PESTICIDES EXPOSURE AND THE MANAGEMENT OF ACUTE HEPATIC INJURY

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[Esposizione a pesticidi e la gestione del danno epatico acuto]

ABSTRACT

The use of pesticides on a large scale began in the '40s, when it was discovered that DDT (dichlorodiphenyltrichloroethane) was a very effective pesticide. The discovery marked the beginning of a massive boom in the production of chemical pesticides and the so-called "green revolution". But over time the risks have emerged and have become increasingly clear. Pesticides producers and farm workers are those who are most at risk, because they directly manipulate these substances. However, all individuals are exposed since pesticides are present in our food, drinking water and are used in gardens. Pesticides may cause cancer and damage the nervous, reproductive, and immune systems, and induce hepatotoxicity in both acute and chronic form.

Abnormal liver enzyme levels may signal liver damage or alteration in bile flow. Liver enzyme alteration may be either the accompanying biochemical picture in a patient with symptoms or the sign suggestive of hepatotoxicity.

Key words: Hepatotoxicity, pesticides, steatosis, transaminase.

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Introduction

Pesticides are ubiquitous contaminants of our environment and have been found in air, soil, water, and human and animal tissues samples from all over the world.

Studies regarding pesticides are considered important in order to decrease pesticide risks and help to improve public health policies[(1)].

Previous studies have indicated that the unsafe use of pesticides is common in developing countries. The worldwide consumption of pesticides is about two million tonnes per year, of which 24% is consumed in the USA alone, 45% in Europe and 25% in the rest of the world.

Pesticides cover a wide range of compounds used in pest control, including insecticides (arthropods), fungicides (fungi), herbicides (weeds), rodenticides (rats), molluscicides (snails), and others.

The heavy metal and pesticide contamination has attracted the attention of researchers all over the world and has increased in the last decades due to their extensive use in agricultural, chemical, and industrial processes[(2)].

Hepatotoxic pesticide exposure occurs both during everyday life and working life. The latter is definitely important, both for the multiple routes of exposure and absorption, both for the number and complexity of the exposure conditions, which add to the extraprofessional ones[(3)].

Pesticide use is very broad:

• in agriculture: as devices to control weeds, to improve production conditions and the protection of food in the following conservation stage,

• in relation to human health: to combat direct or indirect carriers of disease (molluscs, insects, arachnids, reptiles, rodents, etc.),

• in the field of preservation of different mate-

rials: such as varnishes, resins and preservative coatings of different sorts to prevent any impairment from biological pollutants.

The most exposed individuals are those who work in the production of active ingredients and formulations, and those involved in the transportation and storage of pesticides. In agriculture, the workers involved in the mixing and loading of pesticides, in the treatment of crops, or in the harvesting stage and those working in confined spaces, such as greenhouses.

The general population may also be exposed through the residues in food and drinks.

Another source of exposure is pollution in confined spaces, resulting from the dispersion generated from treated materials (wood, carpets and furnishings).

Pesticide uptake occurs mainly through the skin and eyes, by inhalation, or by ingestion. The fat-soluble pesticides and, to some extent, the water-soluble pesticides are absorbed through intact skin. Sores and abrasions may facilitate uptake through the skin. The vapors of pesticides or aerosol droplets smaller than 5 pm in diameter are absorbed extensively through the lungs.

Larger inhaled particles or droplets are likely to be swallowed after being cleared from the airways. Ingestion can also occur from the consumption of contaminated food or from using contaminated utensils. Contaminated hands may also lead to an intake of pesticides, such as smoking a cigarette.

The liver is an important target organ for the toxicity of drugs and xenobiotics due to its connection with the gastrointestinal tract as well as for the singularity and complexity of its anatomical structure and metabolic functions. It can be considered a target organ of numerous chemicals both in the workplace or at home. The liver damage can not be considered as a single entity, in fact the lesions observed depend not only on the type of substance involved in the process but also on the duration of exposure. After an acute exposure, the most frequently observed types of damage are steatotic, necrotic or hepatobiliary dysfunction. On the other hand, chronic exposure is usually followed by a cirrhotic or neoplastic damage[4, 5].

The liver function is essential in maintaining the well-being of the individual, therefore all the conditions that affect its operation have an important impact on the individuals' health. Consequently, preventing conditions which may be risk factors for liver functions is important.

When, however, a liver disease has developed, appropriate therapeutic strategies require an accurate diagnosis, in particular in the case of liver damage from toxic substances. Moreover, an effective therapeutic approach, in this case, must rule out other causes of liver disease[6, 7].

Diagnostic approach

In a recent review by Manno et al.[8] biomonitoring is defined as "the repeated, controlled measurement of chemical or biological markers in fluids, tissues or other accessible samples from subjects exposed or exposed in the past or to be exposed to chemical, physical or biological risk factors in the workplace and/or the general environment".

Pesticides can cause liver damage, which can manifest in different forms, from hepatic steatosis to inflammation, or a serious chronic damage. For hepatic steatosis it is meant the accumulation of lipids, generally constituted by triglycerides, within hepatocyte; this accumulation is not pathological if it is less than 5% of the liver weight[9].

Steatosis is generally caused by a series of alterations in lipid metabolism of toxic, metabolic, nutritional or hereditary origin. A distinction must be made to differentiate these secondary forms from the primitive (NAFLD proper) one.

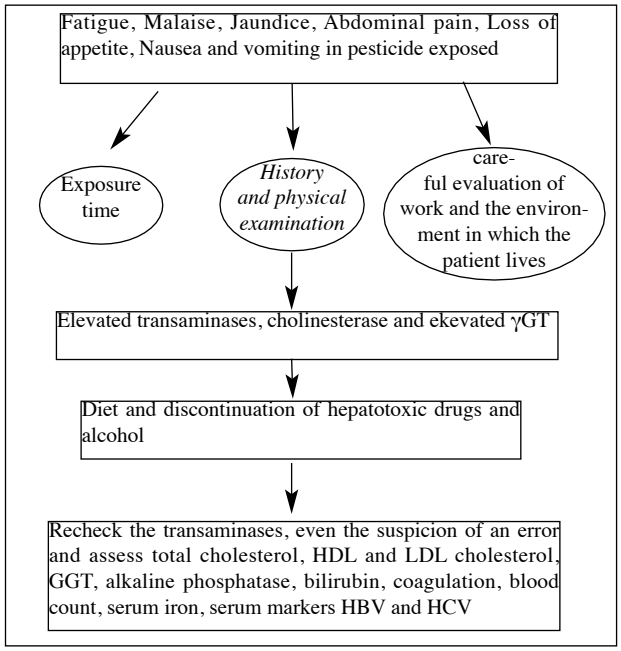
Hepatotoxicity may occur in many ways, such as changes in the levels of serum cholesterol, serum levels of liver enzymes, γGT, or bilirubin, which account for many indices of liver function[10]. The first diagnostic approach is in fact facilitated by the availability of sensitive and reliable tests, which are used to evaluate the function and the damage of the liver. For the initial assessment, it is required to measure serum alanine and aspartate aminotransferase, alkaline phosphatase, bilirubin, direct and indirect, of glutamyltranspeptidase (γGT).

The results of these tests indicate whether there is liver impairment, if the disease is acute or chronic, if the disease is a parenchymal and/or colestasic one, if cirrhosis or liver failure are already present.

Altered liver enzyme activities have been reported among pesticide workers exposed to organophosphorus pesticide alone or in combination with other pesticides[11].

Hepatotoxicity by pesticides can have two different diagnoses: a first level and a more specific one and they can be performed only in specialized laboratories.

The first level diagnosis is anamnestic and of exclusion. The markers of the most common liver diseases are then investigated, in addition to the levels of glucose, HDL-cholesterol, triglycerides and the general evaluation with complete blood count, creatinine, bilirubin, gamma-GT, alkaline phosphatase, total protein and protein electrophoresis, and ultrasound of the upper abdomen. The enzymatic alterations can be cholestatic (mainly elevation of alkaline phosphatase and gamma-GT), hepatocellular (predominantly transaminase elevation) or mixed. In the second level diagnosis acetylcholinesterase and bile acids can be assessed (Tab. 1).



Tab. 1: Management of acute pesticides poisoning.

➢ *Transaminase*

Patients with acute liver damage may not have a history of exposure to risk factors and may have nonspecific (fatigue, arthralgias, low-grade fever) or specific (jaundice) symptoms of liver disease; the diagnosis may be made by following routine analysis even with asymptomatic hypertransaminasemia.

Injury to the liver, whether acute or chronic, eventually results in an increase in serum concentrations of aminotransferases. AST and ALT are enzymes that catalyze the transfer of the α-amino groups from aspartate and alanine to the α-keto group of ketoglutaric acid to generate oxalacetic and pyruvic acids respectively, which are important contributors to the citric acid cycle. Both enzymes require pyridoxal-5'-phosphate (vitamin B6) in order to carry out this reaction, although the effect of pyridoxal-5'-phosphate deficiency is greater on ALT activity than on that of AST[12, 13].

Both aminotransferases are highly concentrated in the liver. AST is also diffusely present in the heart, skeletal muscle, kidneys, brain and red blood cells, while ALT has low concentrations in skeletal muscle and kidneys[14]; an increase in ALT serum levels is, therefore, more specific for liver damage. In the liver, ALT is localized solely in the cellular cytoplasm, whereas AST is both cytosolic (20% of total activity) and mitochondrial (80% of total activity)[15]. Pesticides may damage liver cells and liver transaminases may be used to monitor liver damage after combined or mixed exposure. In a toxicological study of a toxic mixture, alanine aminotransferase (ALT) activity in rat blood plasma was measured and combined with a histopathological assessment. In this case data suggested that the two substances had an antagonistic effect[16].

In a study of workers exposed to a mixture of toxics, different biochemical parameters of liver function were measured, such as alkaline phosphatase (ALP), total bilirubin (TB) and aspartate aminotransferase (AST). These markers showed significantly higher levels in the workers compared to a control group. Furthermore, the authors found that serum bile acids were the most sensitive markers for detecting liver injury, suggesting that serum bile acids could be a valuable biomarker of hepatotoxicity caused by toxics[17].

The cumulative (over time) exposure of toxics and liver biomarkers was evaluated in 29 exposed workers. The study reports that higher liver enzymes activities of AST and ALT in blood related to exposure during the past 5 years, while higher levels of triglycerides reflected the total lifetime cumulative toxics exposure.

The study of Özgu¨r Fırat[18] showed changes in the activities of serum enzymes, metabolites and ions between fish exposed to pollution by pesticides and those exposed to heavy metals. These changes were considered to be the manifestation of the biochemical actions of these toxics. In fact, the activities of serum ALT, AST, ALP, and LDH have been commonly used in the diagnosis of fish diseases as well as in the detection of tissue damage caused by environmental pollution. An increase of these enzyme activities in the extracellular fluid or serum is a sensitive indicator of even minor cellular damage[19] and indicates stress-based tissue impairment.

Generally, the results of ALT, AST, ALP, and LDH may indicate degeneration changes and hypo-

function of liver as the toxicants effects on the hepatocytes are in the form of tissue damage in which cellular enzymes are released from the cells into the blood serum.

In this study, very high transaminase levels in fish exposed to pesticides have been identified. The levels were higher than those of fish exposed to heavy metals. It is assumed that this condition is linked to the fact that the liver may be a greater target for the toxic action of pesticides.

The magnitude of aminotransferase alteration can be classified as "mild" (< 5 times the upper reference limit), "moderate" (5–10 times the upper reference limit) or "marked" (> 10 times the upper reference limit). This classification is somewhat arbitrary, since no uniform definition exists and various reviews of the subject use different cut-off points. Very high aminotransferase levels (> 75 times the upper reference limit) indicate ischemic or toxic liver injury in more than 90% of cases in acute hepatic injury.

➢ *γGT and alkaline phosphatase (ALP)*

In the cholestatic pattern, ALP and bilirubin levels are routinely assessed, and the level of γGT is often measured as an additional aid toward diagnosis in specific situations because of its high sensitivity but low specificity. γGT and alkaline phosphatase are liver enzymes capable of signaling liver damage or alteration of bile flow. Alterations in liver enzymes may be the corresponding biochemical condition to accompanying symptoms or signs (such as jaundice), suggestive of liver disease.

ALP is an enzyme that transports metabolites across cell membranes. Liver and bone diseases are the most common causes of pathological elevation of ALP levels, although ALP may originate from other tissue, such as the placenta, kidneys or intestines, or from leukocytes[20].

The degree and rate of enzyme alteration may provide minor and nonspecific clues to diagnosis, but the presence of symptoms and the patient's history, with particular emphasis on comorbid conditions, may provide fundamental clues. In various cases, these laboratory data must be combined with a careful history. Great attention must be paid to discretionary factors and work of the patient. The history in a subject exposed to pesticides becomes crucial and must be associated with an objective laboratory work-up. Subsequently, liver ultrasound may reveal the presence of bile duct dilation, demonstrate signs of chronic liver disease or even liver cirrhosis, and identify hepatic masses[21].

ALP alterations due to common bile duct obstruction may be heralded by a peak in aminotransferase levels, typical symptoms and conjugated hyperbilirubinemia, especially in the acute setting, or else the ALP levels may have a fluctuating pattern (± GGT alteration) with normal serum bilirubin in "valve" choledocholithiasis[22].

In these patients, liver ultrasound may show dilated bile ducts, and endoscopic retrograde cholangiopancreatography can be used to remove the obstacle. One example of biomonitoring the effects of relevance to the nervous system is the measurement of acetylcholinesterase activity in red blood cells following complex exposure to organophosphorus insecticides.

GGT is an enzyme that is present in hepatocytes and biliary epithelial cells, renal tubules, as well as the pancreas and intestine. GGT is a microsomal enzyme, and its activity can be induced by several drugs, such as anticonvulsants, oral contraceptives and toxics[23].

In patients exposed to pesticides, GGT serum levels can be markedly altered (> 10 times the upper reference value).

➢ *Bilirubin*

Bilirubin is the product of hemoglobin catabolism within the reticuloendothelial system. Heme breakdown determines the formation of unconjugated bilirubin, which is then transported to the liver. In the liver, UDP-glucuronyltransferase conjugates the water-insoluble unconjugated bilirubin to glucuronic acid, and conjugated bilirubin is then excreted into the bile[24].

Unconjugated bilirubin may increase because of augmented bilirubin production or decreased hepatic uptake or conjugation or both.

Biliary obstruction can cause various degrees of conjugated hyperbilirubinemia. The severity of alteration depends upon the degree and duraction of obstruction and the functional reserve of the liver.

Biliary obstruction may have an abrupt onset and be preceded by typical symptoms (right upper quadrant pain, nausea) or may be silent and progressive. With the presence or absence of concomitant aminotransferase alteration, a liver ultrasound is essential to identify and locate the obstacle to bile flow[25, 26].

➢ *Cholinesterase*

Organophosphates exert toxicity via acetylcholinesterases inhibition that under acute exposure leads to Ach accumulation at nerve synapses.

Cholinesterase is one of many important

enzymes needed for the proper functioning of the nervous systems of humans, other vertebrates, and insects. Certain chemical classes of pesticides, such as organophosphates (OPs) and carbamates (CMs) work against undesirable bugs by interfering with, or 'inhibiting' cholinesterase. While the effects of cholinesterase inhibiting products are intended for insect pests, these chemicals can also be poisonous, or toxic, to humans in some situations.

Cholinesterases (ChE) are specialized carboxylic ester hydrolases that catalyze the hydrolysis of choline esters. Two types of ChE activity have been identified in mammalian blood and tissues. These are distinguished according to their substrate specificity and sensitivity to the selective inhibitors. The first is acetylcholinesterase,

which is systematically called acetylcholine acetylhydrolase. Other names include true cholinesterase, specific cholinesterase, red blood cell cholinesterase, erythrocyte cholinesterase and cholinesterase I.

The second is butyrylcholinesterase, which is referred to systematically as acylcholine acylhydrolase. Other names include cholinesterase, pseudocholinesterase, non-specific cholinesterase, plasma cholinesterase, serum cholinesterase, propionylcholinesterase, benzoylcholinesterase and cholinesterase II[27, 28].

Both AChE and BChE serve a pivotal role in regulating the transmission of nerve impulses by rapid hydrolysis of the neurotransmitter ACh. It appears that AChE

is the predominate enzyme performing this function, since AChE catalyzes the hydrolysis of ACh much more rapidly than BChE does. The widespread use of organophosphorus and carbamate pesticides and the dangers associated with their application have resulted in cholinesterase (ChE) activities being used as biomarkers for evaluating both exposure to and the effect of these pesticides[29].

The most characteristic feature of acute exposure to pesticides is the reduction of cholinesterase. There is also a reduction of pseudocholinesterase. But it should be noted that the test is not sensitive because there may be other underlying conditions such as liver disease, malnutrition, fasting, and drugs such as codeine.

The reduction of up to 50% can be considered the expression of a latent exposure, but a further drop is almost always concomitant with the presence of clinical signs.

If the reduction is between 50 and 20% the patient will have a mild intoxication, between 10 and 20% intoxication of intermediate severity, and less than 10% severe intoxication[30].

The types and severity of cholinesterase inhibition symptoms depend on:

a. the toxicity of pesticide,

b. the amount of pesticide involved in the exposure,

c. the route of exposure,

d. the duration of exposure.

A cholinesterase depression may involve destructive sampling (brain acetylcholinesterase) or nondestructive sampling (serum butyrylcholinesterase). With a specific antibody it is possible to measure the concentration of a particular esterase in plasma or serum[31]. Measurement of AchE in red blood cells is used to assess cholinergic effects whereas inhibition of neuropathy target esterase in lymphocytes might be used to assess the delayed neurotoxic effects of some organophosphate insecticides[32, 33, 34].

As noted by Wilson et al.[35], determining ChE activities may form the basis for establishing safe levels of such pesticides in food and the environment. Further studies are necessary, both under experimental and field conditions, in order to improve and strengthen these results and to increase our knowledge about this very interesting enzyme as a potential biochemical marker for intoxication by pesticide compounds.

➢ *Paraoxonases (PONS)*

PONs are responsible for the metabolism of organophosphates-based insecticides[36]. Serum paraoxonases activity plays a major role in the metabolism of organophosphates.

Individuals with low PON1 activity are more susceptible to parathion poisoning than subjects with higher PON1 activity. Isoforms of serum paraoxanes exhibit a substrate-dependent polymorphism characterized by a different efficiency in metabolising different chemical compounds belonging to the organophosphates class.

➢ *Bile acids*

The pesticides can induce hepatotoxicity. Understanding mechanisms of hepatotoxicity, and in vitro models to predict hepatotoxicity in humans, are essential for pesticides development and patient safety. One hypothesized mechanism for pesticides hepatotoxicity is impairment of bile acid (BA) transport, causing cholestasis and subsequent hepatocellular apoptosis or necrosis[37].

While drug-induced hepatotoxicity can resemble all forms of liver injury, most often it shows characteristics of hepatocellular, cholestatic, or mixed injury, depending on serum biochemical markers[38, 39]. Hepatocellular injury is marked by an initial elevation of alanine aminotransferase (ALT) levels, cholestatic injury is characterized by an increase in serum alkaline phosphatase (ALP) levels, and mixed injury displays features of both types of injury[40].

Bile acids (BAs) are the major organic solutes in bile, and are involved in several important functions in the liver and the intestine. Primary BAs are directly synthesized from cholesterol by hepatocytes, by the addition of hydroxyl groups and the oxidation of its side chain to form a more water soluble end product. The hydroxylation is always on one side of the molecule resulting in an amphipathic molecule. In humans, the most abundant BA species are the primary BAs cholic acid (CA) and chenodeoxycholic acid (CDCA), and the secondary BAs deoxycholic acid (DCA) and lithocholic acid (LCA), generated in the intestine by bacterial biotransformation of CA and CDCA, respectively.

Small amounts of other secondary BAs such as ursodeoxycholic acid, currently used in the treatment of cholestatic liver diseases, are also present in the human BA pool[41, 42]. Cholestasis is defined as the impairment of bile flow, and can be caused by intrahepatic or extrahepatic mechanisms. Intrahepatic cholestasis occurs at the cellular level and is caused by the perturbation of bile formation, while extrahepatic cholestasis occurs at the bile duct level as a result of mechanical obstruction or impairment of bile secretion and flow[43]. Generally, pesticides can cause a type of noninflammatory cholestasis through the inhibition of BA transporters or the retrieval of transporters from the plasma membrane, but also "cholestatic hepatitis" or inflammatory cholestasis. It occurs as a result of repression of transporter expression or function due to pro-inflammatory cytokine release in response to toxicity, sepsis, viral hepatitis, alcohol, and drugs[43].

Retention of bile constituents within the hepatocyte during cholestasis is associated with hepatocyte apoptosis. Although the mechanisms of cholestasis associated with hepatocyte apoptosis are likely to be complex and multifactorial, hydrophobic bile acids are especially hepatotoxic, and they accumulate in the liver in cholestatic disorders[44]. But the mechanisms involved in this toxicity are not fully understood.

Several mechanisms may account for the cytotoxicity associated with the most hydrophobic BAs in cholestatic liver diseases[41].

BAs could disrupt cell membranes through their detergent action on lipid components[45] and promote the generation of reactive oxygen species (ROS) that, in turn, oxidatively modify lipids, proteins, and nucleic acids, and eventually cause hepatocyte apoptosis[46]. Additionally, they can activate Kupffer cells to generate ROS, which may further contribute to the liver cell insult[47].

Various environmental toxins have been shown to perturb liver physiology. However, the influences of pollutants on the molecular networkers, which govern the physiological function of hepatocytes, remain poorly resolved.

The response of cellular systems to stimuli is complex with numerous functionally diverse networks interacting to manifest an appropriate response. Investigating individual pathways has successfully uncovered the various components involved in the adjustment of cell behaviour to different conditions.

Conclusions

In agriculture and in everyday life, to produce more and to get more profits, toxic chemicals, such as pesticides, herbicides, insecticides and chemical fertilizers are used. But they are generating harmful consequences to animals, the environment and human health. Approximately 40% of the pesticides produced worldwide is used in North America, 25% in Western Europe and the rest in other continents.

In Italy about 2-3% of world production is used. In recent years, the consumption of pesticides in Italy, expressed as the quantity used, has increased.

Damage to health and the environment arising from excessive and growing agriculture is increasingly eviden[t(48)]. They are a consequence of the accumulation of toxic and carcinogenic residues in the tissues of humans and animals, from both poisoning of soil, groundwater and surface water, gardens and food (especially from greenhouses). While it is true that you can not underestimate the economic benefits on productivity and "marketability" of the product, many studies show the harmful effects that pesticides and fertilizers have caused on the environment and on the health of man. The liver plays a central role in the metabolism of substances

and it can be considered a target organ of numerous chemicals used in the workplace. After an acute exposure, the most frequently observed types of damage are steatotic, necrotic or hepatobiliary dysfunction, while the chronic exposure is usually followed by cirrhotic or neoplastic types of damage.

Many pesticides behave experimentally by inducers of the enzymatic complex of cytochrome P-450 enzymes which, as it is known, play a central role in the metabolism of drugs, steroid hormones, fatty acids, endogenous and exogenous toxins as well as of carcinogenic substances[49].

Many insecticides seem to be able to cause enzyme induction with the modification of the hepatic metabolism of drugs and hormones, as well as significant changes in the liver.

Therefore, it is important to know the most appropriate diagnostic management for a patient with a suspected hepatotoxicity by pesticides. This can facilitate and speed up the therapeutic clinical path needed, also in view of the great interest aroused by these substances, suspected to be involved in the pathogenesis of neurodegenerative diseases (Parkinson's disease and progressive supranuclear palsy)[50-51].

References

1) Ecobichon DJ. *Pesticide use in developing countries*. Toxicology. 2001 Mar 7; 160(1-3): *27-33*, Review.
2) Salomone S, Drago F. *Effects of PPARγ Ligands on Vascular Tone*. Curr Mol Pharmacol. 2012 Jun 1; 5(2): *282-91*.
3) Wesseling C, Hogstedt C, Picado A, Johansson L. *Unintentional fatal paraquat poisonings among agricultural workers in Costa Rica: report of 15 cases*. Am J Ind Med. 1997 Nov; 32(5): *433-41*.
4) Arafa UA, Fujiwara Y, Higuchi K, Shiba M, Uchida T, Watanabe T, Tominaga K, Oshitani N, Matsumoto T, Arakawa T. *No additive effect between Helicobacter pylori infection and portal hypertensive gastropathy on inducible nitric oxide synthase expression in gastric mucosa of cirrhotic patients*. Dig Dis Sci. 2003 Jan; 48(1): *162-8*.
5) Piñeiro-Carrero VM, Piñeiro EO. *Liver*. Pediatrics. 2004 Apr; 113(4 Suppl): *1097-106*. Review.
6) Tomei F, Baccolo TP, Tomao E, Palmi S, Rosati MV. *Chronic venous disorders and occupation*. Am J Ind Med. 1999 Dec; 36(6): *653-65*.
7) Straubinger RM, Balasubramanian SV. *Preparation and characterization of taxane-containing liposomes*. Methods Enzymol. 2005; 391: *97-117*.
8) Manno M., Viau C., Cocker, J., Colosio C., Lowry L., Mutti A., Nordberg M., Wang S. *Biomonitoring for occupational health risk assessment (BOHRA)*. Toxicol. Lett. 2010, 192, *3-16*.
9) Burkitt HG, Young B, Heath JW. W*heater's functional histology: a text and colour atlas*. 3rd ed. Edinburgh: Churchill Livingston, 1993.
10) Malaguarnera G, Cataudella E, Giordano M, Nunnari G, Chisari G, Malaguarnera M. *Toxic hepatitis in occupational exposure to solvents*. World J Gastroenterol. 2012 Jun 14; 18(22): *2756-66*.
11) al-Saleh IA. *Pesticides: a review article*. J Environ Pathol Toxicol Oncol. 1994; 13(3): *151-61*. Review.
12) Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. *Diagnosis and monitoring of hepatic injury. II. Recommendations for use of laboratory tests in screening, diagnosis, and monitoring*. Clin Chem. 2000 Dec; 46(12): *2050-68*. Review.
13) Vanderlinde RE. *Review of pyridoxal phosphate and the transaminases in liver disease*. Ann Clin Lab Sci. 1986 Mar-Apr; 16(2): *79-93*.
14) Wroblewski F. T*he clinical significance of alterations in transaminase activities of serum and other body fluids*. Adv Clin Chem. 1958; Vol. 1(2): *313-51*.
15. Rej R. *Aminotransferases in disease*. Clin Lab Med. 1989 Dec; 9(4): *667-87*.
16) Anand SS, Bruckner JV, Haines WT, Muralidhara S, Fisher JW, Padilla S. *Characterization of deltamethrin metabolism by rat plasma and liver microsomes*. Toxicol Appl Pharmacol. 2006 Apr 15; 212(2): *156-66*. Epub 2005 Sep 16.
17) Nunes de Paiva MJ, Pereira Bastos de Siqueira ME *Increased serum bile acids as a possible biomarker of hepatotoxicity in Brazilian workers exposed to solvents in car repainting shops*. Biomarkers. 2005 Nov-Dec; 10(6): *456-63*.
18) Fırat O, Cogun HY, Yüzereroğlu TA, Gök G, Fırat O, Kargin F, Kötemen Y. *A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, Oreochromis niloticus*. Fish Physiol Biochem. 2011 Sep; 37(3): *657-66*. Epub 2011 Jan 13.
19) Palanivelu C, Jani K, Malladi V, Senthilkumar R, Rajan PS, Sendhilkumar K, Parthasarthi R, Kavalakat A. *Laparoscopic management of hepatic hydatid disease*. JSLS. 2006 Jan-Mar; 10(1): *56-62*. Review.
20) Fishman WH. *Alkaline phosphatase isozymes: recent progress*. Clin Biochem. 1990 Apr; 23(2): *99-104*. Review.
21) Uccello M, Malaguarnera G, Pelligra EM, Biondi A, Basile F, Motta M. *Lipoprotein(a) as a potential marker of residual liver function in hepatocellular carcinoma*. Indian J Med Paediatr Oncol. 2011 Apr; 32(2): *71-5*.
22) Hosono-Fukao T, Hosono T, Seki T, Ariga T. *Diallyl trisulfide protects rats from carbon tetrachloride-induced liver injury*. J Nutr. 2009 Dec; 139(12): *2252-6*. Epub 2009 Oct 7.
23) Rosalki SB, Foo AY, Dooley JS. *Benign familial hyperphosphatasaemia as a cause of unexplained increase in plasma alkaline phosphatase activity*. J Clin Pathol. 1993 Aug; 46(8): *738-41*. Review.
24) Berk PD, Zhou SL, Stump D, Kiang CL, Isola LM. *Recent studies of the cellular uptake of long chain free fatty acids*. Trans Am Clin Climatol Assoc. 1994; 105: *179-89*. Review.
25) Fevery J, Blanckaert N, Heirwegh KP, Préaux AM, Berthelot P. *Unconjugated bilirubin and an increased proportion of bilirubin monoconjugates in the bile of patients with Gilbert's syndrome and Crigler-Najjar disease*. J Clin Invest. 1977 Nov; 60(5): *970-9*.
26) Fevery J, Heirwegh KP, De Groote J. *Unconjugated hyperbilirubinaemia in achalasia*. Gut. 1974 Feb;

15(2): *121-4*.
27) Wilson BW, Arrieta DE, Henderson JD. Monitoring cholinesterases to detect pesticide exposure. Chem Biol Interact. 2005 Dec 15;157-158:253-6. Epub 2005 Nov 18.
28) A Chatonnet and O Lockridge. *Comparison of butyrylcholinesterase and acetylcholinesterase*. Biochem. J. (1989) 260 (*625–0*).
29) Wilson BW, Henderson JD, Furman JL, Zeller BE, Michaelsen D. *Blood cholinesterases from Washington State orchard workers*. Bull Environ Contam Toxicol. 2009 Jul; 83(1): 59-61. Epub 2009 Apr 23. Erratum in: Bull Environ Contam Toxicol. 2009 Sep; 83(3): *462*. Michaelson, David [corrected to Michaelsen, David].
30) Wilson BW, Walker CR. *Regulation of newly synthesized acetylcholinesterase in muscle cultures treated with diisopropylfluorophosphate*. Proc Natl Acad Sci U S A. 1974 Aug; 71(8): *3194-8*.
31) Walker CH. *Biochemical responses as indicators of toxic effects of chemicals in ecosystems*. Toxicol Lett. 1992 Dec; *64-65,* Spec No: *527-33*. Review.
32) Hayes AL, Wise RA, Weir FW. *Assessment of occupational exposure to organophosphates in pest control operators*. Am Ind Hyg Assoc J. 1980 Aug; 41(8): *568-75*.
33) Ames RG, Brown SK, Rosenberg J, Jackson RJ, Stratton JW, Quenon SG. *Health symptoms and occupational exposure to flea control products among California pet handlers*. Am Ind Hyg Assoc J. 1989 Sep; 50(9): *466-72*.
34) Ciesielski S, Esposito D, Protiva J, Piehl M. *The incidence of tuberculosis among North Carolina migrant farmworkers*, 1991. Am J Public Health. 1994 Nov; 84(11): *1836-8*.
35) Wilson BW 2001. *Acetylcholinesterases* in Handbook of Pesticide Toxicology (2nd edition) ed R.E. Krieger. New York, Academic Press pp *967-985*.
36) Costa LG, Li WF, Richter RJ, Shih DM, Lusis A, Furlong CE. *The role of paraoxonase (PON1) in the detoxication of organophosphates and its human polymorphism*. Chem Biol Interact. 1999 May 14;119-120: *429-38*. Review.
37) Polson J, Lee WM. *Etiologies of acute liver failure: location, location, location!* Liver Transpl. 2007 Oct; 13(10): *1362-3*.
38) Abboud G, Kaplowitz N. *Drug-induced liver injury*. Drug Saf. 2007; 30(4): *277-94*.
39) Benichou G, Takizawa PA, Ho PT, Killion CC, Olson CA, McMillan M, Sercarz EE. *Immunogenicity and tolerogenicity of self-major histocompatibility complex peptides*. J Exp Med. 1990 Nov 1; 172(5): *1341-6*.
40) Navarro VJ, Senior JR. *Drug-related hepatotoxicity*. N Engl J Med. 2006 Feb 16; 354(7): *731-9*. Review.
41) Attili AF, Angelico M, Cantafora A, Alvaro D, Capocaccia L. *Bile acid-induced liver toxicity: relation to the hydrophobic-hydrophilic balance of bile acids*. Med Hypotheses. 1986 Jan; 19(1): *57-69*.
42) Paumgartner G, Beuers U. Ursodeoxycholic acid in cholestatic liver disease: mechanisms of action and therapeutic use revisited. Hepatology. 2002 Sep;36(3):525-31. Review
43) Zollner G, Trauner M. *Mechanisms of cholestasis*. Clin Liver Dis. 2008 Feb; 12(1): *1-26,* vii. Review.
44) Rodrigues DC, Silva SS, Prata AM, Felipe MG. *Biotechnological production of xylitol from agroindustrial residues. Evaluation of bioprocesses*. Appl Biochem Biotechnol. 1998 Spring; 70-72: *869-75*.
45) Billington D, Evans CE, Godfrey PP, Coleman R. *Effects of bile salts on the plasma membranes of isolated rat hepatocytes*. Biochem J. 1980 May 15; 188(2): *321-7*.
46) Sokol RJ, Mack C. *Etiopathogenesis of biliary atresia*. Semin Liver Dis. 2001 Nov; 21(4): *517-24*.
47) Ljubuncic P, Fuhrman B, Oiknine J, Aviram M, Bomzon A. *Effect of deoxycholic acid and ursodeoxycholic acid on lipid peroxidation in cultured macrophages*. Gut. 1996 Sep; 39(3): *475-8*.
48) Vecchio M, Malaguarnera G, Giordano M, Malaguarnera M, Li Volti G, Galvano F, Drago F, Basile F, Malaguarnera M. A musician's dystonia. Lancet. 2012 Jun 2;379(9831):2116.
49) Caraci F, Leggio GM, Drago F, Salomone S. *Epigenetic drugs for Alzheimer's Disease: hopes and challenges*. Br J Clin Pharmacol. 2012 Aug 21.
50) Rampello L, Buttà V, Raffaele R, Vecchio I, Battaglia G, Cormaci G, Alvano A. *Progressive supranuclear palsy: a systematic review*. Neurobiol Dis. 2005 Nov; 20(2): *179-86*. Review.
51) Rampello L., Ruggieri M., Vecchio I., Battaglia G., Chisari C.G.,Malaguarnera M., Zelante G., Catalano A., Rampello L. *Sclerosi laterale amiotrofica: diagnosi differenziale con la mieloradicolopatia cervicale*. Acta medica mediterranea, 2011, 27: *149-52*.

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