_4H_3ClN_2O$	130	4.261	569 971 ± 0.102	73, 130	pyridazinone
	3	trimethylsilyl-aziridine	$C_8H_{18}F_3NOSi_2$	257	4.300	1 399 500 ± 0.07	45, 59, 73, 100, 115	organochlorine
	4	1,2,4 triazolol	$C_6H_5N_3S$	151	4.624	45 239 ± 0.07	93, 151	triazole fungicides
	5	*N*,*N*-diethyl-trimethylsilyl-carbamate	$C_8H_{19}NO_2Si$	189	5.249	151 670 413 ± 0.022	54, 59, 73, 100, 130, 174	carbamates
	6	pyrrolidine 1-nitroso	$C_4H_8N_2O$	100	5.604	15 5871 ± 0.099	41, 69, 100	*N*-nitroso-compounds
	7	*N*,*N*-diethyl-trimethylsilyl-carbamate	$C_8H_{19}NO_2Si$	189	5.829	1 009 547 ± 0.001	54, 59, 73, 100, 130, 174	carbamates
	8	2,2-bi-1,3-dioxolane	$C_6H_{10}O_4$	146	5.523	136 183 ± 0.064	45, 73	carbamates
					5.907			
	9	*N*-benzyloxy-2,2-bis(trifluoromethyl)aziridine	$C_{11}H_7F_6NO_2$	299	6.081	158 136 ± 0.002	51, 105	organochlorine
	10	xylo-hexos-5-ulose, 2,3,4,6,tetrakis-*O*-trimethylsilyl-bis(*O*-methyl oxime)	$C_{20}H_{48}N_2O_6Si_4$	524	6.277	2 460 678 ± 0.003	73, 103, 147, 202, 364	carbamates
sample 2	1	oxime, methoxy-phenyl	$C_8H_9NO_2$	151	3.993	62 239 086 ± 0.06	40, 42, 55, 74	carbamates
	2	L-(2-cyanobicyclo-(2,2,1)-hept-5-en-2-yl), dimethyl-ethyl ester carbamate	$C_{13}H_{18}N_2O_2$	235	5.251	126 400 865 ± 0.005	57, 59, 66, 113, 134, 178	carbamates
sample 3	1	galacto-hexodialdose, 2,3,4,5 tetrakis-*O*-trimethylsilyl-bis(*O*-methyl oxime)	$C_{20}H_{48}N_2O_6Si_4$	525	4.098	12 824 737 ± 0.042	28, 73, 147, 160, 364	carbamates
	2	carbamate, *N*-(2 naphtyl)-3 pentynyl ester	$C_{16}H_{15}NO_2$	253	5.257	151 670 413 ± 0.002	41, 55, 67, 115, 143, 169, 253	carbamates

[a]MW, molecular weight. [b]RT, retention time. [c]SD, standard deviation.

in their metabolic patterns. Additionally, a two-component PLS-DA model was subsequently constructed, showing the fundamental separation between groups (Figures S13−S16). R^2 and Q^2 values derived from permuted data were lower than the original values, and the regression of the Q^2 line intersected at below zero indicates the validation of the PLS-DA model (Figure 8). The differential metabolites accountable for separation obtained from the VIP values of the PLS-DA model were statistically different by the threshold of their P values (<0.01, t-test).

4. DISCUSSION

4.1. Development of a GC-MS Analytical Method for Pesticide Metabolite Research in Biological Samples.

4.1.1. Sample Preparation. Sample preparation is a crucial step in any analysis due not only due to the complexity of the matrix but also due to the low concentrations of metabolites that can be deteriorated by solvents. Besides, an MS analysis requires modified extraction steps as well as cleanup procedures according to the matrix and type of analytes. Thus, in our study, we have first focused on the generally described protocols based on extraction, oximation, and silylation prior to any analysis using GC methods in order to avoid time waste and the use of various extraction solvents with the risk of absence of any traces of metabolites.

Second, we opted to use the lyophilization of our tissue samples. Although this technique is cost-effective, restricted to industrial use, and has not become a routine method in research laboratories yet,[33,34] it was adapted in order to facilitate and better extract different components from our samples transformed into powder. Indeed, lyophilization or freeze-drying is described as the first-rate preservation method that produces high-quality products. It is used not only to improve validity and reproducibility but also to reduce scatter in molecular biology research based on previous works proved by quantity and quality measurements and biomolecular research methods, viz. real-time-polymerase chain reaction (RT-qPCR), copy number variation analysis, Western blot, gelatin zymography, SDS-PAGE, or even enzyme activity analysis in which both nucleic acids and proteins are well preserved in lyophilized samples.[35,36] A recent study conducted by Filho et al. has shown that recoveries for lyophilized samples were higher than those calculated for the extracted samples and that lyophilization is a suitable preparation method for sample analysis by GC in aqueous matrices since it allows the determination of more compounds.[37] Another study has also revealed that freeze-drying is an alternative method for analyzing volatile organic compounds by GC-MS.[38]

Third, our sample tissues were extracted using dichloromethane, which is a commonly used solvent to remove organic compounds.[39] Afterward, we opted for derivatization to improve the volatile nature of compounds and ameliorate the analyses of those that cannot be directly analyzed by GC due to either their high boiling point or their used temperature instability. In fact, derivatization plays a crucial role in GC analyses that protect substances from any thermal decomposition and guarantee an increase in their volatility and/or apolarity to be analyzed in a chemical reaction. Also, it decreases the adsorption of not only polar compounds in column walls but also hydrogen-bond formation between different compounds.[40] Trimethylsilylation is the most commonly used method for derivatization that involves a reaction adding a trimethylsilyl (TMS) functional group to the compound. It is performed by the use of *N*,*O*-

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

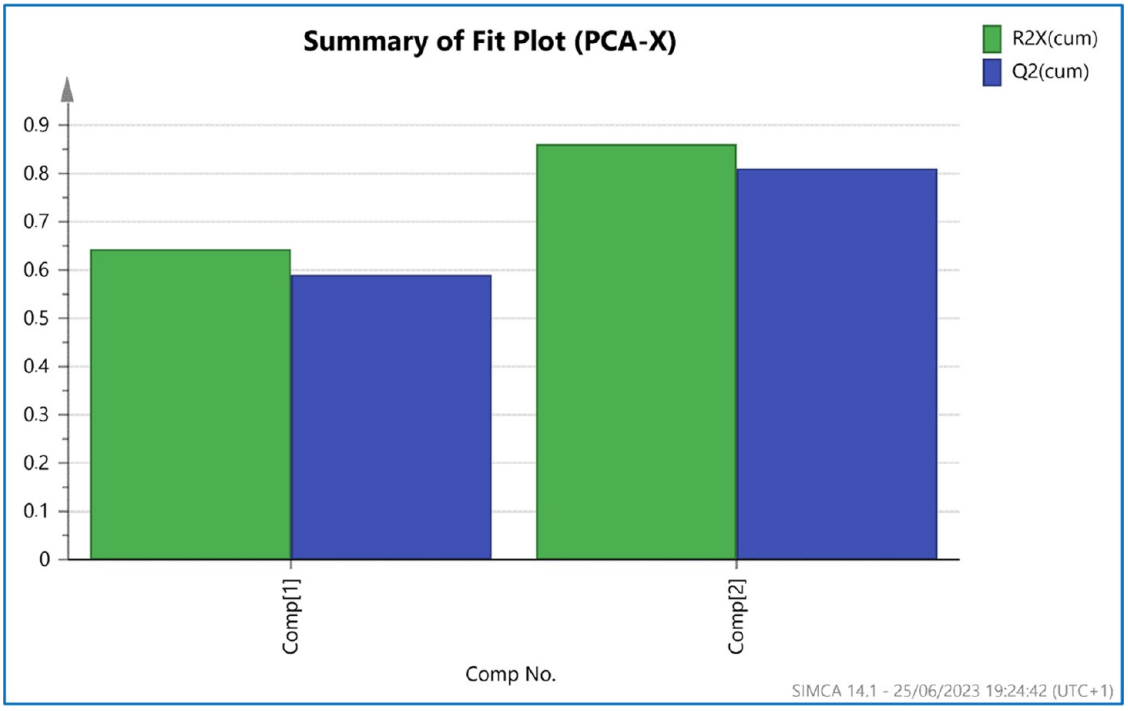


Figure 5. Summary of the fit plot in the PCA model. Component No. 1: $R^2 = 0.643$, $Q^2 = 0.589$. Component No. 2: $R^2 = 0.860$, $Q^2 = 0.810$. For both components, R^2 values, the measure of fit, are close to 1 proving a good model and $Q^2 > 0.5$, suggesting a reliable predictive ability.

bis(trimethylsilyl)-trifluoroacetamide, also called BSTFA, or N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA), as the most suitable reagents for many analytes.[41] For moderately hindered or slowly reacting compounds, a catalyst as a pyridine may be added to the BSTFA.[42,43]

Finally, in order to prove the recovery of our results, we performed some preliminary assays on brain tumor control samples by making a comparison between lyophilized and nonlyophilized tissues in the yielded metabolites. A total of six experiments were conducted by liquid−liquid extraction (LLE) and then by GC-MS separation in the same analytical conditions in order to assess the effect of lyophilization on the separation quality. Thereafter, a two-component PLS model relating the design matrix X to the GC/MS peak area (matrix Y) was performed by SIMCA software. Our results illustrated in Figures 9 and 10 showed that the left quadrant of the plot contains experiments that yielded the highest peak areas and numbers. Thus, the majority of metabolites including amino acids and organic acids are strongly and positively correlated with lyophilized samples. In contrast, only a minority of peaks were identified in nonlyophilized samples.

Consequently, we can conclude that lyophilization does not alter the quality of the sample or lead to the majority loss of volatile compounds. Indeed, in our study, we have detected at RT = 5.523 min and RT = 5.907 min, metabolite 1,3-dioxolan, reported as a volatile organic compound belonging to pesticide formulations.[44]

Hence, the use of this technique can not only afford reproducible and efficient results but also minimize organic solvents and steps in sample preparation.

Moreover, the LLE method was revealed to be as efficient as the other developed methods associated with GC-MS, namely, solid-phase extraction (SPE) and solid-phase microextraction (SPE), according to some recent studies in the literature (Table SII).[45−50] Although these new methods have increased effectiveness in separation, the classic method by LLE remains the simplest and the less expensive one.

4.1.2. Optimization of the Analytical Method by GC-MS. GC-MS has been recently employed as the most effective technique to detect multianalytes in complex matrixes. However, the trouble-shooting and time-consuming nature could be attributed to the adaptive method for detecting multiple residues in a complex matrix by using a single injection.[51−54]

It seems hard to optimize a reliable and sensitive screening method for diverse classes of pesticides in a complex tissue.[55]

The most described methods for pesticide separation in many different matrices, such as plasma, urine, soil, etc.,[56] were based on sophisticated and expensive chromatographic techniques that may not be available in most laboratories in many countries. Thus, our aim was to develop a simple and rapid method by GC-MS that will be adapted for detecting different groups of pesticides in human brain tissues by optimizing experimental chromatographic conditions. Our preliminary trials were conducted using the available GC column by changing analytical parameters, such as the oven temperature program, and the flow rate in order to obtain a fast method that separates all classes of metabolites in the same run without any interference peak. We succeeded in separating the majority of metabolites in a single run and within a short period of time (20 min).

Thus, our study reveals the possibility of estimating different pesticide metabolites using the least expensive technique of chromatographic devices, known as GC-MS, which may be found in most laboratories worldwide.

4.2. Identification of Implicated Classes of Pesticides. Pesticides are groups of artificially synthesized substances that are nonbiodegradable in the environment and persist after application. They are subject to some chemical processes of degradation, hydrolysis, oxidation, and photolysis by the

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

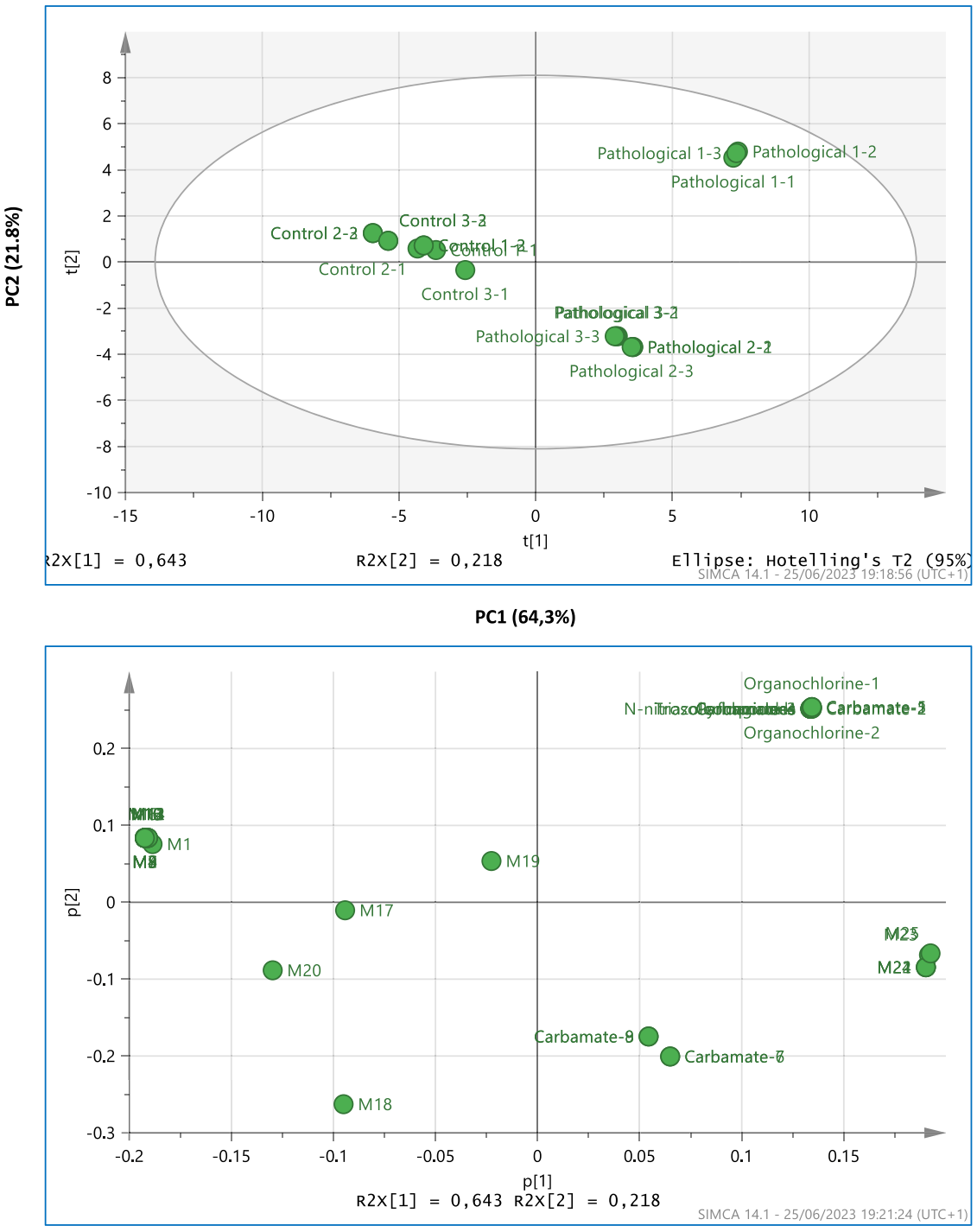


Figure 6. Chemometric analysis: principal component analysis score map (PC1/PC2) and the corresponding loading plot of the CPG-MS analysis of human brain tumor tissues (pathological samples versus control samples). Three replicate analyses derived from each sample were performed. M1, M2, ···, M*x*: other metabolites including organic acids, amino acids, sugars, and lipids.

ecosystem. Most studies have principally focused on primary residues rather than their transformation products.[57,58]

In our study, the major identified metabolites were extracted from the CAR class known for their significant cerebral permeation with a chemical profile favorable for crossing the blood−brain barrier to cause alterations in the CNS.[59] US-EPA has classified human carcinogens into four CARs, namely, carbaryl, fenoxycarb, pyrimicarb, and thiodicarb.

Most insecticidal CAR subgroups are substituted phenol esters (e.g., carbaryl, carbofuran, or pyrimicarb), while others such as aldicarb are oxime esters that exhibit a strong inhibitory activity. The identified oxime compounds (bis-*O*-methyl oxime, butanol-*O*-methyl-oxime, methoxy-phenyl-oxime, acetamide oxime) could be issued from the alkaline or acidic hydrolysis of aldicarb.[60]

Based on a study carried out by Zeinali et al., the metabolite 1,3-dioxolan was identified by the use of GC-MS as a volatile organic compound belonging to pesticide formulations.[44] Thus, our metabolite 2,2-bi-1,3-dioxolane could be issued from the catalytic degradation of dioxocarb, [2-(1,3-dioxolan-2-yl)-

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

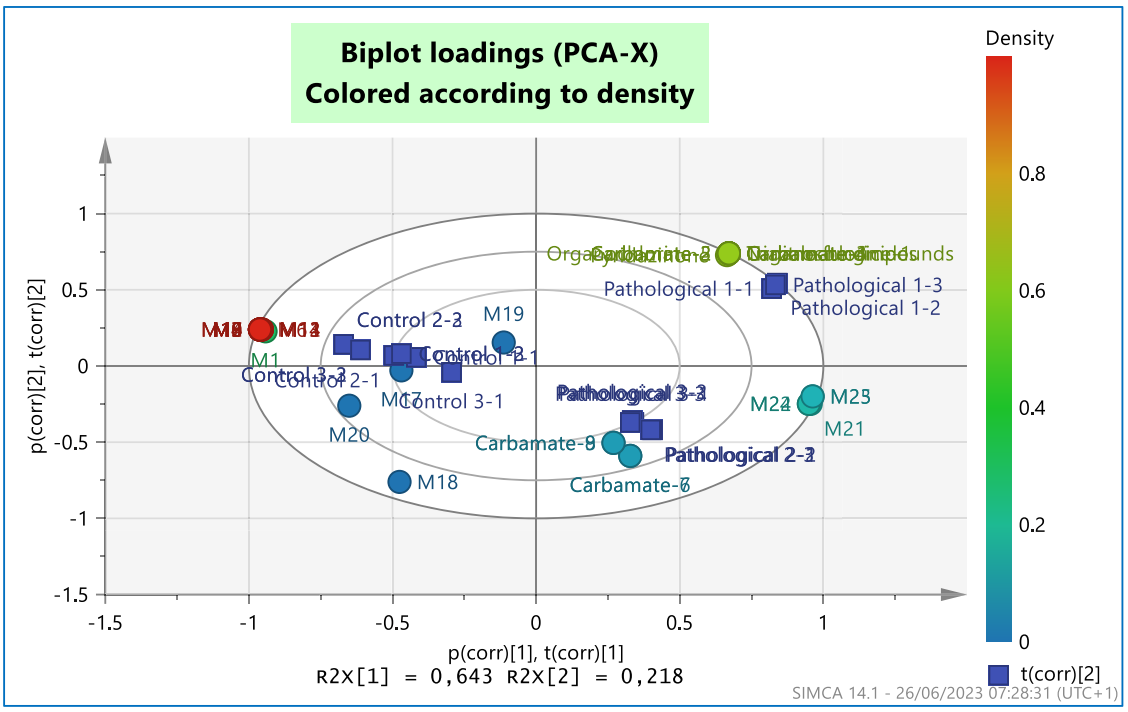


Figure 7. Chemometric analysis. Biplot loadings by the PCA score plot: observations (pesticide metabolites) situated near pathological variables are high in these variables and low or absent in opposite variables. Three replicate analyses derived from each sample were performed. M1, M2, ···, M*x*: other metabolites including organic acids, amino acids, sugars, and lipids.

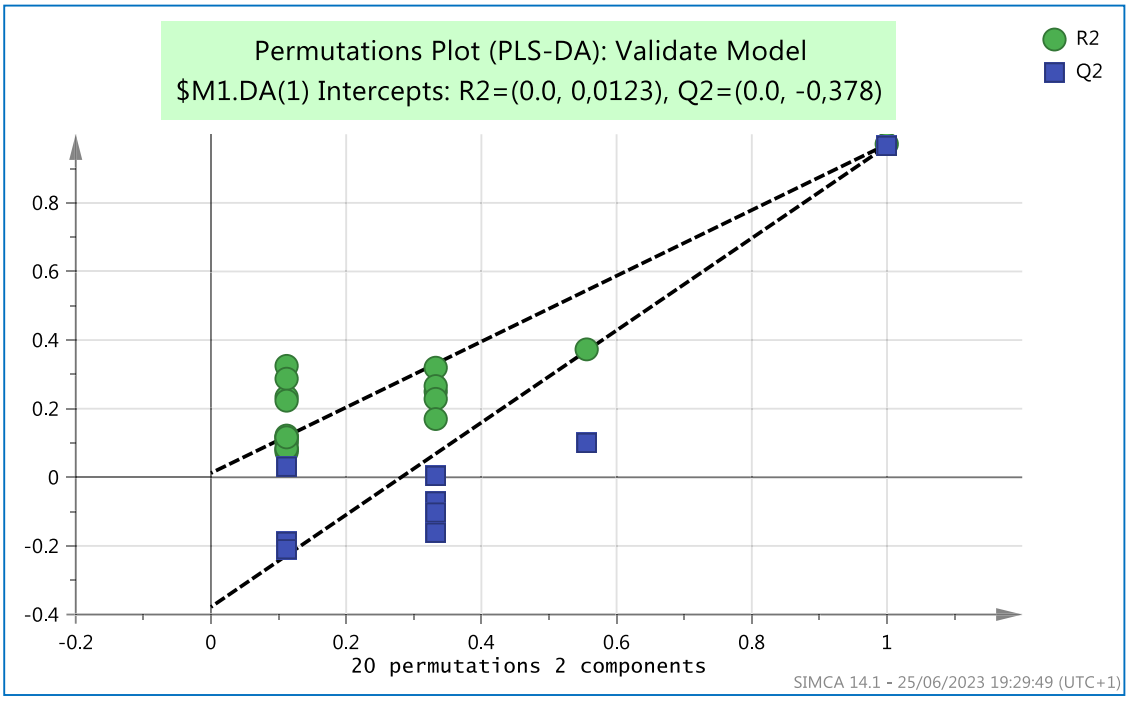
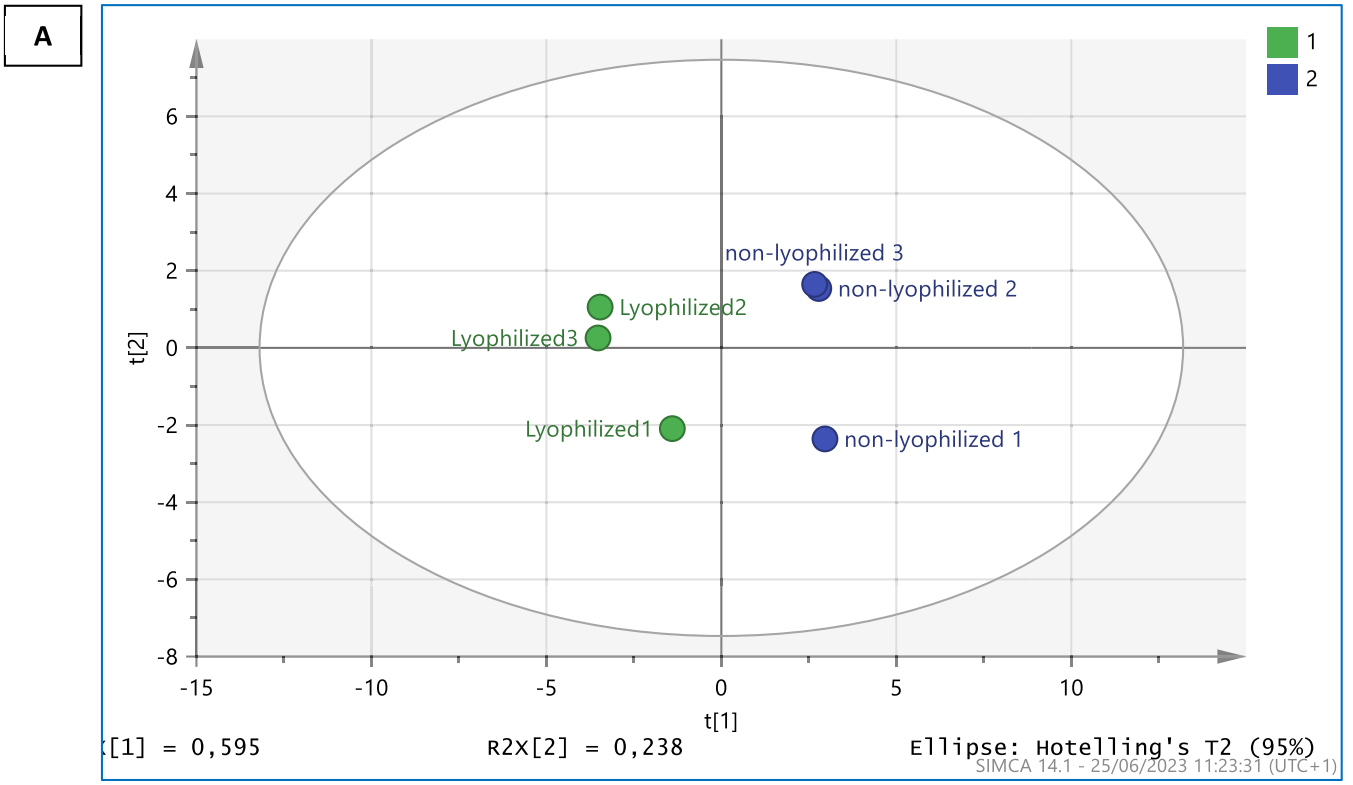


Figure 8. Chemometric analysis: permutation test for the validation plot of pathological and control groups.

phenyl] N-methyl carbamate, described for its cytotoxic and genotoxic effects on human peripheral blood lymphocytes.[61] The inhalation pathway may be implicated in the toxicity effect of this pesticide.

N-nitroso compounds (NOCs) carry the "*N*-nitroso" chemical function. They are formed by a chemical and/or enzymatic reaction (called nitrosation) from a nitrosable precursor (essentially secondary amines) and a nitrosating agent (e.g., nitrites). Several N-nitroso compounds are known as human carcinogens.[62] In fact, about 20 *N*-nitroso compounds are classified as carcinogens by IARC (groups 1, 2A, or 2B) for various organs, including some in the brain. Among these compounds, we have identified pyrrolodine-1-nitroso (IARC monograph 17, sup 7). According to the literature, *N*-nitrosoureas (e.g., N-ethyl-*N*-nitrosourea) show a strong carcinogenic potential in animals. They have been used since

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835



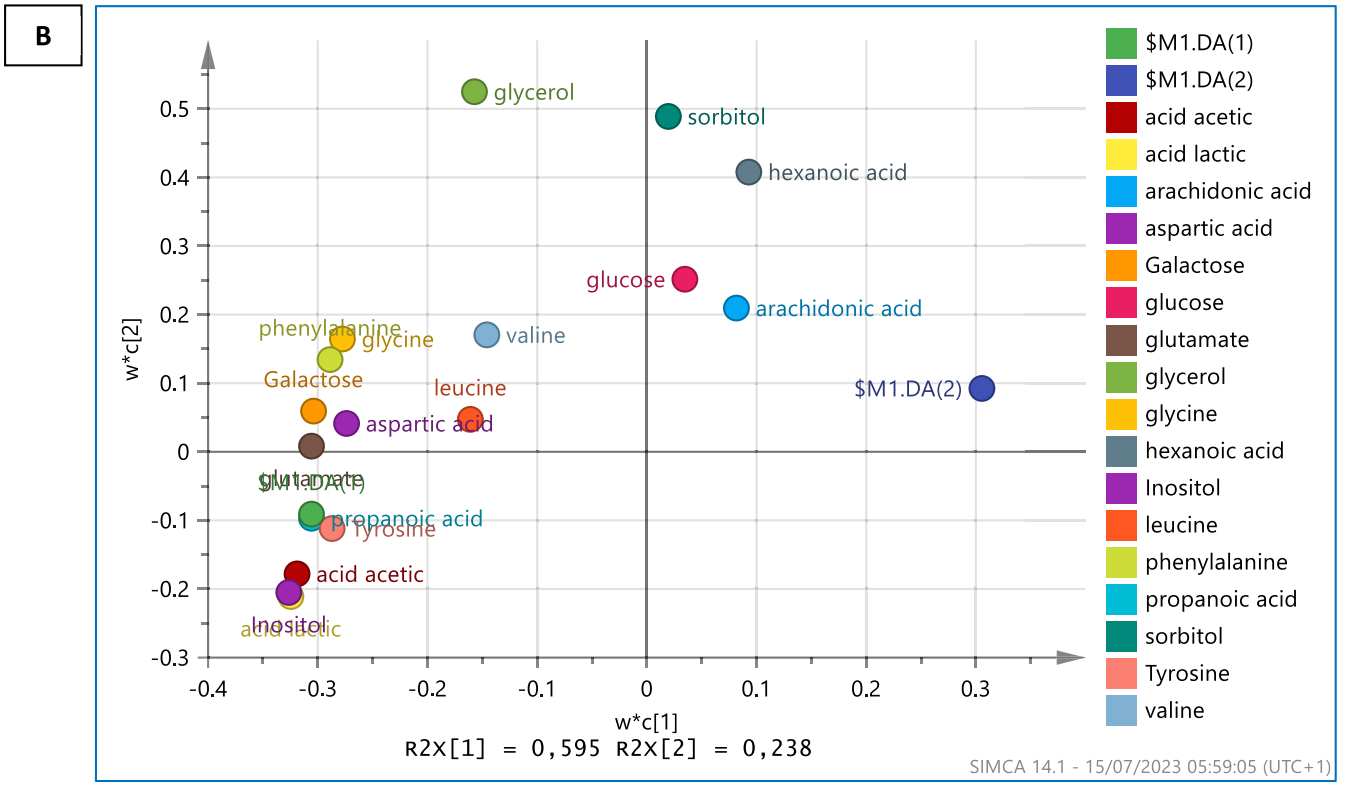


Figure 9. Chemometric analysis: the determination of the optimal method for extracting components from human brain tissues. (A) PLS score plot ($t[1]/t[2]$) shows the inner correlation structure between the X matrix (experimental conditions: lyophilized/nonlyophilized) and the Y matrix (areas of the resolved peaks). (B) PLS loading plot summarizing the influence and correlation between variables in both the X matrix and the Y matrix. Lyophilized samples are strongly positively correlated with the majority of resolved peaks.

the 1970s to induce brain tumors in rats and mice in the laboratory.[63−65] Human exposure to *N*-nitroso compounds via smoking and food in particular is also suspected to increase the risk of CNS tumors. However, evidence supporting human exposure to NOCs remains limited.[66−68] In agriculture, several studies have shown that pesticides contain secondary or tertiary

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

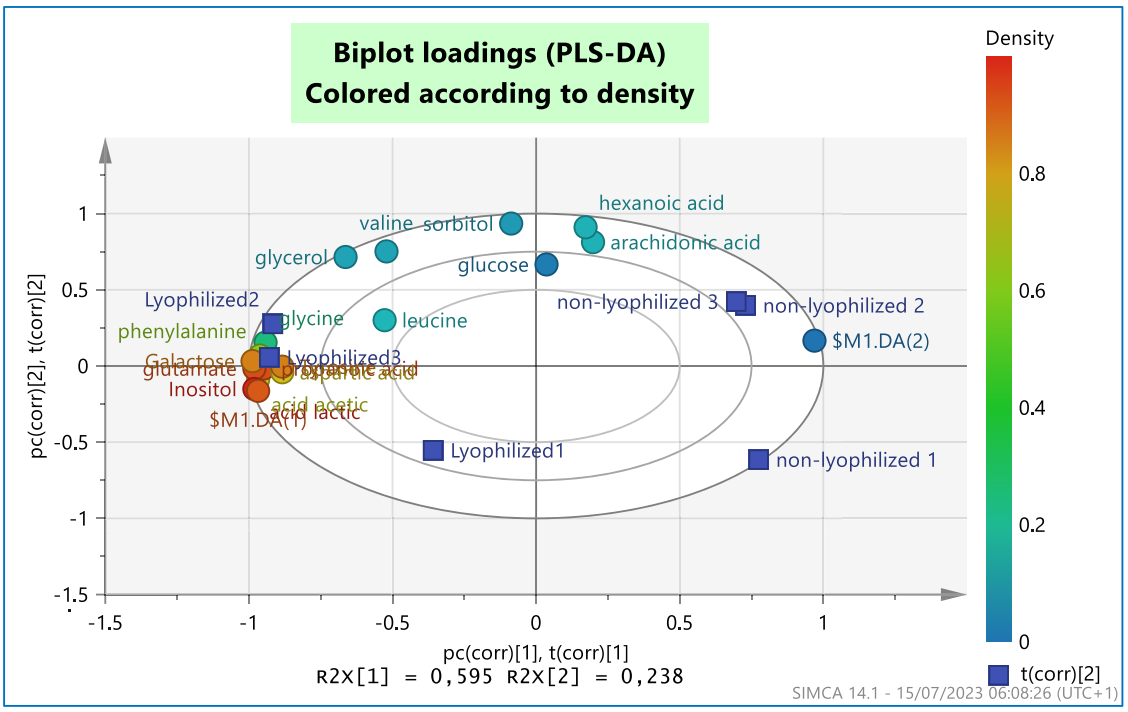


Figure 10. Biplot loadings by PLS-DA: The majority of observations (metabolites) are situated near the "lyophilization status" as variables.

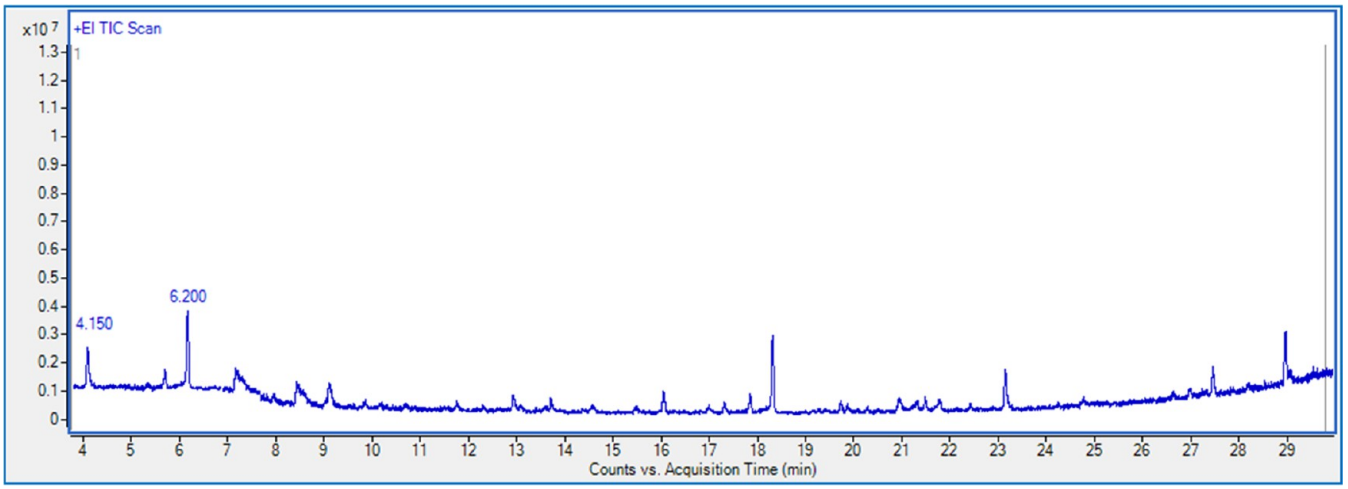


Figure 11. GC-MS analysis of aldicarb degradation products induced by stress degradation in an oxidative medium.

amine groups that can react with nitrites to form NOCs.[69−71] Farmers could therefore be occupationally exposed to NOCs through contact with "nitrosable" pesticides (nitrosation in vivo after exposure and/or storage of the product).

In a case-control study of gliomas in Nebraska, Lee and his collaborators investigated the hypothesis of the link between CNS tumors and nitrosable pesticides.[72] The authors reported an increased risk of gliomas related to occupational exposure to nitrosable pesticides. The list of pesticides likely to lead to *N*-nitroso compounds has been published by IARC.[69−71]

OCs have been associated with a variety of cancers in humans.[73−76] These chemicals are highly resistant to breakdown and persist for longer times in the environment and bodies of living organisms. The identified metabolites, aziridine and benzyloxy bis-trifluoro-methyl aziridine, can be issued from the degradation of the 1,3-dichloropropene pesticide,[77] which is a halogenated hydrocarbon from OCs, used as a soil fumigant to kill nematodes, insects, and weeds in vegetables and orchard crops. Its toxicity has been proved based on sufficient evidence of carcinogenicity from studies in experimental animals and has been listed in the Fifth Annual Report on Carcinogens (1989).[78]

The 1,2,4 triazolol could be a metabolite from the triazole antifungal agent used for controlling ascomycetes, fungi imperfecti, and basidiomycetes in a wide variety of crops,[79] and its cytotoxicity was evaluated following many studies.[80,81] It was described to induce chromosomal aberrations, sister chromatid exchanges, and DNA fragmentation in cultured bovine lymphocytes and inhibit cytochrome P450 enzymes. Transcriptional analysis of liver tissues from the genomic studies of triazole antifungal agents suggests that these compounds induce constitutive androstane receptor and pregnane × receptor activation, CYP induction, oxidative stress, dysregulation of cholesterol biosynthesis, and alteration in cell signaling, cell growth, cell proliferation, and apoptosis pathways.[82,83]

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

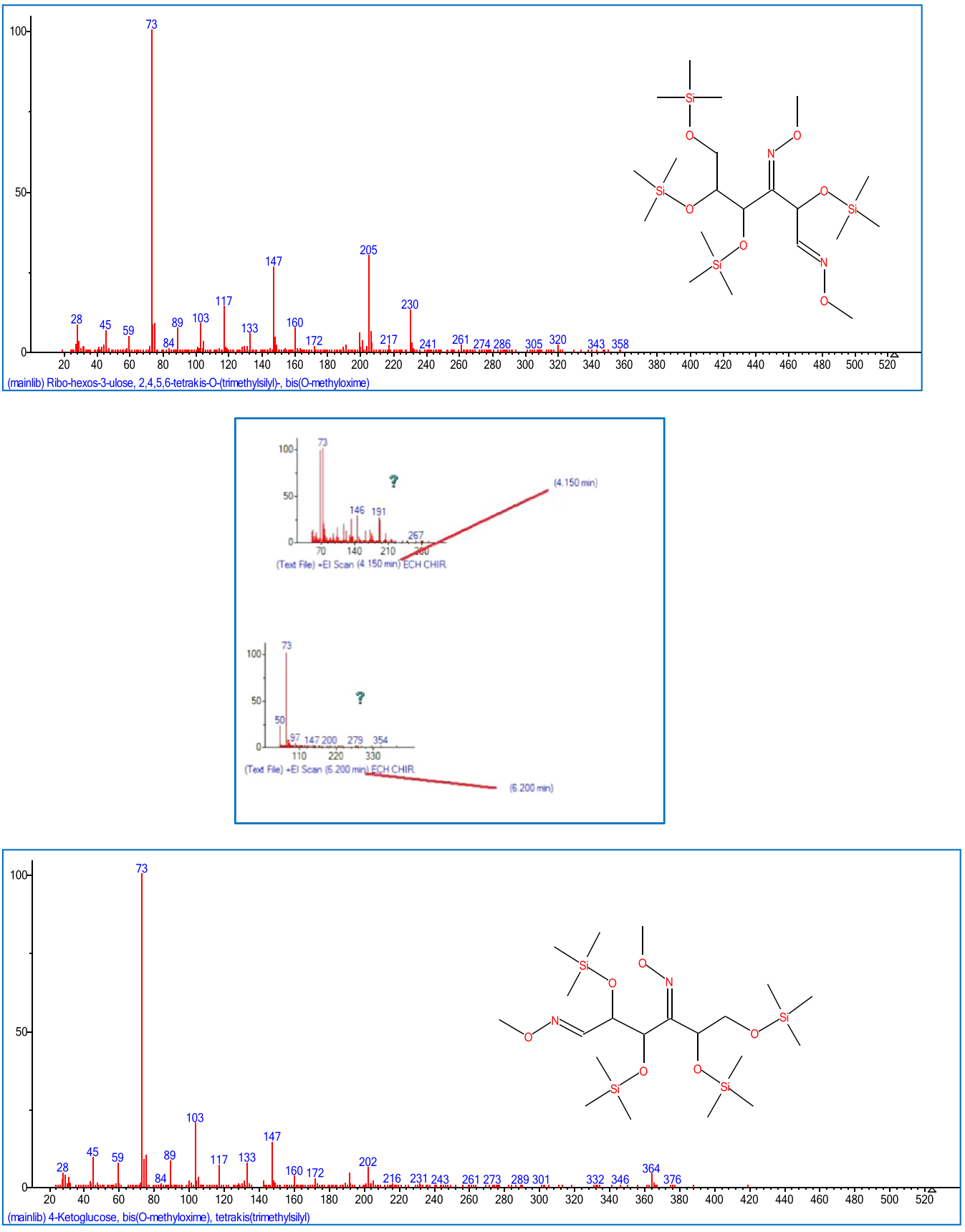


Figure 12. Mass spectrum identification of oxime compounds at RT = 4.150 and 6.200 min. The identified m/z 73 ion is the most important characteristic of oxime metabolites.

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

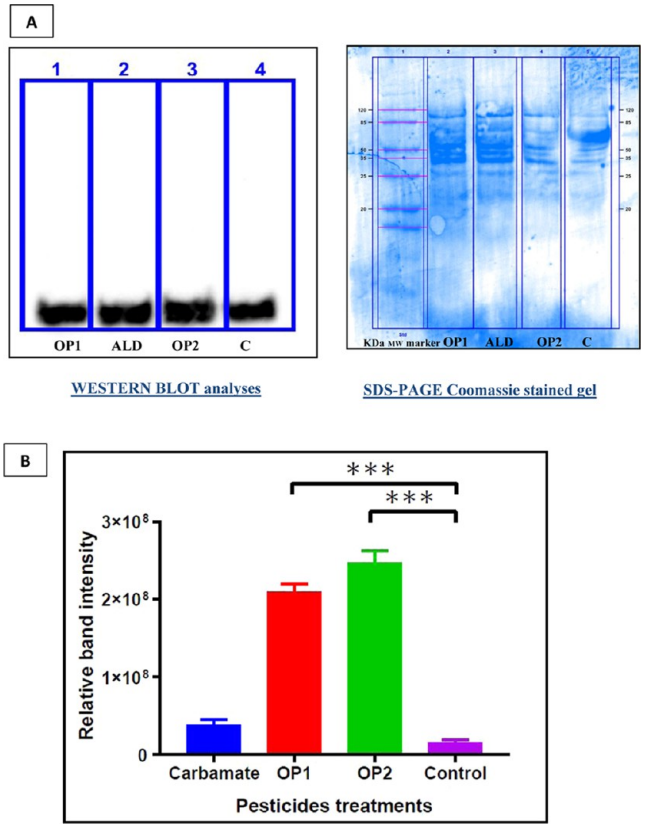


Figure 13. 2D-OXYBLOT analysis. (A) Representative SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) obtained for both control and treated *SH-SY5Y* neuroblastoma cells: Immunoprecipitation of protein carbonyls with Western blotting and colometric analysis on PVDF (poly(vinylidene difluoride)) membranes probed with mouse monoclonal anti-DNP (dinitro-phenyl-hydrazone) antibody. OPs (organophosphorus), ALD (aldicarb), C (control cells). (B) Histograms presenting the proteins' carbonyl level after 24 h treatment by each type of pesticide at IC_{50}. The measured value is normalized with the mean of control cells. Bars represent mean± SD; ***: statistically significant difference versus control (by ANOVA test), $P < 0.05$, $n = 3$ replicates per treatment.

The metabolite 6-chloro-pyridazinone could be issued from the chloridazon herbicide that belongs to the pyridazinone class described in the occurrence of pregnancy complications according to a study conducted by Manangama et al.[84]

In order to provide evidence for some of the identified metabolites, we added some preliminary assays by a pesticide stress degradation study in a low oxidative medium (H_2O_2 10% for 4 h) with the aim to induce oxidative stress and hence generate some major metabolites for their identification. Aldicarb was selected since the majority of our identified metabolites were issued from the CAR class. In addition, it is available in our laboratory where we conducted our further 2D-OXYBLOT analysis. Afterward, we applied the same analytical method by GC-MS for the identification of pesticide metabolites. As shown in Figure 11, we detected some minor degradation products, particularly the oxime compounds identified at RT 4.150 and 6.200 min, with their major ion *m*/*z* 73 (Figure 12), which may best confirm the traces of pesticide metabolites in our positive case study of a 10-year-old child with brain tumor.

4.3. Correlation between Pesticide Exposure and the Development of Human Brain Tumors. Pesticides with their biotransformation products have become an unavoidable part of the environment due to their high persistence and pervasiveness. They circulate in ecosystems and are considered persistent organic pollutants possessing long half-lives that can accumulate in the environment and in living organisms in several ways. They are transferred throughout the food chain until they reach human beings according to their nature, chemical structures, and targets.[83,84] High pesticide exposures can be attributed to the use of large amounts of chemicals or an increasing application rate of pest outbreak periods. Pesticide residues can remain in carpets, furniture, and toys without being degraded by processes that exist outdoors (e.g., rain and sun), which may lead to long-lasting exposures.[85]

In our study, we want to test our hypothesis for the correlation between pesticide exposure and the risk factor for human CNS tumors, especially in childhood. This causal link has been documented in the literature based essentially on epidemiological studies carried out in the agricultural population using indirect approaches such as questionnaires or job-exposure matrices, presenting that this is more advantageous than conducting research of trace metabolites in urine or blood since this type of measurement entails a high cost and requires the agreement and the presence of participants. Therefore, it cannot be used for large-scale studies.

Medulloblastoma is a rare cerebellar embryonal neoplasm that occurs almost exclusively in children, and in our case study, it was identified in a 10-year-old child. Relatively little is known about the etiology of childhood brain tumor (CBT). However, pesticide exposure is hypothesized as one of the risk factors as it is known that children absorb, more readily than adults, toxins via the respiratory and gastrointestinal systems that are accumulated and slowly removed despite higher metabolic rates. Thus, children constitute a population group particularly vulnerable to environmental exposure to xenobiotics. In our case study, children from poor families living in rural regions usually keep playing in agricultural areas or are sometimes carried on parent's backs during agricultural activities, which represents the main way for their direct exposure to pesticides.

Moreover, numerous studies have been published supporting the role of pesticides in the CBT etiology and showing a correlation between occupational and/or residential proximity to agricultural applications and CBT risk due to pesticide exposures before pregnancy, during pregnancy, and during childhood.[86,87] This can be explained by the fact that some pesticides due to their persistence in the human body can lead to in utero and breastfeeding exposure in children.[88] Also, many neurotoxicant pesticides have been found in cord blood indicating placental transfer, so that any disruption in signaling factors necessary for brain development leads to neurotoxicity.[89]

A recent study carried out by Lombardi et al. had revealed that three types of cancer, namely, medulloblastoma, ependymoma, and astrocytoma, were associated with specific pesticides, suggesting that exposure to certain pesticides through residential proximity to agriculture applications during pregnancy may increase the risk of CBT.[89] A systematic review conducted by Manangama et al.,[84] for assessing pesticide exposures and adverse pregnancy outcomes, has shown that pesticide families including OCs, OPs, and atrazine were associated with unfavorable pregnancy outcomes. Consequently, a strong presumption of a causal link was perceived and pesticide families involving neonicotinoid, bipyridium, pyridazinone, and strobilurin have been related to the occurrence of pregnancy complications.

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

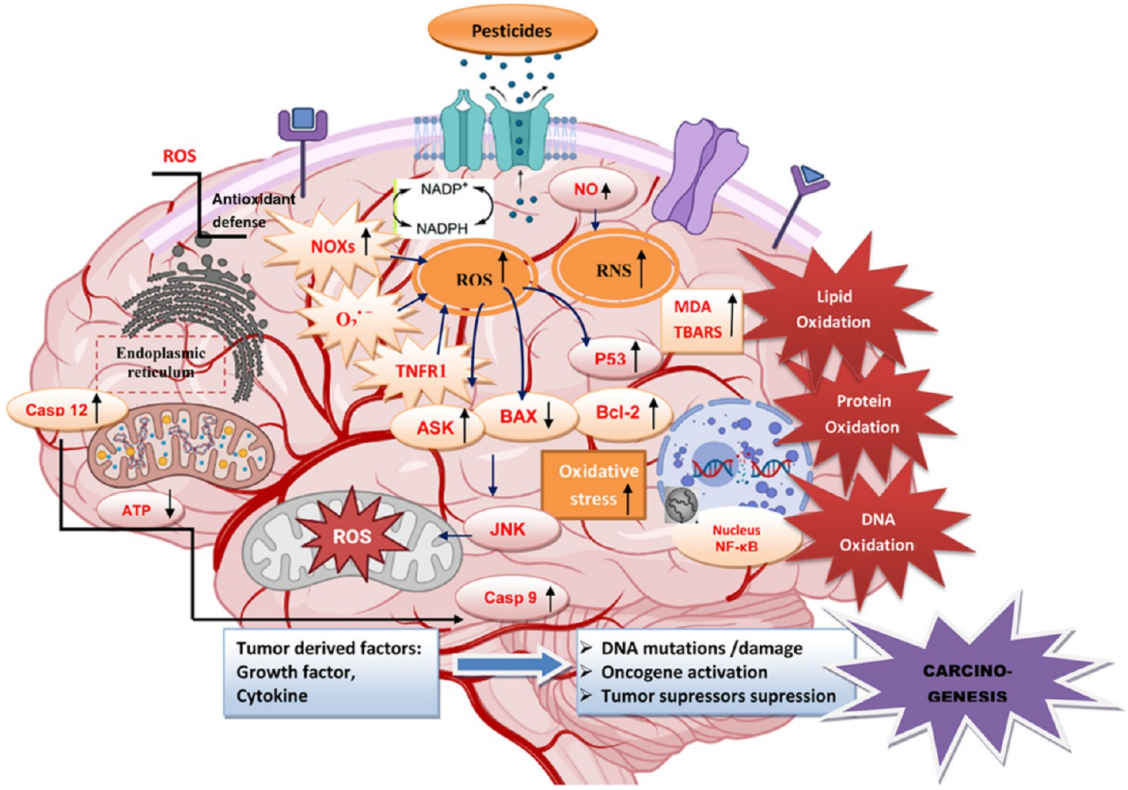


Figure 14. Signaling pathways involved in pesticide-induced oxidative stress implicated in the physiopathological mechanism of brain cancer: Pesticides increase NADPH oxidase (NOX), superoxide ($O_2^{\bullet-}$), and nitric oxide (NO) levels, which leads to an increase in reactive oxygen species (ROS) and reactive nitrogen species (RNS) signaling in the cell. The stressors activate TNFR1/TNF-α, MAPKs, and NF-κb and act as signaling molecules to drive cancer cell motility/invasion and tumor progression. Some common characteristics of oxidative stress include increased protein oxidation, lipid peroxidation, nucleic acid oxidation, and changes in the levels of antioxidants such as glutathione and the activities of antioxidant enzymes leading to various toxicities. ROS: Reactive oxygen species that includes superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), singlet oxygen ($1O_2$), peroxyl radical ($ROO^{\bullet}$), alkoxyl radical ($RO^{\bullet}$), lipid hydroperoxide (LOOH), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl), and ozone (O_3); TNFR1, tumor necrosis factor receptor 1; TNFα, tumor necrosis factor-α; NF-κB, nuclear factor κB; MDA, malondialdehyde; ASK1, apoptosis signal-regulating kinase 1, also known as MAPK5; BAX, apoptosis regulator (bcl-2-like protein 4); Bcl-2, B-cell lymphoma 2; P53, transcription factor playing an important role in cancer; Casp 9, cysteine protease from the caspase family associated with the mitochondrial apoptosis pathway; Casp 12, cysteine protease from the caspase family; TBARS, thiobarbituric acid reactive substances; and JNK, c-Jun N-terminal kinase, a protein kinase playing a crucial role in stress signaling pathways.

The Cox proportional hazards model revealed that the risk of a human brain tumor occurs especially beyond the age of 50 (graph in Figure 1), which gives a bird's eye view that carcinogenicity is a major risk factor for the long-term effects of pesticide toxicity as it was substantially proved from a wide range of well-designed studies.[90,91] Nevertheless, the detection of traces of pesticide metabolites at an early age could be explained by the fact that some molecules may have a long lifespan that can be retained for several years in lipophilic tissues and that the process of their degradation is achieved over many years, the time that is probably necessary for the development or the expression of some specific metabolic enzymes. In fact, drug metabolism enzyme activity changes profoundly throughout the human development continuum, which results in different disposition pathways through age. In addition, infants may exhibit silencing enzymes or their expressions at low levels as low concentrations of circulating plasma proteins reduce protein binding and the process of xenobiotic metabolic degradation.[92,93]

Moreover, pesticides belonging to the organochlorine (OC) class are notoriously persistent chemicals in tissues of mammals due to their lipophilic characteristics that are bioaccumulated in the adipose tissue,[94] and the rate of their biotransformation can be extremely slow (years to decades). A large number of these pesticides, such as DDT (dichloro-diphenyl-trichloroethane), is characterized by their retention for several years in soils (5−8 years).[1] In our case study, the identification of "aziridine" metabolites extracted from the OC class may best emphasize the theory for such an identification at an early age, although adult patients have been directly exposed to pesticides throughout their life.

Food and environmental safety necessarily involve the proper use of pesticides. However, in our population study, most patients were farmers from interior regions having low educational levels, which can unravel the correlation between the educational level and the occurrence of brain tumor since a higher level of education increases the awareness of chemical usage and a higher probability of chemical labels being read as well as used with greater caution. A report written by our National Agency for Sanitary and Environmental Control of Products (ANCSEP) has noted that a wide range of Tunisian farmers were struggling to protect themselves properly from pesticides.[95,96] This is consistent with other findings referring to the level of education as a potential factor in reducing exposure to different toxins.[97]

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

4.4. Mechanisms of Pesticide Toxicity. There are numerous mechanisms by which pesticides cause cancer, but these mechanisms are still under investigation and are not incompletely understood. It appears that pesticides can act on the three phases of cancer development: initiation, promotion, and progression. During initiation, direct genotoxicity is an important mechanism in which the cellular genetic material is damaged by the action of genotoxic agents causing cell transformation.[98−100] Several pesticides exhibit genotoxic properties, either intrinsically or through their metabolites. During tumor promotion, when the transformed cell grows and proliferates forming a group of identical transformed cells, some pesticides will act by unbalancing the processes of cell survival and cell death. Finally, in a third step, when the cell acquires the characteristics of a cancerous cell by being multiplied in an anarchic way, some pesticides can promote their progression by acting on hormone receptors.

Many studies in the literature have put forward oxidative stress as the most important mechanism, whereas inflammatory and aberrant epigenetic mechanisms such as DNA methylation, histone modifications, and micro-RNA expression are still in the process of development.[98,100−102] We are going to consider only the role of oxidative stress implicated in a large number of physiopathological mechanisms and described as a component of numerous neurological disorders.[103,104]

The brain tissue is particularly vulnerable to oxidative attack because of different variables, namely, its relatively low antioxidant capacity, high consumption of oxygen, glucose, and energy, huge amounts of fatty acids, high content of easily oxidized substrates, relatively low levels of antioxidants, and the accumulation of redox-active transition metals, such as iron and copper.[105] Furthermore, oxidative stress can prove detrimental by several interacting mechanisms including direct damage of crucial molecular species, increase in intracellular free Ca^{2+}, and release of excitatory amino acids.

Chronic exposure to low pesticide doses can cause toxicity at the cellular level through alteration in redox homeostasis by the generation of oxidative stress following the overproduction of reactive oxygen species (ROS) either by products of detoxification pathways, altering the functioning of mitochondrial transport and endoplasmic reticulum electron chains, or by entering redox cycles.[106−110] ROS are free radical species that are highly unstable in the chemical sense[106,107] and could conceivably be protumorigenic or cytotoxic at high levels. Oxidative stress can also be induced by reactive nitrogen species (RNS) produced during interaction between exogenously and endogenously created nitric oxide (NO) and oxidants such as H_2O_2 and $[O_2^{\bullet-}]$.[108,109] Toxicological studies have included oxidative stress, ROS, and RNS as culprits in protein oxidation, lipid peroxidation, and nucleic acid oxidation (8-OH-deoxyguanosine) and in the changing levels of antioxidants.[103−108,111]

Consequently, the disruption of normal cells state can deregulate several signaling pathways,[112] alter tight junctions,[101,113−115] or damage all cell constituents.[103−108] Proteins, as one of the major targets of oxygen free radicals and other reactive species, may encounter post-translational modifications (PTMs) or be covalently modified by adduct formation on its key amino acid side chains to initiate toxicity via the modification of their structure and/or cellular function and damage cytological processes.[116,117]

PTMs are involved in most signaling cellular processes such as cell growth, differentiation, apoptosis, maintenance of protein structure and integrity, the regulation of metabolism and defense processes, as well as cellular recognition events and morphology changes. They are good candidates with increased research interest in recent years for their application as oxidative stress biomarkers or putative predictors of toxicity. In fact, PTMs symbolize changes in the polypeptide chain as a result of either the addition or removal of chemical moieties from amino acid residues, the proteolytic processing of the protein termini, or covalent crosslinks between protein domains. Thus, PTM characterization is of great interest for an in-depth identification of their role in the pathogenesis as well as the elucidation of the toxicity mechanism, which helps answer clinical questions on novel strategies of diagnosis and therapy.[117]

Protein carbonyl content is one of the most widely used markers of protein oxidative damage due to its early formation and stability by irreversible protein modification. It is used as a strong connection between oxidative stress and the onset of disease progression for many human disorders[118] by the use of redox proteomic approaches,[104,119,120] emerging as a key tool to provide new insights into protein modification with relevance to diseases.[121]

We conducted some preliminary assays to assess carbonylated proteins damaged by oxidative stress in response to environmental toxic agents such as pesticides. We adopted a redox proteomic approach using *SH-SY5Y* neuroblastoma cells, which are widely used in medical research to model the main types of human CNS in different disease contexts, as well as to explore toxic cellular mechanisms and neurotoxin screening.[122,123] Our results by 2D-OXYBLOT as shown in Figure 13 have proved the neurotoxicity effect of both OP and CAR insecticides including aldicarb on neuroblastoma cells found to be significantly altered at the level of protein carbonyls compared to control samples (Figure 13B).

The generation of oxidative stress for possible carcinogenicity of pesticides was highlighted by multiple mechanisms described in the literature.[59,107,108,124,125] Herein, we supply Table SIII to resume some reported studies.

Figure 14 illustrates most of the signaling pathways involved in pesticide-induced ROS and RNS, consequently to oxidative stress implicated in the physiopathological mechanism of human brain tumor.

The overexpression of the antiapoptotic protein BCL-2 leads to apoptosis disruption at the mitochondrial level by the inhibition of caspase activation.[126] Consequently, carcinogenicity occurs when there is an imbalance of proapoptotic proteins such as BAX versus antiapoptotic BCL-2 proteins, which plays a critical role in regulating the intrinsic apoptosis process.

Even though these hypotheses with oxidative stress generation are attractive mechanisms, several issues remain questionable and many details need to be filled.[127]

5. CONCLUSIONS

Cancer risk assessment of environmental pesticide exposures is quite challenging, and strong causal links have not been confirmed yet as most estimations are based on epidemiological studies. To the best of our knowledge, our study is the first conducted research in this field to investigate human brain tumors that may better explain the results of previous studies, which reported the relationships between broader ranges of pesticide types and CNS tumors.

Our findings reveal two major strengths: first, scrutinizing pesticide metabolites in human brain tumor tissues and their separation by a simple and rapid method using GC-MS as the

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

highest analytical tool in metabolomics studies, with the lyophilization and LLE techniques that proved to be suitable preparation methods by preserving the sample quality without altering its composition or losing the majority of its volatile compounds; and second, showing the strongest links between pesticide exposures by occupational and/or residential proximity to agricultural applications and the risk of human brain tumor development mainly in childhood. This, in turn, suggests that preconception pesticide exposure and possibly exposure during pregnancy are associated with an increased risk for CBT.

Our hypothesis was enhanced by the three case identifications of pesticide metabolites detected at an early age and mostly issued from the CAR insecticide class known for their neurotoxicity. The other trace metabolites were issued from pyridazinone, OCs, triazole fungicide, and *N*-nitroso compounds, also known for their carcinogenicity.

The 2D-OXYBLOT analysis confirmed the neurotoxicity effect of insecticides to induce oxidative damage in CNS cells. Aldicarb was implicated in brain carcinogenicity confirmed by the identification of oxime metabolites in a stress degradation study.

Revealing "aziridine" metabolites from the OC class may better emphasize the theory to detect traces of pesticide metabolites at an early age although the Cox proportional hazards model highlights the risk of human brain tumor development beyond the age of 50 years as a long-term effect of pesticide toxicity.

Our study aims to identify gaps in knowledge and areas for future research, for which collaborative work will be needed on human cohort studies with larger sample sizes and different areas in order to achieve more conclusive results and display other possible mechanisms involved in human brain cancer.

Overall, given the widespread use of pesticides and the fact that we continue to find health effects, we need to be vigilant in the postmarketing surveillance of these agrochemicals and to provide effective spraying equipment with PPE in order to decrease exposure risks and consequently the hazardous related diseases.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c04592.

> Additional description of pesticide classification; additional experimental method of gel-based redox proteomics approaches; Table SI, additional table as mentioned in the text; Figure S1, additional photographs as mentioned in the text; Figures S2−S12, additional MS spectra identification of pesticide metabolites in the positive case samples as mentioned in the text; chemometric analysis by a two-component PLS-DA model with additional Figures S13−S16 as mentioned in the text; Tables SII and SIII, additional tables as mentioned in the text; and references (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Kaouthar Louati − *Faculty of Pharmacy, Laboratory of Pharmacology, Analytics and Galenic Drug Development, LR12ES09, University of Monastir, 5000 Monastir, Tunisia;* orcid.org/0000-0001-5085-4766; Phone: + 216 92 761 162; Email: kaoutharlouati66@gmail.com, kaouthar.louati@fphm.u-monastir.tn

Authors

Fatma Kolsi − *Department of Neurosurgery, Habib Bourguiba University Hospital, 3089 Sfax, Tunisia; Faculty of Medicine, University of Sfax, 3029 Sfax, Tunisia*

Rim Kallel − *Laboratory of Pathological Anatomy and Cytology, Habib Bourguiba University Hospital, 3089 Sfax, Tunisia; Faculty of Medicine, University of Sfax, 3029 Sfax, Tunisia*

Yassine Gdoura − *Department of Neurosurgery, Habib Bourguiba University Hospital, 3089 Sfax, Tunisia; Faculty of Medicine, University of Sfax, 3029 Sfax, Tunisia*

Mahdi Borni − *Department of Neurosurgery, Habib Bourguiba University Hospital, 3089 Sfax, Tunisia; Faculty of Medicine, University of Sfax, 3029 Sfax, Tunisia*

Leila Sellami Hakim − *Laboratory of Pathological Anatomy and Cytology, Habib Bourguiba University Hospital, 3089 Sfax, Tunisia; Faculty of Medicine, University of Sfax, 3029 Sfax, Tunisia*

Rania Zribi − *Higher Institute of Applied Studies to Humanities of Tunis (ISEAHT), University of Tunis, 1005 Tunis, Tunisia*

Sirine Choura − *Laboratory of Environmental Bioprocesses, Centre of Biotechnology of Sfax, 3018 Sfax, Tunisia*

Amina Maalej − *Laboratory of Environmental Bioprocesses, Centre of Biotechnology of Sfax, 3018 Sfax, Tunisia*

Sami Sayadi − *Biotechnology Program, Center for Sustainable Development, College of Arts and Sciences, Qatar University, 2713 Doha, Qatar*

Mohamed Chamkha − *Laboratory of Environmental Bioprocesses, Centre of Biotechnology of Sfax, 3018 Sfax, Tunisia*

Basma Mnif − *Faculty of Medicine, University of Sfax, 3029 Sfax, Tunisia*

Zouheir Khemakhem − *Legal Medicine Department, Habib Bourguiba University Hospital, 3089 Sfax, Tunisia; Faculty of Medicine, University of Sfax, 3029 Sfax, Tunisia*

Tahya Sellami Boudawara − *Laboratory of Pathological Anatomy and Cytology, Habib Bourguiba University Hospital, 3089 Sfax, Tunisia; Faculty of Medicine, University of Sfax, 3029 Sfax, Tunisia*

Mohamed Zaher Boudawara − *Department of Neurosurgery, Habib Bourguiba University Hospital, 3089 Sfax, Tunisia; Faculty of Medicine, University of Sfax, 3029 Sfax, Tunisia*

Fathi Safta − *Faculty of Pharmacy, Laboratory of Pharmacology, Analytics and Galenic Drug Development, LR12ES09, University of Monastir, 5000 Monastir, Tunisia*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c04592

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors express their deepest and most sincere gratitude to (1) the representative authorities of the Medical Ethics Committee; (2) the head of the Pathological Anatomy Department and Cytology Laboratory in Habib Bourguiba medium hospital and her team due to their support in the histological diagnosis; (3) neurosurgeons: all neurosurgical doctors who performed successful surgeries by taking into consideration their patients' safety, and all of the team in the

neurosurgery department for their help; (4) Sirine Choura, Amina Maalej, and Mohamed Chemkha for their technical assistance and help in GC-MS analysis and cell culture at the biotechnology center, Sfax, Tunisia; (5) Rania Zribi, for proofreading the English in this article; and (6) the pharmaceutical team of Habib Bourguiba Medium Hospital for providing the necessary surgical materials. The authors are also immensely grateful to Dr. A. Wijesekara, A. Kocon, and Dr. W. Carter for their precious help and support while conducting the first experimental analysis in assessing pesticides' toxicity in the school of medicine, Royal Derby Hospital Center, University of Nottingham, U.K.

ABBREVIATIONS

AChE:acetylcholinesterase
ACh:acetylcholine
ALD:aldicarbe
ANOVA:one-way analysis of variance
Anti-NSE:antineuron specific enolase
AZO:azamethiphos-oxon
BSTFA:*N*,*O*-bis(trimethylsilyl)trifluoroacetamide trimethylsilyl
CAT:catalase
CBT:childhood brain tumor
CARs:carbamates
CNS:central nervous system
CPO:chlorpyrifos-oxon
2D-PAGE:2-dimensional polyacrylamide gel electrophoresis
DDT:dichlorodiphenyltrichloroethane
ECD:electron capture detection
FAO:The Food and Agriculture Organization
FFPE:formalin-fixed paraffin-embedded
GC-MS:gas chromatography−mass spectrometry
GFAP:glial fibrillary acidic protein
GPx:glutathione peroxidase
GSH:glutathione
GST:glutathione-S-transferase
H_2O_2:hydrogen peroxide
IHS:immunohistochamical staining
IARC:International Agency for Research on Cancer
LD:lethal dose
MSTFA:*N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide
NOCs:*N*-nitroso compounds
NO:nitric oxide
NS:nervous system
OPs:organophosphates
OCs:organochlorines
OR:odds ratio
PCA:principal component analysis
PLS:partial least-squares projection to latent structures
PLS-DA:PLS-discriminant analysis
PTM:post-translational modifications
PPE:personal protective equipment
PPs:phenylpyrazoles
PYPs:pyrethroids
RNA:ribonucleic acid
RNS:reactive nitrogen species
RR:risk ratio
RT:retention time
RT-qPCR:quantitative reverse-transcription PCR
ROS:reactive oxygen species
SDS-PAGE:sodium dodecyl sulfate-polyacrylamide gel
SD:standard deviation
SOD:superoxide dismutase
TMS:trimethylsilyl
US-EPA:U.S. Environmental Protection Agency
WHO:World Health Organization

REFERENCES

(1) Lushchak, V. I.; Matviishyn, T. M.; Husak, V. V.; Storey, J. M.; Storey, K. B. Pesticide toxicity: a mechanistic approach. *EXCLI J.* **2018**, *17*, 1101−1136.
(2) Osman, K. A. Pesticides and Human Health. In *Pesticides in the Modern World − Effects of Pesticides Exposure*; Stoytcheva, M., Ed.; InTech, 2011; pp 206−230.
(3) Schleiffer, M.; Speiser, B. Presence of pesticides in the environment, transition into organic food, and implications for quality assurance along the European organic food chain - A review. *Environ Pollut.* **2022**, *313*, 120116.
(4) Cabras, P.; Angioni, A. Pesticide residues in grapes, wine, and their processing products. *J. Agric. Food Chem.* **2000**, *48*, 967−973.
(5) Betarbet, R.; Sherer, T. B.; MacKenzie, G.; Garcia-Osuna, M.; Panov, A. V.; Greenamyre, J. T. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat. Neurosci.* **2000**, *3*, 1301−1306.
(6) Bjørling-Poulsen, M.; Andersen, H. R.; Grandjean, P. Potential developmental neurotoxicity of pesticides used in Europe. *Environ. Health* **2008**, *7*, 50.
(7) Roeleveld, N.; Bretveld, R. The impact of pesticides on male fertility. *Curr. Opin. Obstet. Gynecol.* **2008**, *20*, 229−233.
(8) Bretveld, R. W.; Thomas, C. M. G.; Scheepers, P. T. J.; Zielhuis, G. A.; Roeleveld, N. Pesticide exposure: the hormonal function of the female reproductive system disrupted? *Reprod. Biol. Endocrinol.* **2006**, *4*, 30.
(9) Salameh, P.; Waked, M.; Baldi, I.; Brochard, P.; Saleh, B. A. Respiratory diseases and pesticide exposure: a case-control study in Lebanon. *J. Epidemiol. Community Health* **2006**, *60*, 256−261.
(10) Bassil, K. L.; Vakil, C.; Sanborn, M.; Cole, D. C.; Kaur, J. S.; Kerr, K. J. Cancer health effects of pesticides. Systematic review. *Can. Fam. Physician.* **2007**, *53*, 1704−1711.
(11) Silva, A. M. C.; Campos, P. H. N.; Mattos, I. E.; Hajat, S.; Lacerda, E. M.; Ferreira, M. J. M. Environmental Exposure to Pesticides and Breast Cancer in a Region of Intensive Agribusiness Activity in Brazil: A Case-Control Study. *Int. J. Environ. Res. Public Health* **2019**, *16*, 3951.
(12) Vettorazzi, G. State of the art of the toxicological evaluation carried out by the Joint FAO/WHO Meeting on Pesticide Residues. II. Carbamate and organophosphorus pesticides used in agriculture and public health. *Rev. Environ. Contam. Toxicol.* **1976**, *63*, 1−44.
(13) Schroeder, C.; Evans, E. The U.S. Environmental Protection Agency's Pesticide Worker Safety Program–implementation of the National Strategies for Health Care Providers: Pesticides Initiative. *J. Environ. Health* **2009**, *72*, 64−65.
(14) Kalyabina, V. P.; Esimbekova, N. E.; Kopylova, K. V.; Kratasyuk, V. A. Pesticides: formulants, distribution pathways and effects on human health − a review. *Toxicol. Rep.* **2021**, *8*, 1179−1192.
(15) Keifer, M. C.; Firestone, J. Neurotoxicity of pesticides. *J. Agromed.* **2007**, *12*, 17−25.
(16) Keifer, M. C.; Mahurin, R. K. Chronic neurologic effects of pesticide overexposure. *Occup. Med.* **1997**, *12*, 291−304.
(17) Stewart, P. A.; Prince, J. K.; Colt, J. S.; Ward, M. H. A method for assessing occupational pesticide exposures of farmworkers. *Am. J. Ind. Med.* **2001**, *40*, 561−570.
(18) Dereumeaux, C.; Fillol, C.; Quenel, P.; Denys, S. Pesticide exposures for residents living close to agricultural lands: A review. *Environ. Int.* **2020**, *134*, No. 105210.
(19) Gatto, N. M.; Ogata, P.; Lytle, B. Farming, Pesticides, and Brain Cancer: A 20-Year Updated Systematic Literature Review and Meta-Analysis. *Cancers* **2021**, *13*, 4477.
(20) Sturm, E. T.; Castro, C.; Mendez-Colmenares, A.; Duffy, J.; Burzynska, A. A. Z.; Stallones, L.; Thomas, M. L. Risk Factors for Brain

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

Health in Agricultural Work: A Systematic Review. *Int. J. Environ. Res. Public Health* **2022**, *19*, 3373.
(21) Amoatey, P.; Al-Mayahi, A.; Omidvarborna, H.; Baawain, M. S.; Sulaiman, H. Occupational exposure to pesticides and associated health effects among greenhouse farm workers. *Environ. Sci. Pollut. Res Int.* **2020**, *27*, 22251−22270.
(22) Greenop, K. R.; Peters, S.; Bailey, H. D.; Fritschi, L.; Attia, J.; Scott, R. J.; Glass, D. C.; de Klerk, N. H.; Alvaro, F.; Armstrong, B. K.; Milne, E. Exposure to pesticides and the risk of childhood brain tumors. *Cancer Causes Control* **2013**, *24*, 1269−1278.
(23) Pope, W. B.; Prins, R. M.; Thomas, M. A.; Nagarajan, R.; Yen, K. E.; Bittinger, M. A.; Salamon, N.; Chou, A. P.; Yong, W. H.; Soto, H.; Wilson, N.; Driggers, E.; Jang, H. G.; Su, S. M.; Schenkein, D. P.; Lai, A.; Cloughesy, T. F.; Kornblum, H. I.; Wu, H.; Fantin, V. R.; Liau, L. M. Non-invasive detection of 2-hydroxyglutarate and other metabolites in IDH1 mutant glioma patients using magnetic resonance spectroscopy. *J. Neurooncol.* **2012**, *107*, 197−205.
(24) Lehnhardt, F. G.; Bock, C.; Röhn, G.; Ernestus, R. I.; Hoehn, M. Metabolic differences between primary and recurrent human brain tumors: a ^{1}H NMR spectroscopic investigation. *NMR Biomed.* **2005**, *18*, 371−382.
(25) Emwas, A. H. The strengths and weaknesses of NMR spectroscopy and mass spectrometry with particular focus on metabolomics research. *Methods Mol. Biol.* **2015**, *1277*, 161−193.
(26) Fiehn, O. Metabolomics by Gas Chromatography-Mass Spectrometry: Combined Targeted and Untargeted Profiling. *Curr. Protoc. Mol. Biol.* **2016**, *114*, 30.4.1−30.4.32.
(27) Zhou, B.; Xiao, J. F.; Tuli, L.; Ressom, H. W. LC-MS-based metabolomics. *Mol. Biosyst.* **2012**, *8*, 470−481.
(28) Engel, L. S.; Keifer, M. C.; Zahm, S. H. Comparison of a traditional questionnaire with an icon/calendar-based questionnaire to assess occupational history. *Am. J. Ind. Med.* **2001**, *40*, 502−511.
(29) Zahm, S. H.; Colt, J. S.; Engel, L. S.; Keifer, M. C.; Alvarado, A. J.; Burau, K.; Butterfield, P.; Caldera, S.; Cooper, S. P.; Garcia, D.; Hanis, C.; Hendrikson, E.; Heyer, N.; Hunt, L. M.; Krauska, M.; MacNaughton, N.; McDonnell, C. J.; Mills, P. K.; Mull, L. D.; Nordstrom, D. L.; Outterson, B.; Slesinger, D. P.; Smith, M. A.; Stallones, L.; Stephens, C.; Sweeney, A.; Sweitzer, K.; Vernon, S. W.; Blair, A. Development of a life events/icon calendar questionnaire to ascertain occupational histories and other characteristics of migrant farmworkers. *Am. J. Ind. Med.* **2001**, *40*, 490−501.
(30) Bell, E. M.; Hertz-Picciotto, I.; Beaumont, J. J. Case-cohort analysis of agricultural pesticide applications near maternal residence and selected causes of fetal death. *Am. J. Epidemiol.* **2001**, *154*, 702−710.
(31) Ostrom, Q. T.; Gittleman, H.; de Blank, P. M.; Finlay, J. L.; Gurney, J. G.; McKean-Cowdin, R.; Stearns, D. S.; Wolff, J. E.; Liu, M.; Wolinsky, Y.; Kruchko, C.; Barnholtz-Sloan, J. S. American Brain Tumor Association Adolescent and Young Adult Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012. *Neuro Oncol.* **2016**, *18*, i1−i50.
(32) Polisetty, R. V.; Gautam, P.; Sharma, R.; Harsha, H. C.; Nair, S. C.; Gupta, M. K.; Uppin, M. S.; Challa, S.; Puligopu, A. K.; Ankathi, P.; Purohit, A. K.; Chandak, G. R.; Pandey, A.; Sirdeshmukh, R. LC-MS/MS analysis of differentially expressed glioblastoma membrane proteome reveals altered calcium signaling and other protein groups of regulatory functions. *Mol. Cell. Proteomics* **2012**, *11*, No. M111.013565.
(33) Fonte, P.; Reis, S.; Sarmento, B. Facts and evidences on the lyophilization of polymeric nanoparticles for drug delivery. *J. Controlled Release* **2016**, *225*, 75−86.
(34) Fornaguera, C.; Castells-Sala, C.; Lázaro, M. A.; Cascante, A.; Borrós, S. Development of an optimized freeze-drying protocol for OM-PBAE nucleic acid polyplexes. *Int. J. Pharm.* **2019**, *569*, No. 118612.
(35) Damsteegt, E. L.; McHugh, N.; Lokman, P. M. Storage by lyophilization − Resulting RNA quality is tissue dependent. *Anal. Biochem.* **2016**, *511*, 92−96.
(36) Mareninov, S.; De Jesus, J.; Sanchez, D. E.; Kay, A. B.; Wilson, R. W.; Babic, I.; Chen, W.; Telesca, D.; Lou, J. J.; Mirsadraei, L.; Gardner, T. P.; Khanlou, N.; Vinters, H. V.; Shafa, B. B.; Lai, A.; Liau, L. M.; Mischel, P. S.; Cloughesy, T. F.; Yong, W. H. Lyophilized brain tumor specimens can be used for histologic, nucleic acid, and protein analyses after 1 year of room temperature storage. *J. Neurooncol.* **2013**, *113*, 365−373.
(37) Filho, P. J. S.; Silveira, L. A.; Betemps, G. R.; Oliveira, P. K.; Sampaio, D. M.; Santos, D. G. L. Use of lyophilization as analytical strategy for chromatographic characterization of aqueous phase of bio-oil produced by rice husk pyrolysis. *Microchem. J.* **2020**, *152*, No. 104457.
(38) Aggio, R. B. M.; Mayor, A.; Coyle, S.; Reade, S.; Khalid, T.; Ratcliffe, N. M.; Probert, C. S. Freeze-drying: an alternative method for the analysis of volatile organic compounds in the headspace of urine samples using solid phase micro-extraction coupled to gas chromatography - mass spectrometry. *Chem. Cent. J.* **2016**, *10*, 9.
(39) Lugemwa, F. N. Extraction of Betulin, Trimyristin, Eugenol and Carnosic Acid Using Water-Organic Solvent Mixtures. *Molecules* **2012**, *17*, 9274−9282.
(40) Zaikin, V. G.; Halket, J. M. Review: Derivatization in mass spectrometry: Acylation. *Eur. J. Mass Spectrom.* **2003**, *9*, 421−434.
(41) Schummer, C.; Delhomme, O.; Appenzeller, B. M. R.; Wennig, R.; Millet, M. Comparison of MTBSTFA and BSTFA in derivatization reactions of polar compounds prior to GC/MS analysis. *Talanta* **2009**, *77*, 1473−1482.
(42) AliHabotta, O.; Abouelmagd, M. M.; Halawa, A. A.; Uno, S. GC-MS based metabolic profile of brain tissue in common carp following acute exposure to cypermethrin. *Int. J. Adv. Res.* **2019**, *7*, 188−194.
(43) Wu, H.; Wei, Q.; Zheng, Y.; Fang, S.; Fu, Y.; Liao, L. Metabolomic analysis of the brain and blood from rats exposed to high-dose Chlorpyrifos. *J. Forensic Sci. Med.* **2019**, *5*, 1−6.
(44) Zeinali, M.; McConnell, L. L.; Hapeman, C. J.; Nguyen, A.; Schmidt, W. F.; Howard, C. J. Volatile organic compounds in pesticide formulations: Methods to estimate ozone formation potential. *Atmos. Environ.* **2011**, *45*, 2404−2412.
(45) Robles-Molina, J.; Gilbert-López, B.; García-Reyes, J. F.; Molina-Díaz, A. Comparative evaluation of liquid-liquid extraction, solid-phase extraction and solid-phase microextraction for the gas chromatography-mass spectrometry determination of multiclass priority organic contaminants in wastewater. *Talanta* **2013**, *117*, 382−391.
(46) Muñoz-González, C.; Pozo-Bayón, M. A.; Rodríguez-Bencomo, J. J.; Martín-Álvarez, P. J.; Cueva, C.; Bartolomé, B.; Moreno-Arribas, M. V. Comparison of Liquid−Liquid Extraction and Solid Phase Extraction for the GC-MS Analysis of Phenolic Acids Resulting from the Degradation of Wine Polyphenols by Faecalmicrobiota, 2010. https://digital.csic.es/handle/10261/113935.
(47) Canbay, H. S. Effectiveness of Liquid-Liquid Extraction, Solid Phase Extraction, and Headspace Technique for Determination of Some Volatile Water-Soluble Compounds of Rose Aromatic Water. *Int. J. Anal. Chem.* **2017**, *2017*, No. 4870671.
(48) Thorstensen, C. W.; Lode, O.; Christiansen, A. L. Development of a solid-phase extraction method for phenoxy acids and bentazone in water and comparison to a liquid-liquid extraction method. *J. Agric. Food Chem.* **2000**, *48*, 5829−5833.
(49) Kumari, C.; Varughese, B.; Ramji, S.; Kapoor, S. Liquid-Liquid Extraction and Solid Phase Extraction for Urinary Organic Acids: A Comparative Study from a Resource Constraint Setting. *Indian J. Clin. Biochem.* **2016**, *31*, 414−422.
(50) Williams, C.; Buica, A. Comparison of an Offline SPE-GC-MS and Online HS-SPME-GC-MS Method for the Analysis of Volatile Terpenoids in Wine. *Molecules* **2020**, *25*, 657.
(51) Niu, Z.; Yu, C.; He, X.; Zhang, J.; Wen, Y. Salting-out assisted liquid−liquid extraction combined with Gas Chromatography-Mass Spectrometry for the determination of pyrethroid insecticides in high salinity and biological samples. *J. Pharm. Biomed. Anal.* **2017**, *143*, 222−227.
(52) Stefanelli, P.; Santilio, A.; Cataldi, L.; Dommarco, R. Multi-residue analysis of organochlorine and pyrethroid pesticides in ground

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

beef meat by Gas Chromatography-Mass Spectrometry. *J. Environ. Sci. Health, Part B* **2009**, *44*, 350−356.
(53) Kiranmayi, C. B.; Krishnaiah, N.; Kumar, M. M.; Kumar, M. S.; Subhashini, N.; Rao, T. M.; Pradesh, A. Multiresidue Analysis of Pesticides in Beef and Mutton Samples and Study on Effect of Cooking on Residual Levels of Aldrin and Dieldrin. *Int. J. Sci. Environ. Technol.* **2016**, *5*, 195−203.
(54) Letta, B. D.; Attah, L. E. Residue levels of organochlorine pesticides in cattle meat and organs slaughtered in selected towns in West Shoa Zone, Ethiopia. *J. Environ. Sci. Health, Part B* **2013**, *48*, 23−32.
(55) Cao, L.; Chen, B.; Zheng, L.; Wang, D.; Liu, F.; Huang, Q. Assessment of potential dermal and inhalation exposure of workers to the insecticide imidacloprid using whole-body dosimetry in China. *J. Environ. Sci.* **2015**, *27*, 139−146.
(56) Li, S.; Yu, P.; Zhou, C.; Tong, L.; Li, D.; Yu, Z.; Zhao, Y. Analysis of pesticide residues in commercially available chenpi using a modified QuEChERS method and GC-MS/MS determination. *J. Pharm. Anal.* **2020**, *10*, 60−69.
(57) La Rocca, C.; Mantovani, A. From environment to food: The case of PCB. *Ann. Ist. Super. Sanita.* **2006**, *42*, 410−416.
(58) Gullick, D. R.; Mott, K. B.; Bartlett, M. G. Chromatographic methods for the bioanalysis of pyrethroid pesticides. *Biomed. Chromatogr.* **2016**, *30*, 772−789.
(59) Bagchi, M.; zafra, S.; Bagchi, D.DNA Damage, Gene Expression, and Carcinogenesis by Organophosphates and Carbamates. In *Toxicology of Organophosphate & Carbamate Compounds*; Academic Press, 2006; Chapter 37, pp 533−548.
(60) Bank, S.; Tyrell, R. J. Kinetics and mechanism of alkaline and acidic hydrolysis of aldicarb. *J. Agric. Food Chem.* **1984**, *32*, 1223−1232.
(61) Eren, Y.; Erdoğmuş, F. S.; Akyıl, D.; Özkara, A.; Konuk, M.; Sağlam, E. Cytotoxic and genotoxic effects of dioxacarb by human peripheral blood lymphocytes CAs and Allium test. *Cytotechnology* **2015**, *67*, 1023−1030.
(62) Loh, Y. H.; Jakszyn, P.; Luben, R. N.; Mulligan, A. A.; Mitrou, P. N.; Khaw, K. T. N-nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)−Norfolk Study. *Am. J. Clin. Nutr.* **2011**, *93*, 1053−1061.
(63) Rustia, M.; Shubik, P. Prenatal Induction of Neurogenic Tumors in Hamsters by Precursors Ethylurea and Sodium Nitrite. *J. Natl. Cancer Inst.* **1974**, *52*, 605−608.
(64) And, A. M.; Mitsumori, K. Spontaneous Occurrence and Chemical Induction of Neurogenic Tumors in Rats-Influence of Host Factors and Specificity of Chemical Structure. *Crit. Rev. Toxicol.* **1990**, *20*, 287−310.
(65) Koestner, A. Characterization of N-Nitrosourea-Induced Tumors of the Nervous System; Their Prospective Value for Studies of Neurocarcinogenesis and Brain Tumor Therapy. *Toxicol. Pathol.* **1990**, *18*, 186−192.
(66) Bondy, M. L.; Scheurer, M. E.; Malmer, B.; Barnholtz-Sloan, J. S.; Davis, F. G.; Il'yasova, D.; Kruchko, C.; McCarthy, B. J.; Rajaraman, P.; Schwartzbaum, J. A.; Sadetzki, S.; Schlehofer, B.; Tihan, T.; Wiemels, J. L.; Wrensch, M.; Buffler, P. A. Brain tumor epidemiology: consensus from the Brain Tumor Epidemiology Consortium. *Cancer* **2008**, *113*, 1953−1968.
(67) Baldi, I.; Robert, C.; Piantoni, F.; Tual, S.; Bouvier, G.; Lebailly, P.; Raherison, C. Agricultural exposure and asthma risk in the AGRICAN French cohort. *Int. J. Hyg. Environ. Health* **2014**, *217*, 435−442.
(68) Pouchieu, C.; Baldi, I.; Grüber, A.; Berteaud, E.; Carles, C.; Loiseau, H. Descriptive Epidemiology and Risk Factors of Primary Central Nervous System Tumors: Current Knowledge. *Rev. Neurol.* **2016**, *172*, 46−55.
(69) Cohen, S. Z.; Zweig, G. Analytical Determination of N-Nitroso Compounds in Pesticides by the United States Environmental Protection Agency-a Preliminary Study. *IARC Sci. Publ.* **1978**, *19*, 333−342.
(70) Bontoyan, W. R.; Law, M. W.; Wright, D. P. Nitrosamines in Agricultural and Home-Use Pesticides. *J. Agric. Food Chem.* **1979**, *27*, 631−635.
(71) Zweig, G.; Selim, S.; Hummel, R.; Mittelman, A.; Wright, D. P., Jr.; Law, C., Jr.; Regelman, E. Analytical Survey of N-Nitroso Contaminants in Pesticide Products. *IARC Sci.* **1980**, *31*, 555−564.
(72) Lee, W. J.; Colt, J. S.; Heineman, E. F.; McComb, R.; Weisenburger, D. D.; Lijinsky, W.; Ward, M. H. Agricultural pesticide use and risk of glioma in Nebraska, United States. *Occup. Environ. Med.* **2005**, *62*, 786−792.
(73) Arrebola, J. P.; Belhassen, H.; Artacho-Cordon, F.; Ghali, R.; Ghorbel, H.; Boussen, H.; Perez Carrascosa, F. M.; Exposito, J.; Hedhili, A.; Olea, N. Risk of female breast cancer and serum concentrations of organochlorine pesticides and polychlorinated biphenyls: a case−control study in Tunisia. *Sci. Total Environ.* **2015**, *520*, 106−113.
(74) Koutros, S.; Langseth, H.; Grimsrud, T.; Barr, D.; Vermeulen, R.; Portengen, L.; Wacholder, S.; Wacholder, S.; Freeman, L. B.; Freeman, L. E. B.; Blair, A.; Blair, A.; Hayes, R.; Hayes, R. B.; Rothman, N.; Rothman, N.; Engel, L. Prediagnostic serum organochlorine concentrations and metastatic prostate cancer: a nested case−control study in the Norwegian Janus Serum Bank cohort. *Environ. Health Perspect.* **2015**, *123*, 867−872.
(75) Parada, H.; Wolff, M. S.; Engel, L. S.; White, A. J.; Eng, S. M.; Cleveland, R. J.; Khankari, N. K.; Teitelbaum, S. L.; Neugut, A. I.; Gammon, M. D. Organochlorine insecticides DDT and chlordane in relation to survival following breast cancer. *Int. J. Cancer* **2016**, *138*, 565−575.
(76) Louis, L. M.; Lerro, C. C.; Friesen, M. C.; Andreotti, G.; Koutros, S.; Sandler, D. P.; Blair, A.; Robson, M. G.; Freeman, L. E. B. A prospective study of cancer risk among agricultural health study farm spouses associated with personal use of organochlorine insecticides. *Environ. Health* **2017**, *16*, 95.
(77) Horvat, C. M.; Wolfenden, R. V. A persistent pesticide residue and the unusual catalytic proficiency of a dehalogenating enzyme. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 16199−16202.
(78) *Report on Carcinogens, Fifteenth Edition. 1,3-Dichloropropene (Technical Grade), CAS No. 542-75-6* National Toxicology Program, Department of Health and Human Services; 2021.
(79) Soler-Rodríguez, F.; Oropesa Jiménez, A. L. Triazole Fungicide. In *Encyclopedia of Toxicology*, 3rd ed.; Wexler, P., Ed.; Elsevier B.V., 2014.
(80) Schwarzbacherová, V.; Šiviková, K.; Drážovská, M.; Dianovský, J. Evaluation of DNA damage and cytotoxicity induced by triazole fungicide in cultured bovine lymphocytes. *Int. J. Cytol., Cytosyst. Cytogenet.* **2015**, *68*, 233−238.
(81) Brancato, A.; Brocca, D.; Carrasco Cabrera, L.; Chiusolo, A.; Civitella, C.; Court Marques, D.; Crivellente, F.; De Lentdecker, C.; Erdös, Z.; Ferreira, L.; Goumenou, M.; Greco, L.; Istace, F.; Jarrah, S.; Kardassi, D.; Leuschner, R.; Medina, P.; Mineo, D.; Miron, I.; Molnar, T.; Nave, S.; Parra Morte, J. M.; Pedersen, R.; Reich, H.; Sacchi, A.; Santos, M.; Stanek, A.; Sturma, J.; Tarazona, J.; Terron, A.; Theobald, A.; Vagenende, B.; Villamar-Bouza, L. Peer review of the pesticide risk assessment for the triazole derivative metabolites in light of confirmatory data submitted. *EFSA J.* **2018**, *16*, No. e05376.
(82) Goetz, A. K.; Dix, D. J. Toxicogenomic effects common to triazole antifungals and conserved between rats and humans. *Toxicol. Appl. Pharmacol.* **2009**, *238*, 80−89.
(83) Nesnow, S.; Ward, W.; Tanya Moore, T.; Ren, H.; Hester, S. D. Discrimination of Tumorigenic Triazole Conazoles from Phenobarbital by Transcriptional Analyses of Mouse Liver Gene Expression. *Toxicol. Sci.* **2009**, *110*, 68−83.
(84) Manangama, G.; Teysseire, T.; Brochard, P.; et al. Exposition aux pesticides et issues défavorables de grossesse: revue systématique. *Arch. Maladies Professionnelles l'Environ.* **2018**, *79*, 308−309.
(85) Zahm, S. H.; Ward, M. H. Pesticides and childhood cancer. *Environ. Health Perspect.* **1998**, *106*, 893−908.

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

(86) Chen, S.; Gu, S.; Wang, Y.; Yao, Y.; Wang, G.; Jin, Y.; Wu, Y. Exposure to pyrethroid pesticides and the risk of childhood brain tumors in East China. *Environ. Pollut.* **2016**, *218*, 1128−1134.
(87) Van Maele-Fabry, G.; Gamet-Payrastre, L.; Lison, D. Residential exposure to pesticides as risk factor for childhood and young adult brain tumors: A systematic review and meta-analysis. *Environ Int.* **2017**, *106*, 69−90.
(88) Ribas-Fitó, N.; Torrent, M.; Carrizo, D.; Muñoz-Ortiz, L.; Júlvez, J.; Grimalt, J. O.; Sunyer, J. In utero exposure to background concentrations of DDT and cognitive functioning among preschoolers. *Am. J. Epidemiol.* **2006**, *164*, 955−962.
(89) Lombardi, C.; Thompsonb, S.; Ritzb, B.; Cockburn, M.; E Heck, J. Residential proximity to pesticide application as a risk factor for childhood central nervous system tumors. *Environ. Res.* **2021**, *197*, No. 111078.
(90) Kaonga, C. C.; Takeda, K.; Sakugawa, H. Concentration and degradation of alternative biocides and an insecticide in surface waters and their major sinks in a semi-enclosed sea, Japan. *Chemosphere* **2016**, *145*, 256−264.
(91) Alavanja, M. C.; Hoppin, J. A.; Kamel, F. Health effects of chronic pesticide exposure: cancer and neurotoxicity. *Annu. Rev. Public Health* **2004**, *25*, 155−197.
(92) De Wildt, S. N. Profound changes in drug metabolism enzymes and possible effects on drug therapy in neonates and children. *Expert Opin. Drug Metab. Toxicol.* **2011**, *7*, 935−948.
(93) Hines, R. N. The ontogeny of drug metabolism enzymes and implications for adverse drug events. *Pharmacol Ther.* **2008**, *118*, 250−267.
(94) Richardson, J. R.; Fitsanakis, V.; Westerink, R. H. S.; Kanthasamy, A. G. Neurotoxicity of pesticides. *Acta Neuropathol.* **2019**, *138*, 343−362.
(95) National Report on the Situation of Highly Hazardous Pesticides (HHPs), 2020. https://ipen.org/sites/default/files/documents/final_2july2020_tunisia_national_report_hhps.pdf.
(96) Delpuech, A. Toxic Active Substances Imported Into Tunisia, 2021. https://inkyfada.com/fr/2021/06/25/pesticides-exportations-toxiques-ue-tunisie/.
(97) Gaber, S.; Abdel-Latif, S. H. Effect of education and health locus of control on safe use of pesticides: a cross sectional random study. *J. Occup. Med. Toxicol.* **2012**, *7*, 3.
(98) Alavanja, M. C. R.; Ross, M. K.; Bonne, M. R. Increased cancer burden among pesticide applicators and others due to pesticide exposure. *CA, Cancer J. Clin.* **2013**, *63*, 120−142.
(99) Aiassa, D. E.; Mañas, F. J.; Gentile, N. E.; Bosch, B.; Salinero, M. C.; Gorla, B. M. N. Evaluation of genetic damage in pesticides applicators from the province of Córdoba, Argentina. *Environ. Sci. Pollut. Res.* **2019**, *26*, 20981−20988.
(100) Doğanlar, Z. B.; Doğanlar, O.; Tozkir, H.; Gökalp, F. D.; Doğan, A.; Yamaç, F.; Aşkın, O. O.; Aktaş, U. E. Nonoccupational Exposure of Agricultural Area Residents to Pesticides: Pesticide Accumulation and Evaluation of Genotoxicity. *Arch. Environ. Contam. Toxicol.* **2018**, *75*, 530−544.
(101) García-García, C. R.; Parrón, T.; Requena, M.; Alarcón, R.; Tsatsakis, A. M.; Hernández, A. F. Occupational pesticide exposure and adverse health effects at the clinical, hematological and biochemical level. *Life Sci.* **2016**, *145*, 274−283.
(102) Collotta, M.; Bertazzi, P. A.; Bollati, V. Epigenetics and pesticides. *Toxicology* **2013**, *307*, 35−41.
(103) Stadtman, E. R. Protein oxidation and aging. *Science* **1992**, *257*, 1220.
(104) Shen, L.; Chen, C.; Yang, A.; Chen, Y.; Liu, Q.; Ni, J. Redox proteomics identification of specifically carbonylated proteins in the hippocampi of triple transgenic Alzheimer's disease mice at its earliest pathological stage. *J Proteomics* **2015**, *123*, 101−113.
(105) Berlett, B. S.; Stadtman, E. R. Protein oxidation in aging, disease, and oxidative stress. *J. Biol. Chem.* **1997**, *272*, 20313−20316.
(106) Halliwell, B. Oxidative stress and neurodegeneration: where are we now? *J. Neurochem.* **2006**, *97*, 1634−1658.
(107) Sanders, L. H.; Timothy Greenamyre, J. Oxidative damage to macromolecules in human Parkinson disease and the rotenone model. *Free Radical Biol. Med.* **2013**, *62*, 111−120.
(108) Sule, R. O.; Condon, L.; Gomes, A. V. A Common Feature of Pesticides: Oxidative Stress: The Role of Oxidative Stress in Pesticide-Induced Toxicity. *Oxid. Med. Cell. Longevity* **2022**, *2022*, No. 5563759.
(109) Reczek, C. R.; Birsoy, K.; Kong, H.; Martinez-Reyes, I.; Wang, T.; Gao, P.; Sabatini, D. M.; Chandel, N. S. A CRISPR screen identifies a pathway required for paraquat-induced cell death. *Nat. Chem. Biol.* **2017**, *13*, 1274−1279.
(110) Weitzman, S. A.; Gordon, L. I. Inflammation and cancer: role of phagocyte-generated oxidants in carcinogenesis. *Blood* **1990**, *76*, 655−663.
(111) Hwang, N. R.; Yim, S. H.; Kim, Y. M.; Jeong, J.; Song, E. J.; Lee, Y.; Lee, J. H.; Choi, S.; Lee, K. J. Oxidative modifications of glyceraldehyde-3-phosphate dehydrogenase play a key role in its multiple cellular functions. *Biochem. J.* **2009**, *423*, 253−264.
(112) Tebourbi, O.; Sakly, M.; Ben Rhouma, K. Molecular Mechanisms of Pesticide Toxicity. In *Pesticides in the Modern World − Pests Control and Pesticides Exposure and Toxicity Assessment*; InTech: Tunisia, 2011.
(113) Lee, H. S.; Namkoong, K.; Kim, D. H.; Kim, K. J.; Cheong, Y. H.; Kim, S. S.; Lee, W. B.; Kim, K. Y. Hydrogen peroxide-induced alterations of tight junction proteins in bovine brain microvascular endothelial cells. *Microvasc. Res.* **2004**, *68*, 231−238.
(114) Doğanlar, Z. B.; Doğanlar, O.; Tozkir, H.; Gökalp, F. D.; Doğan, A.; Yamaç, F.; Aşkın, O. O.; Aktaş, Ü.E. Nonoccupational Exposure of Agricultural Area Residents to Pesticides: Pesticide Accumulation and Evaluation of Genotoxicity. *Arch. Environ. Contam. Toxicol.* **2018**, *75*, 530−544.
(115) Abdel-Halim, K. Y.; Osman, S. R. Cytotoxicity and Oxidative Stress Responses of Imidacloprid and Glyphosate in Human Prostate Epithelial WPM-Y.1 Cell Line. *J. Toxicol.* **2020**, *2020*, No. 4364650.
(116) Yang, X.; Bartlett, M. G. Identification of protein adduction using mass spectrometry: Protein adducts as biomarkers and predictors of toxicity mechanisms. *Rapid Commun. Mass Spectrom.* **2016**, *30*, 652−664.
(117) Ramazi, S.; Zahiri, J. Post-translational modifications in proteins: resources, tools and prediction methods. *Database* **2021**, *2021*, No. baab012.
(118) Dalle-Donne, I.; Rossi, R.; Giustarini, D.; Milzani, A.; Colombo, R. Protein carbonyl groups as biomarkers of oxidative stress. *Clin. Chim. Acta* **2003**, *329*, 23−38.
(119) Chiaradia, E.; Renzone, G.; Scaloni, A.; Caputo, M.; Costanzi, E.; Gambelunghe, A.; Muzi, G.; Avellini, L.; Emiliani, C.; Buratta, S. Protein carbonylation in dopamaminergic cells exposed to rotenone. *Toxicol. Lett.* **2019**, *309*, 20−32.
(120) Butterfield, D. A.; Gu, L.; Domenico, F. D.; Robinson, R. A. S. Mass spectrometry and redox proteomics: applications in disease. *Mass Spectrom. Rev.* **2014**, *33*, 277−301.
(121) Butterfield, D. A.; Perluigi, M. Redox Proteomics: A Key Tool for New Insights into Protein Modification with Relevance to Disease. *Antioxid. Redox Signal.* **2017**, *26*, 277−279.
(122) Yang, W.; Tiffany-Castiglioni, E.; Lee, M. Y.; Son, I. H. Paraquat induces cyclooxygenase-2 (COX-2) implicated toxicity in human neuroblastoma *SHSY5Y* cells. *Toxicol. Lett.* **2010**, *199*, 239−246.
(123) Zou, D. J.; Wang, G.; Liu, J. C.; Dong, M. X.; Li, X. M.; Zhang, C.; Zhou, L.; Wang, R.; Niu, Y. C. Beta-asarone attenuates beta-amyloid-induced apoptosis through the inhibition of the activation of apoptosis signal-regulating kinase-1 in *SH-SY5Y* cells. *Pharmazie* **2011**, *66*, 44−51.
(124) Milatovic, D.; Gupta, R. C.; Aschner, M. Anticholinesterase toxicity and oxidative stress. *Sci. World J.* **2006**, *6*, 295−310.
(125) Chang, P.-A.; Wu, Y. J.; Li, W.; Leng, X. F. Effect of carbamate esters on neurite outgrowth in differentiating human SK-N-SH neuroblastoma cells. *Chem.−Biol. Interact.* **2006**, *159*, 65−72.
(126) Stegh, A. H.; Kesari, S.; Mahoney, J. E.; Jenq, H. T.; Forloney, K. L.; Protopopov, A.; Louis, D. N.; Chin, L.; DePinho, R. A. BCl2L12-mediated inhibition of effector caspase-3 and caspase-7 via distinct

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835

mechanisms in glioblastoma. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 10703−10708.
(127) Hofmann, J. N.; Beane Freeman, L. E.; Lynch, C. F.; Andreotti, G.; Thomas, K. W.; Sandler, D. P.; Savage, S. A.; Alavanja, M. C. The Biomarkers of Exposure and Effect in Agriculture (BEEA) Study: Rationale, design, methods, and participant characteristics. *J. Toxicol. Environ. Health, Part A* **2015**, *78*, 1338−1347.

https://doi.org/10.1021/acsomega.3c04592
ACS Omega 2023, 8, 29812−29835