

## RESULTS OF A STUDY ON THE CHEMICAL COMPOSITION OF WOOD DUST AND THE ETIOLOGY OF BRONCHIAL ASTHMA IN WOODWORKERS

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The possible biological actions of wood dust are of an irritant, allergic, toxic, and carcinogenic nature. Besides the cutaneous disturbances, the most common pathologies found in workers at risk are respiratory and oculo-rhinitic; specifically, in the respiratory system wood dust can cause bronchial asthma, either of an allergic or pharmacirritant nature. Therefore, studies have developed concerning: 1) the chemical composition of the type of wood, 2) *in vivo* and *in vitro* experiments regarding the toxicity of wood dust or its extracts and derivatives, 3) clinical and epidemiological observations of subjects at risk, and 4) environmental investigations in lumber workshops and yards.

This present work is related to the first group of research investigations in that it studies the chemical composition of the different types of wood through the use of TLC (thin layer chromatography); in relationship to the third group of studies it is related to clinical observations carried out in our occupational allergology out-patient clinic.

### METHODS USED IN THE STUDY OF THE WOOD CHEMICAL COMPOSITION

Woods examined were chosen on the basis of information obtained from wood workers who came under our observation for respiratory disturbances, choosing those types of wood that were most often used or most often thought by the patients themselves to be responsible for their problems.

Before carrying out TLC, samples of the different types of wood were pulverized manually by rasping. Approximately 2 grams of finely ground and well dried wood dust were added to a solution of 95% ethanol and allowed to stand for 24 hours, to obtain an extract; an aqueous 1% HCl solution was added for the determination of alkaloids. We carried out chromatographic studies on these wood extracts, adapting a method recently proposed by the W.H.O. for phytopharmacological research,<sup>6,7</sup> which employs TLC. We used, for the TLC, plates of silica gel (Merk HF 254). For the elution of compounds containing different charges, a system of solvents analogous to that found in the literature<sup>9</sup> was used, according to the degree of polarity. After elution and subsequent drying in an oven, the plates were sprayed with several reagents in order to determine the main classes of compounds: Polyphenols, Terpenoids, Cardenolids, Alkaloids, Anthranoids and Coumarins. The eluents and the reagents used are listed in Table I. Furthermore, in lieu of specific reagents, the plates were sprayed with 2N H<sub>2</sub>SO<sub>4</sub> to detect eventual organic compounds different from those

studied above and which we shall call "non identified compounds"; at the moment, these shall not be taken into consideration.

It is noteworthy that TLC represents one of the most sensitive analytical techniques, capable of detecting quantities of substances on the order of a few micrograms (whereas the detection of compounds present in still smaller quantities requires a concentration of the extract). It reveals information not only regarding the categories of the substances extracted (polyphenols, alkaloids, etc.), but also their numbers (number of spots). At first, TLC can orient us as to the type of substance under examination, on the basis of its differential motion due to a difference of polarity (for example, if a terpene compound can be eluted with a low polar solvent, then it will be identified as a steroid terpene, while a saponine will be eluted with more polar solvents). Therefore, TLC analysis can be considered a preliminary step for deeper subsequent examinations.

The classes of compounds that we considered make up the major part of the secondary metabolites of woody plants that can be considered harmful for the organism: in fact, Polyphenols have irritative and sensitizing properties; Cardenolids have cardioactive effects; Alkaloids have systemic toxic effects and can bring about the liberation of histamine; Terpenoids have irritative and sensitizing effects and, in particular, saponins have hemolytic properties; some Coumarins have photosensitizing effects.<sup>4</sup>

### RESULTS OF THE CHROMATOGRAPHIC ANALYSIS OF THE DIFFERENT TYPES OF WOOD AND COMMENTS

The results of the chromatographic examinations are contained in Table II and III. The woods tested are indicated with their commercial names followed by their scientific names and, in parenthesis, their family names. The results are expressed as the number of significant spots revealed in the nonconcentrated extracts; these spots are marked with an "x".

We grouped the woods into exotic and domestic and further distinguished the softwoods (conifers) from the hardwoods (latifolia). In general, exotic woods contain a moderate amount of Polyphenols and Cardenolids as well as Terpenoids, while the Alkaloids are present only in Asian Rosewood, Mansonian Walnut and Teak, and the Anthranoids only in Mansonian Walnut. Among the domestic woods there was a widespread presence of Polyphenols and

Table I

Eluents and Reagents Used for the Study of the Chemical Composition of Wood Dust Through the Use of TLC

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<b>ELUENTS (v:v) :</b>	
<b>E1</b>	<b>Toluene : Ethyl Acetate (1:1)</b>
<b>E2</b>	<b>Chloroform : Ethyl Acetate (1:1)</b>
<b>E3</b>	<b>Chloroform : Methanol (9:1)</b>
<b>E4</b>	<b>Chloroform : Acetic Acid : Water (50:45:5)</b>
<b>E5</b>	<b>1-Butanol : Acetic Acid : Water (4:1:5)</b>
<b>E6</b>	<b>Methyl Ethyl Ketone : Toluene : Methanol : Acetic Acid : Water (80:10:5:2:6)</b>
<b>E7</b>	<b>Toluene : Ethyl Acetate : Diethylamine (5:4:1)</b>
<b>REAGENTS :</b>	
<b>R1</b>	<b>Potassium Ferricyanide : Ferric Chloride (for Polyphenols: phenols, phenolic acids, flavonoids, tannins, catechins, coumarins, quinones, and stilbenes)</b>
<b>R2</b>	<b>Acetic Anhydride : Sulfuric Acid (for Terpenoids: terpenes, steroids, sterols, and saponins)</b>
<b>R3</b>	<b>Phosphomolybdic Acid (for reducing compounds: mainly appropriate for terpenes if associated with R2)</b>
<b>R4</b>	<b>3,5-Dinitrobenzoic Acid : Potassium Hydroxide (for Cardenolids)</b>
<b>R5</b>	<b>Basic Bismuth Nitrate : Acetic Acid : Potassium Iodide (for Alkaloids)</b>
<b>R6</b>	<b>Sulfuric Anisaldehyde (for Coumarins and Anthranoids, especially if associated with R1)</b>

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Terpenoids and a constant lack of Cardenolids, Alkaloids, and Anthranoids, that is, those classes of compounds having potential systemic pharmacological activity. In general, the larger amount of active substances contained in exotic woods with respect to domestic woods seems to account for the fact, at the present, that the former have a greater toxicity. Nevertheless, on the one hand, the presence of large amounts of accessory components in some domestic woods, such as Cherry, and, on the other hand, the scarcity of these components in some exotic woods, such as Obeche, make it unwise to generalize.

Instead, differences are less marked when comparing the presence of Polyphenols or Terpenoids; these were found in all examined except Mahogany, Obeche and Elm. It may be surprising that some softwoods (conifers), both domestic and foreign, examined by us contain no Terpenoids which are the most characteristic substances found in the Pine family; however, it is necessary to bear in mind that the analysis was carried out on well seasoned wood dust and, therefore,

free of resins, and that some volatile terpene fractions can be lost during the initial phases of the techniques involved in chromatography. Furthermore, more work must be done on the "nonidentified substances" found in 3 of the 5 softwoods examined.

In the hardwoods (latifolia), in addition to the Terpenoids contained in Beech, Walnut, and Linden, large amounts of Polyphenols are present especially in Cherry, Walnut, and Olive. Because of the characteristics of such compounds chemical analysis allows us to confirm that these woods have a greater capacity for causing irritative and allergic reactions. Moreover, Colophony, contained in conifers, is a Terpene, and Plicatic Acid, found in Red Cedar, is a Polyphenol.<sup>1,2</sup> Such an observation, together with the results of clinical data, allows us to conclude that even domestic woods, rich in such substances, are to be considered as a potential cause of asthma, no less than exotic woods; in Italy, insurance protection exists only for the latter.

A first consideration that can arise from our study is a

Table II  
Results of the Chromatographic Analysis of the Different Types of Wood (Exotic Woods)

Woods (commercial and scientific names)	Polyphenols	Terpenoids	Cardenolids	Alkaloids	Anthranoids & Coumarins	Non identified compounds
<b>SOFTWOODS</b>						
Douglas Fir						
<i>Pseudotsuga douglasii</i> Carr.	xxx	x	-	-	-	xxx
Pitch Pine						
<i>Pinus</i> spp	xx	-	-	-	-	x
<b>HARDWOODS</b>						
Afromosia						
<i>Afromosia elata</i> Harms.	-	xx	-	-	-	-
Asian Rosewood						
<i>Dalbergia latifolia</i> Roxb.	xxx	-	x	xx	-	-
Iroko						
<i>Chlorophora excelsa</i> Benth.	xxx	-	xx	-	-	-
Mahogany						
<i>Shorea</i> spp	-	-	-	-	-	-
Mahogany						
<i>Entandrophragma utile</i> Sprag.	-	-	x	-	-	-
Mansonian Walnut						
<i>Mansonia altissima</i> A. Chév.	xxxxx	x	xx	xx	x	-
Obeche						
<i>Triplochiton scleroxylon</i> K.	-	-	-	-	-	-
Padouk, Kejatt						
<i>Pterocarpus</i> spp	xx	x	-	-	-	xx
Ramin						
<i>Gonystylus bancanus</i> Kurz	-	xxx	-	-	-	-
Tanganyika Aniegré						
<i>Aningeria altissima</i> Aubr. P.	-	xx	-	-	-	-
Teak						
<i>Tectona grandis</i> L.f.	xxx	xx	-	x	-	-

deeper examination aimed at both woods with greater reactivity (*Mansonia*, Rosewood, Teak, Cherry, Larch, etc.), as well as woods that resulted in practically no reactivity (Such as Obeche), but which were reported in the literature as being responsible for pathological effects.<sup>5</sup> Another consideration that seems to derive from our study is the use of TLC methods in preliminary hygienic and sanitary investigations of work environments where lumber is employed for which adequate bibliographical references are lacking. A third line of development involves the chromatographic separation of the compounds through the use of TLC and the subsequent employment of the various fractions for pharmacological tests on animals, or for allergometric tests or bronchial challenge tests on workers at risk and symptomatic. For example, it is possible, using patch tests, to apply the single spots cut out from the chromatographic plates, and subsequently carry out skin tests or tests involving bronchial exposure with the same preliminary chromatographic fractions, dried and redissolved in controlled solutions. Furthermore, the analysis of different samples of the same wood did not always give rise to identical results (such as the Douglas Fir), and this can be explained, at least in part, by the different origin of

the trees: and this is another reason for using, in the etiological research of the disturbances attributed to wood dust, the components extracted directly from the material supposed to be responsible.

#### CLINICAL CASES

We now report case history data regarding 86 wood workers observed during the past 5 years at the Institute of Occupational Health of the Catholic University of Rome for respiratory pathology problems. Table IV contains a summary of clinical history data concerning the subjects observed. The subjects were all of the male sex with an average age of 43 years and average work seniority of 18 years at that type of job. On the basis of the type of employment, they were divided into 2 groups: those exposed only to wood and those who were also exposed to paints (in this latter group we should take into consideration isocyanates and other components of paints as possible contributors to the genesis of the respiratory symptomatology). All the subjects underwent a specialistic examination by an otorhinolaryngologist, a cranial radiography to evaluate the paranasal sinuses, a radiography of the chest, a spirometric examination, and

Table III  
Results of the Chromatographic Analysis of the Different Types of Wood (Domestic Woods)

Woods (commercial and scientific names)	Polyphenols	Terpenoids	Cardenolids	Alkaloids	Anthranoids & Coumarins	Non identified compounds
<b>SOFTWOODS</b>						
Larch <i>Larix decidua</i> Mill.	xxx	-	-	-	x	x
Northern Redwood <i>Pinus silvestris</i> L.	-	xxx	-	-	-	-
Spruce <i>Picea abies</i> Karst	xx	x	-	-	-	-
<b>HARDWOODS</b>						
Ash <i>Fraxis excelsior</i> L.	xx	-	-	-	x	-
Beech <i>Fagus sylvatica</i> L.	-	xx	-	-	-	-
Cherry <i>Prunus avium</i> L.	xxxxxxx	-	-	-	-	-
Chestnut <i>Castanea sativa</i> Mill.	xx	-	-	-	-	x
Elm <i>Ulmus campestris</i> L.	-	-	-	-	-	xx
Linden <i>Tilia cordata</i> Mill.	-	xx	-	-	-	-
Maple <i>Acer campestre</i> L.	x	-	-	-	-	xx
Oak <i>Quercus petraea</i> Liebl.	x	-	-	-	-	-
Olive <i>Olea europea</i> L.	xxx	-	-	-	-	-
Walnut <i>Juglans regia</i> L.	xx	xxx	-	-	-	-

allergometric tests carried out by pick or i.d. test with allergens provided by the Lofarma Company and containing "Pollens," "Mycophytes," and "Inhalants" which include also 30 extracts of wood dust both from exotic as well as domestic woods. Furthermore, some of these subjects (chosen on the basis of history criteria, type of exposure, and type of referred pathology, and, moreover, excluding those that presented with serious impairment of respiratory functions of the obstructive type even at rest) underwent bronchial challenge tests which were both aspecific (with ultrasonic mist or methacoline) and specific with the more commonly used and suspected wood dust or with toluene diisocyanate (TDI) or both, depending on the circumstances. A reduction of at least 20% of the FEV<sub>1</sub> was considered as a positive result. In the meantime, it was not possible for us to carry out a skin test with TDI conjugated to human albumin. The more significant group of subjects with positive results for wooddust, that is, those 3 that responded to Tanganyika Aniegré both with the skin test as well as the bronchial exposure test, underwent a RAST for this type of wood.

## RESULTS AND COMMENTS

After examining the results of our clinical case studies we can observe that the type of respiratory pathology found in the workers studied was made up of recurrent asthma crises in 45.3% of the cases, less than half of which also had oculorhinitic symptoms; in the remaining 54.7%, the symptoms were mainly bronchial. Among the clinical and functional findings observed in the 86 subjects, we noted: 1) the presence of chronic rhinopharyngitis in 58.2% of the cases; 2) radiological alterations of the paranasal sinuses in 46.7%; 3) an increase in the bronchovasal lung tissue in 73.3% of the cases and hyperdiaphany in 30%; 4) obstructive ventilatory deficit in 50% of the cases; and 5) mixed ventilatory deficit in 15% of the cases.

The cutaneous allergometric tests (Table V) were positive only in 13 subjects, amongst which 7 were positive for non-work related allergens, 2 were positive for both work and nonwork related allergens, and 4 were positive only for work related allergens. The woods that resulted in a positive skin

Table IV  
Clinical Data of Subjects Studied

Number	86
Age (yr, mean and range)	43 (31-62)
Work seniority (yr, mean and range)	18 (5-38)
Current smokers	49 (56.9%)
Exposed only to wood	21 (24.4%)
Also exposed to paints	65 (75.6%)
Duration of symptoms at diagnosis (yr, mean and range)	4.6 (0.6-15)
Prevalence of symptoms (No. and %):	
Chronic cough and phlegm	47 (54.7%)
Asthma	39 (45.3%)
Work related:	
asthma	35 (40.7%)
eye symptoms	16 (18.6%)
nasal symptoms	23 (26.7%)
Clinical and functional findings (No. and %):	
Chronic rhinopharyngitis	50 (58.2%)
Radiological alterations of the paranasal sinuses	41 (46.7%)
Radiographic increase in bronchovascular marking	63 (73.3%)
Obstructive ventilatory deficit (in baseline FEV <sub>1</sub> )	43 (50.0%)

Table V

Prevalence of Skin Reactivity to Common Allergens and Wood Dust Extracts (Prick or Intradermal Tests)

Subjects examined (No.)	86
Positive for work related allergens	4 <sup>(*)</sup> ( 4.6%)
Positive for both work and non-work related allergens	2 <sup>(*)</sup> ( 2.3%)
Positive for non-work related allergens	7 ( 8.1%)
Negative	72 (84.8%)

(\*) 2 positive to Tanganyika; 2 positive to Cherry

(\*) 1 positive to Pine, Oak, Dermatophagoides pt., and some Mycophytes; 1 positive to Tanganyika and Gramineaceous pollen

test in the 4 workers were the Tanganyika Aniegré (in 2 cases) and the Cherry (in 2 cases); in the two with mixed positive results, one case reacted positively to the Tanganyika Aniegré together with Gramineaceous pollen, while the other reacted positively to Pine and Oak together with Dermatophagoides and some Mycophytes.

Table VI indicates those subjects who were positive only to the specific bronchial challenge test, both with woods and

TDI; those subjects positive only to the aspecific bronchial challenge test, both with mist and methacoline; and those positive to both. The number of subjects who underwent the specific test was 25, of which 6 both for woods and TDI. Seven out of 13 subjects examined resulted positive to wood dust, and of these 7, 2 were also positive to ultrasonic mist. There were 10 out of 18 positive results to TDI; of these, 4 were also positive to mist and 1, in addition to mist, was also positive to Mansonian Walnut.

Table VI  
Results of Bronchial Challenge Tests Both Specific (Wood Dust and TDI) and Aspecific  
(Metacholine or Ultrasonic Mist)

Subject examined (No.)	25 (of which 6 both for woods and TDI)		
Wood dust challenge test	Bronchial hyperreactivity test		Total
	positive	negative	
positive	2 (*)	5 (†)	7
negative	3 (‡)	3 (¶)	6
<b>TDI challenge test</b>			
positive	4 (§)	6	10
negative	2	6	8
<b>Total</b>	<b>11</b>	<b>20</b>	<b>31</b>

(\*) 1 positive to Tanganyika; 1 positive both to Mansonia and TDI  
 (†) 2 positive to Tanganyika; 2 positive to Mansonia; 1 positive to Cherry  
 (‡) of which 1 was negative to Pine and Oak, but had positive skin test with the same type of woods  
 (¶) of which 1 was negative to Cherry, but had positive skin test with the same type of wood  
 (§) of which 1 was also positive to Mansonia

The RAST for Tanganyika wood was done, according to the classical method, with an aqueous extract and binding the antigen to a solid polystyrol phase. The test resulted strongly positive in 2 out of 3 subjects (Table VII), while it resulted negative in that subject who had a positive skin reaction also for Gramineaceous pollen and was also positive to the bronchial provocation test with ultrasonic mist. Our RAST results differ from those of other authors.<sup>8</sup>

A first finding of a certain interest obtained in our study is that in wood workers, harmful agents in professional exposure comprise not only wood dust but also many other factors that have irritative and allergic properties for the respiratory airways: in fact, the exposure to paints and solvents was significant in 75% of our cases. More than 50% of the carpenters observed by us presented prevalently with bronchial symptoms; but after an accurate history study involving the conditions of onset and the evolution of the symptomology, it was found that in about 50% of those subjects with bronchitis the disease had begun many years earlier with typical asthma crises, while the onset of the bronchitis was subsequent. We believe that this is due to the long time interval that elapsed between the beginning of the symptomology and the beginning of appropriate diagnostic examinations (on the average of 4.6 years in the entire group): it is worthwhile to emphasize the importance of carrying out allergometric examinations as early as possible, especially in those cases of asthma that arise in work environments.<sup>3</sup>

The clinical and functional findings stress a high incidence of alterations of the rhinopharynx and paranasal sinuses. This is in agreement with data found in the literature concerning damage produced by wood dust on the upper respiratory airways, with possible rhinitis that can also be hemorrhagic. In fact, the average diameter of wood dust is greater than 5  $\mu$  and this justifies its localization in the upper respiratory airways.<sup>11</sup>

In our case studies the allergometric skin tests, considered as a whole, were positive in 33.3% of the asthmatic subjects (excluding all those with bronchitis from the total) and this percentage is lower than that of other case studies conducted on non-professional subjects with asthma (up to 50-60% skin test positive). This could imply a prevalently non allergic cause for asthma due to wood dust. On the other hand, studies on the chemical composition of woods conducted by us have shown that in nearly all the woods examined there was a presence of Polyphenols or Terpenoids, compounds that have properties that are notoriously irritative or allergic. This, together with the consideration that the extracts on the market with which the allergometric tests are carried out are of a protein nature and, therefore, do not contain those substances which also have potential haptenic properties, tends to strengthen the hypothesis that, like for Plicatic Acid,<sup>10</sup> at least a part of the cases is due to sensitization to haptenes and another considerable part is due instead to a pharmacological-irritative type of reaction. In-

Table VII

Serum Rast Values for Tanganyika Extract Obtained in 3 Subjects Who Responded to This Type of Wood Both with the Skin Test as Well as the Bronchial Challenge Test

Subject	Age	Work seniority	Skin reactivity to common allergens	Bronchial hyperreactivity	RAST value
V. C.	43	15	-	-	+++ (15.0%)
S. A.	45	11	-	-	+++ (12.5%)
F. P.	37	12	+	+	- (0.9%)
5 control subjects			+		-

stead, in those cases with a positive skin reaction to the protein extract, we believe that an allergic pathologic mechanism is the most probable.

As far as the bronchial challenge tests are concerned, we note that only one subject had a positive result to mist, Mansonian wood, and TDI; one was positive to mist and Tanganyikan wood; and three were positive to mist and TDI. Five subjects who were positive to mist had negative results with wood dust or TDI; on the other hand, 5 subjects who were positive to wood dust and 6 who were positive to TDI had negative results with mist. Regarding the type of response to the specific test, in 9 cases it was immediate, in 6 it was diphasic, and in 2 it was delayed.

The results of the specific bronchial exposure tests would seem to confirm a certain selectivity in the response, even in those subjects with aspecific bronchial hyperreactivity; nevertheless, they give us no indication as to the pathogenetic mechanism of the bronchospastic attack, which can be either pharmacoirritative or allergic. In 2 out of 3 subjects who had given a positive response both to skin tests and bronchial exposure provocation tests with Tanganyikan wood, the specific IgE dosage by RAST, was strongly positive, bringing us to the conclusion that there are specific IgE's towards protein constituents of the wood. In those subjects with a positive result to the bronchial challenge test with wood dust, but negative to the skin test with an extract of the same type of wood, an irritative cause of the bronchospasm could be an explanation. But another explanation could also be a sensitization to some non protein substance contained in this wood (a haptene). The next step of our research program is to carry

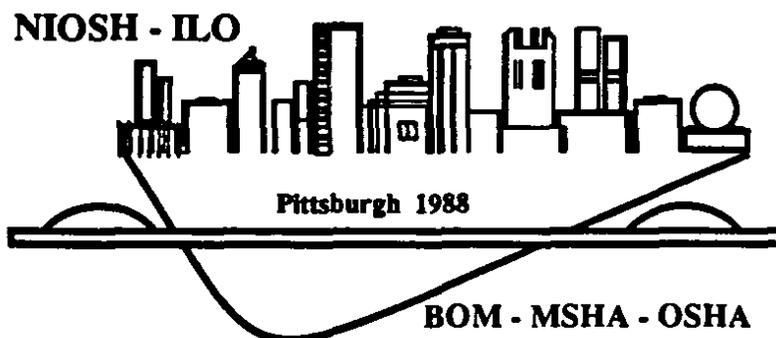
out tests both in vivo (skin reaction, patch tests, bronchial challenge tests) and in vitro (RAST) with the fractions isolated with TLC.

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Tome  
Parte



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