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Envi 102
Prof. Kegley
May 14, 1990

A Study of the Incidence of Polychlorinated Biphenyls (PCBs) in Macroinvertebrates of the Hoosic River

Introduction: In my introductory class for Environmental Studies last fall, I read that certain human-made organic chemicals are particularly dangerous when released into the environment. Substances such as DDT make their way up the food chain, accumulating in greater concentrations with each step to a higher trophic level. The particular danger associated with these compounds is that they can lodge themselves in the fatty tissues of various animals, where it is extremely difficult to excrete them, and this allows for prolonged exposure to their toxic effects. Polychlorinated Biphenyls (PCBs) constitute a group of such toxic, chemically stable organic molecules, and in the Hoosic River valley they present a particular problem. It has been shown that PCBs occur in dangerously high concentrations in the river's trout, and as a result anglers are now warned against eating their catch.[1] The idea of bioaccumulation within the food web attracted me to study the incidence of these toxins in a lower trophic level, the macroinvertebrates (a.k.a. "insect larvae" or "bugs") that live on the river's bottom. Due to their relatively short life cycles, macroinvertebrates provide a good indication of current PCB levels within a given ecosystem.[2] A study of PCB concentrations in this group of animals is also particularly important, given their median position between the first trophic level (microorganisms) and higher levels closer to the human end of the food chain (vertebrates, e.g. fish). Unfortunately, I didn't have the time or the means to spend a relaxing afternoon fishing: otherwise I might have been able to expand upon my data to show possible correlations between trophic levels.

My sampling site was at the point where the river emerges from the concrete flood chutes that conduct the water through North Adams, past the Sprague Electric plant. This area is located directly adjacent to the landfill

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[1] HOORWA Report, PCBs and the Hoosic River, Vol 3, No. 1; Spring 1990.
[2] Bush, pg 96.

once used by Sprague to dispose of their PCB-rich transformer oils, and it has since been determined to be the primary source of the chemicals in the Hoosic. Both times that I visited the site, the warm sun, the cool water, and the plush new growth along the banks made it difficult to remember that I wasn't simply experiencing a refreshing escape from college, but that I was dealing with a

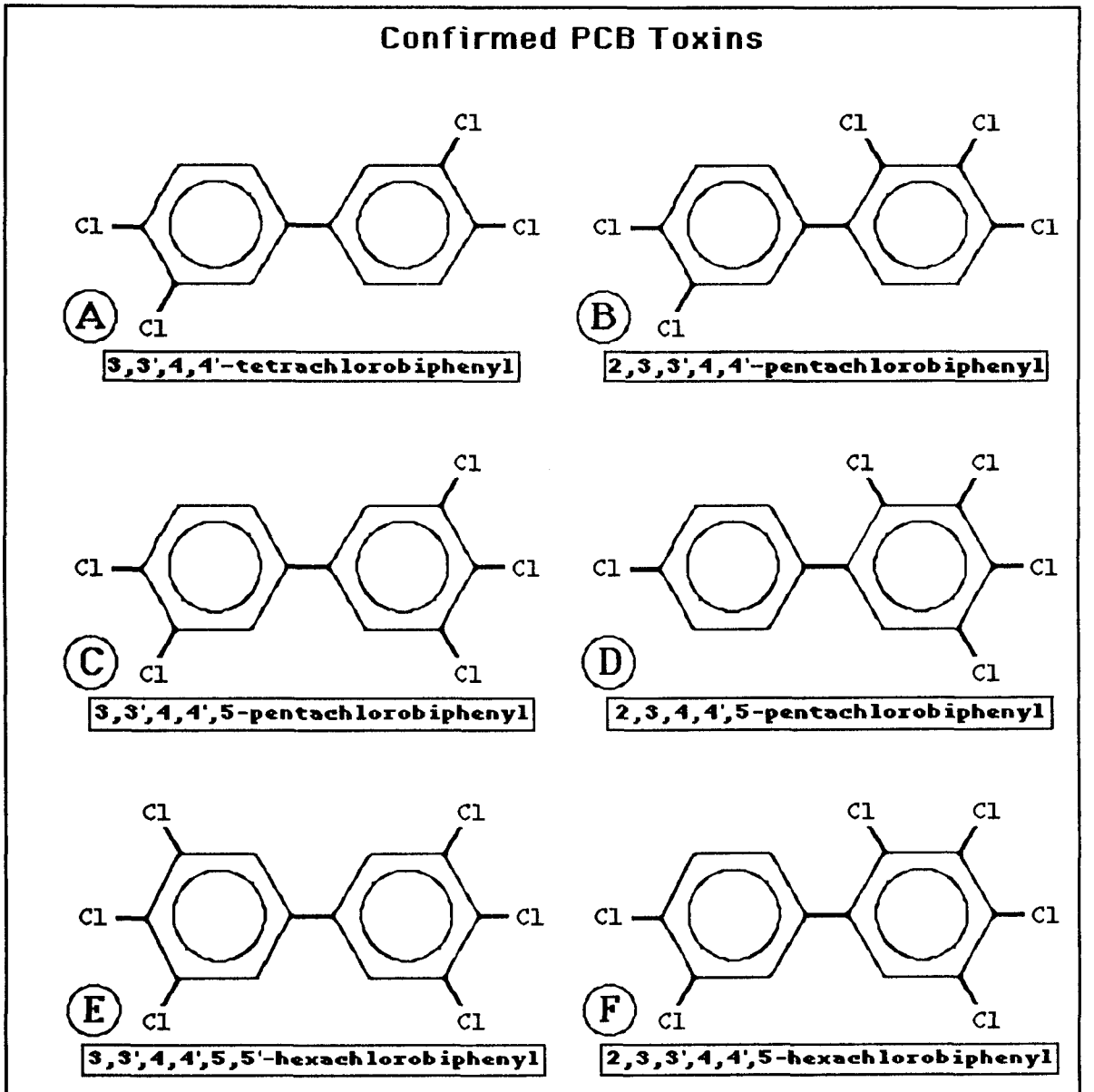

fig 1. Six PCB isomers known to have toxic effects upon the environment.[1] It is common for each ring to have an equal number of chlorine atoms, or to have a difference of one, as is the case in A, B, C, and E.

[1] D'Itri, pg 166.

dangerous pollutant. This project is a grim reminder of that fact, and it provides some further insight as to how PCBs spread and accumulate within the environment.

Nature of PCBs:[1] Polychlorinated biphenyls are organic compounds made up of carbon, hydrogen, and chlorine. Their molecules are made up of two linked rings of six carbons, with a varying number of chlorine atoms bound to each (see fig 1). They are distinguished from each other by the amount of chlorine contained within a given mixture, and are numbered accordingly. For example, in a sample which contains 54% chlorine by weight, the mixture, or *arochlor*,® would be called PCB 1254 (i.e. 1-2, 54%). The large number of possible combinations in which chlorine can bind to the aromatic rings means that a wide variety of molecules can be found within any one arochlor. So long as each of these molecules contains the same number of chlorines, they are all considered isomers of the same compound; otherwise, they are classified as different compounds.

PCBs are extremely stable compounds, an attribute which makes them both extremely safe and useful in one sense, but extremely dangerous and impractical in another. On the one hand, their inert qualities make them excellent cooling lubricants for electrical transformers and capacitors,[2] which was their primary function from the 1940's into the 70's. On the other hand, once PCBs are released into the environment, their chemical stability makes them extremely resistant to biodegradation, so their effects are felt for years after their release.[3] Now it is estimated by the National Academy of Sciences that there are *82 million tons* of PCBs to be dealt with in the entire world.[4] Over the thirty years in which they were produced, PCBs were used in inks, paints, dedusting agents, pesticides, wood coatings, carbonless copy paper, and a wide range of others.[5] Such widespread use has made it extremely difficult to deal with all the different sources of the chemical in today's environment.

The nonpolar nature of these compounds makes them all extremely insoluble in water. This means that, thankfully, PCBs are not found in signifi-

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[1]This section quoted from Ringgold, 1990.

[2]HOORWA Report.

[3]There is some evidence to indicate that the PCBs experience "preferential decomposition" over time, especially those with 5 chlorines or less (Beeton, pg 27-8).

[4]D'Itri, pg 7.

[5]Beeton, pg 2, 16.

cant quantities in drinking water. However, these pollutants attach themselves quite successfully to sediments, which can be introduced into aquatic ecosystems. Here the real damage begins: PCBs are readily incorporated into the living tissues of aquatic microorganisms, allowing them to make their way up the food chain. Once embedded in the fatty tissues of higher organisms such as fish and birds, it is virtually impossible to expel them, so the PCBs simply accumulate to higher and higher levels.

Method -- *Collection*: On two sunny spring afternoons, I had the occasion to set schoolwork aside to collect samples of bugs in the Hoosic river. The first day was a practice run, because I later learned that the plastic bottles I had used to contain my samples were not as inert as I had imagined. In the course of one week's storage in my roommate's fridge, enough plastic would have leached into my bug solutions to interfere with my final readings. The second day I used glass bottles (with plastic caps, mind you!), which seemed to be more effective.

↳ Teflon-lined plastic caps!

Contrary to popular opinion, bug-catching is quite a sophisticated operation. I used hip-length waders to position myself strategically in the middle of the current,[1] and the second day I wore latex gloves, more as a reminder

fig 2. The action shot. The current in this area was much slower than in the other sites, so my *Lab-w* was far lower here. Keeping my boots up and my legs dry was the main challenge. Try as I might, I didn't get a tan. Darn. !

[1]This had more to do with keeping my legs warm than with keeping them dry, as I soon discovered.

↳ You can't trust waders from DPD!

that I shouldn't rub my eyes or wipe my mouth than as protection against my surroundings. I'm afraid the nature of the exercise required that I come into some contact with the samples, though I imagine the risks involved were quite minimal. I collected my macroinvertebrates with the aid of a finely-woven D-frame aquatic net, which I obtained from the Hoosic River Watershed Association (HooRWA). With the net positioned strategically downstream from myself, I could shuffle over the rocks and gravel, dislodging the larvae that clung to their surfaces, thereby sweeping them into the net (see fig 2).

I usually spent about five minutes dancing in the current before coming ashore to harvest my catch and tally the results. To this end, I dumped the contents onto a tray, taking care that none of the larvae were left clinging to the net. A little water splashed on the tray would encourage the bugs to wriggle around, making it easier to find them in the sediment. Using forceps, I

Scott, This is a great picture! How'd you do it?

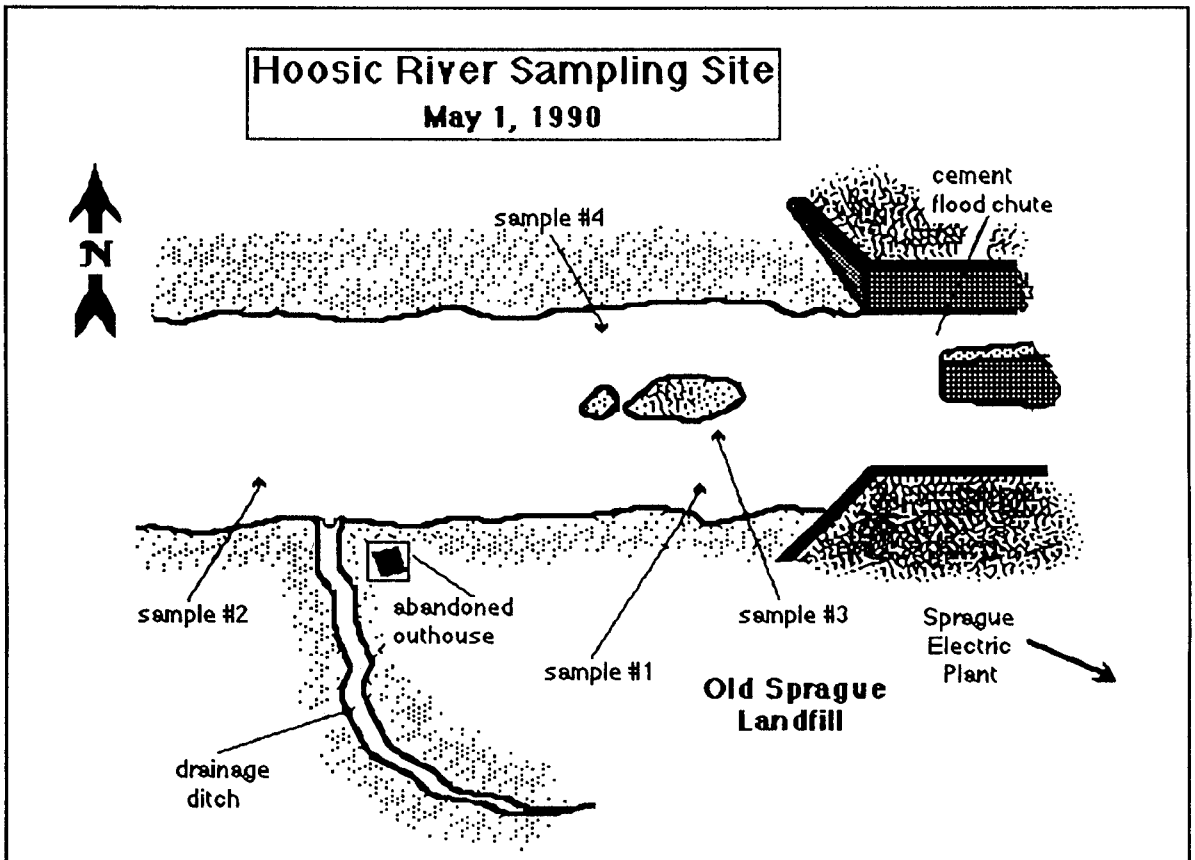

fig 3. Hoosic River sampling site (water flows east to west). The first sample was taken quite far upstream, close to the left bank, adjacent to the old landfill; the second comes from further downstream, below the drainage outlet flowing from the landfill; the third was collected on the left side of the island in the middle of the river; the fourth comes from the opposite side. Map derived from lab handout and personal field measurements.

transferred all the bugs into my jars, keeping track of how many I found and to which taxonomic orders they belonged. If there weren't enough, I spent more time in the same general area, trying to collect a reasonable quantity, (usually about thirty). Each sample took about an hour to collect, isolate, identify, and record. My final four samples were obtained in the sites pictured in figure 3.

This method of collection seemed quite sensible at first, and my first day in the field I was able to collect a reasonable number of larvae in five-minute intervals at each site. However, being the type of person who lacks any patience in a new endeavor, I would pull the net up periodically to check my progress. Often I found that the amount of coarse sediment caught in the net would increase and diminish over the course of the 5-minute period, and once I was surprised not to find one particularly large larva that I was sure I had seen earlier. I eventually concluded that the net was creating an eddy-effect (probably most pronounced when I picked the net up and put it back in the water), sweeping many of my specimens back out of the net, and that a more effective method was simply to pull rocks up from the river bed to pick each larva off individually.

Bug Distribution

Order	sample 1	sample 2	sample 3	sample 4
Mayflies[1]	6	6	6	5
Stoneflies	3	2	2	3
Flies	7	4	12	15
Caddisflies	10	6	24	21
Other	1 tapeworm	1 water penny		2 water pennies

table 1. Distribution of aquatic orders, as found in the four samples taken 5.1.90. It is important to note that these numbers do not reflect the size of each specimen: fly larvae were generally smaller than the others, and caddisfly larvae could be quite large. Most varied considerably, so it is impossible to equate the total number with the final weights.

I am sure that this difference in my collection methods had an effect upon the species composition in my samples. The first two contained a greater percentage of *ephemeroptera* (mayflies), *plecoptera* (stoneflies), and *diptera* (flies), while the second two contained primarily *trichoptera* (caddisflies) -- these orders of insects are depicted in figure 4, and the species composition of each sample is described in table 1. This difference was apparently because

[1]Unlike earlier in April, there were very few mayflies this time, presumably because they were spending Mayday flying around, like they're supposed to!

flies, mayflies, and stoneflies are not fastened as solidly to the rocks, so they tended to be brushed off as I dislodged the stones from the riverbed. Conversely, the caddisflies attach themselves to the rocks with the help of their special cemented cases, which makes them more difficult to brush off the stones and into the net -- unless I had the rocks themselves in my hands, so that I could pry each cage off with my forceps.

There were apparent differences between the species compositions obtained in my first and second trips to the site. The first time, there was a noticeable predominance of mayfly larvae, and the stoneflies and caddisflies all tended to be much smaller. Overall, it was much more difficult to obtain a reasonable amount of bug-mass the second time, even though the larvae I did catch were so much larger than before. It was clear that, in the space of ten days, the river's macroinvertebrate population had been developing to maturity, so that the composition of my samples would certainly have been different had I returned the next week for a third assay.

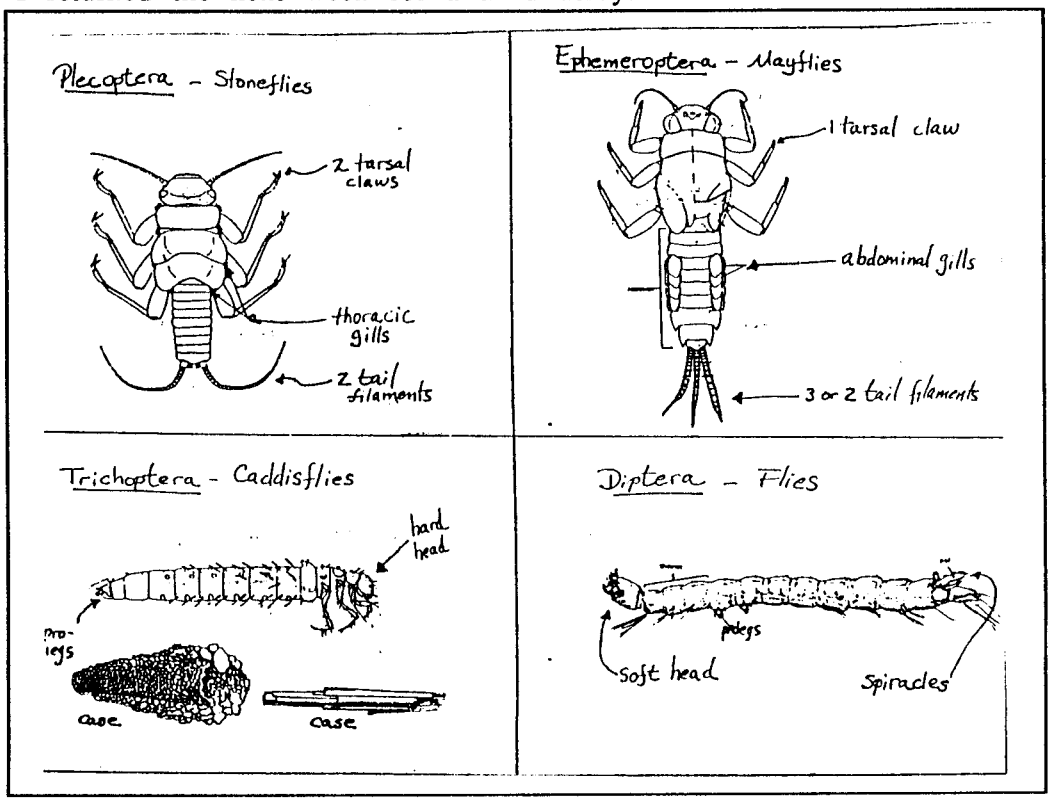

fig. 4. Four orders of aquatic insects (larval stage) found in the Hoosic River.

During a previous lab, in which we determined the PCB levels of sediment in the river's banks, it was a fairly simple matter to isolate 30g of the samples to process in the lab. Unfortunately, it would have cost me at least a

week of sunny afternoons to collect thirty grams of bug-guts, so I had to settle for smaller amounts. A more reasonable weight tended to be 0.5 to 2 grams, though one of my samples measured as low as 0.25 grams.[1] I was afraid that these low weights would provide only indiscernible results in the end. Later, as I was looking through a report of a similar project conducted on the Hudson river, I found that successful results had been obtained with samples as light as 200 mg.[2]

Lab-work: In the lab, a few bugs were spared the mortar, only to be subjected to the desiccator. Their wet weight was first measured with a fine-tuned scale, and after a night of constant baking, they were measured again. Comparing the dry weight to the wet weight, it was clear that my tiny samples were mainly water, and that only 32% was actually bug-tissue. No matter: undeterred, I forged onward.

The first step was to remove any excess water by placing the individual bugs on a kimwipe,® then each of the four samples was mashed into bug-juice with a mortar and pestle. Had I been able to collect more specimens, I would have divided each sample into their respective orders, so that the level of PCBs in each species might give me an indication of their bioaccumulation factors. However, this was not possible given the limitations of my samples, so I ground each one into a homogeneous mixture.

To extract PCBs from the assorted bug parts, 50 ml of methylene chloride was poured into the mortar and stirred, producing a lovely green-yellow suspension. Methylene chloride is a particularly good solvent for PCBs, as it is also a halogenated organic compound: like dissolves like. Three minutes in the sonicator bath also helped to dislodge the PCBs from their previous perches into the solution. This mixture was then passed through a Buchner filter to separate out the larger particles (even though I made sure to rinse the filter through with hexane, it was clear that some of the original solution was lost due to the filter's poor suction). The above process was carried out a second time, sodium ~~nitrate~~ sulfate was added to remove any remaining water, and then the yellowish solution was run through a Florisil® separatory funnel, which re-

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[1] At this site, the current was quite slack, and the bottom was composed primarily of finer sediment. Besides the fact that there was less water to carry bugs into my net, I suppose the larvae prefer to inhabit places where the current is strong, to provide them with more oxygen and nutrients.

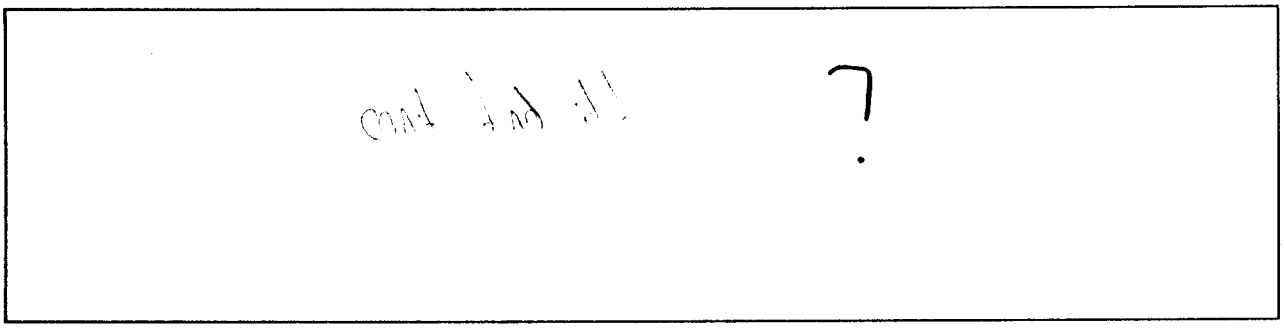
[2] Bush, pg 96.

moved the remaining pigment and turned it clear. Again, I ran hexane through the apparatus to make sure that all the PCBs had passed through.

Now the solution had to be condensed as much as possible to obtain a reasonably concentrated final solution. This was accomplished using a Kuderna-Danish receiver connected to a 4-ball Snyder column, a sophisticated set of glassware that I took special care not to break. First it was necessary to boil off the methylene chloride: a reasonably simple feat, since that particular solvent has a very low boiling point, even when compared to hexane. More hexane was added, and the remaining solution was boiled to down as far as possible, then diluted with hexane to 10 ml. The four tiny little vials that resulted from so many hours of processing were almost worth their weight in gold.

Gas Chromatograph: The final step was to inject the mixture into the gas chromatograph, which divides each PCB-rich sample into its individual isomers, so that each can be quantified and thus compared to other samples. The GC uses helium gas to propel the substances to be measured through a long (30 meter) glass column, which is coiled up inside a specially regulated oven (see figure 5). The substances to be measured are retained within the column for various periods of time, after which they are released to be measured by the detector. Each individual component of the PCB mixture has its own retention time, making it possible to differentiate between various isomers. This is reflected in the final printout, on which each peak shows the amount of a given isomer contained in the sample.

Fig. 5. The Gas Chromatograph.



Our method of quantifying the total concentration of *all* the PCB 1242 and 1254 was to select two fairly isolated peaks for each arochlor, then to find the average the values for each; these average values provided us with an indication of the total PCB concentrations. It seems that our results are based on a dangerous assumption: that the four peaks we chose were in fact characteris-

tic of the total amounts. ~~In appendix B, I have tried to develop a better method for determining overall PCB levels, but these values are tentative at best.~~

Results: I was pleased to see that most of the graphs generated by the Gas Chromatograph displayed pronounced peaks with fairly well-defined valleys and baselines. These graphs, as well as one standard curve, are included and discussed to some extent in appendix A. In some cases, it is clear that the computer misjudged altogether in its calculations, but I have nevertheless accepted all the data in good faith, and have incorporated it all into table 3 below as an indication of what the correct reading *might* have been.

Unfortunately, the one sample that I had expected to have particularly pronounced peaks (sample # 2) had virtually none at all. I had imagined that the PCB levels there would be quite high, due to its location downstream from the landfill's drainage ditch, but perhaps I simply didn't have enough bug-mass to obtain a satisfactory reading. In the first figure of appendix A, it is apparent that there are at least some visible correlations in the incidence of certain isomers: these occur within the bars drawn in red. Discrepancies are pointed out on each individual graph thereafter.

Although specific answers are not readily forthcoming, these results pose some interesting questions. For example, the high PCB levels described in table 3 below show no correlation to those of river sediment located in the same sites: if we are to assume that the larvae absorb their nutrients from their immediate surroundings, then their bioaccumulation factors range anywhere from 22.4 to 463. If, however, we assume that all the PCBs are concentrated from the water itself as it flows over each larva, then those factors might range from 608 to 14,300. It has been determined that certain species of *trichoptera* will concentrate PCBs "several thousand times" from the surrounding water, so these latter values seem within reason.[1] In either case, this sort of calculation requires a considerable degree of random selection, and in the end the true biomagnification factor is certain to be lurking somewhere in the middle of all these values.

River trout in the Hoosic have been shown to accumulate PCBs to an even greater extent. In 1986, the Massachusetts Division of Water Pollution Control detected levels of up to 30.6 ppm in some of the river's fish, when federal limits

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[1] Bush, pg 96.

are 2.0 ppm! In 1989, trout that had been introduced from elsewhere in the spring were caught later that year in the fall, and over their six-month soujourn in the Hoosic they had concentrated 11.5 to 17.25 ppm of PCBs in their tissues. To top that, mature lake trout in Lake Michigan have been shown to concentrate PCBs by a factor of nearly 3.5 million![1] These figures offer a grim warning against eating any of the fish caught in the Hoosic.

what levels are Michigan fish in ppm?

GC analysis of Hoosic River sediments and water

sample	% water	PCB 1242 wet (ppb)	PCB 1242 dry (ppb)	PCB 1254 wet (ppb)	PCB 1254 dry (ppb)
HR-6L	24.1	123	162	14	18
HR-5C	22.8	5.3	6.8	36	34
HR-1R	42.0	1.7	2.9	29	49
HR-3R (aq)	100.0	0	0	0.4	

table 2. PCB concentrations in Hoosic River sediment. HR-6L was taken on the left bank, close to the position where my sample #1 was collected; HR-5C was sampled from the island in the middle of the river, in the same place that I collected my sample #3; and HR-1R represents the levels in site on the right bank, opposite from the Sprague dump (close to my #4). HR-3R is a water sample taken from the right bank of the Hoosic, and therefore it is shown as 100% moisture.

Results from GC analysis (macroinvertebrates)

sample	weight wet (g.)	PCB 1242 wet (ppb)	PCB 1242 dry (ppb)	PCB 1254 wet (ppb)	PCB 1254 dry (ppb)
#1	0.76	1830	5720	379	1180
#2	0.25	414	1290	349	1090
#3	1.80	1010	3150	243	760
#4	0.90	615	1920	1570	4900

table 3. In general, these values are surprisingly high, though it seems there are no obvious trends.

Discussion: The variablity of these results is partly attributable to the constant mobility and change of the sytem itself. The areas in which I did my sampling were within a rather turbulent section of the river, and there is no doubt that results obtained from the same sites later on would have been somewhat different. Also, it has been established that different species incorporate PCBs into their tissues according to distinct bioaccumulation factors, and my samples were far from homogenous in their species. Therefore, I am probably expecting too much when I attempt to find corrolations in my data.

From the graphs in appendix A, it is clear that the lower-chlorinated PCB congeners are either not present or have been depleted significantly. There

[1] Beeton, pg 160.

are a few reasons why this might be so. First, the lower chlorinated PCBs are more likely to be metabolized by micro-organisms, or even by bugs such as these. Second, they are more likely to be decomposed into other substances[1] by ultraviolet light, which has much less of an effect on PCB molecules with more chlorines. Third, and I would claim most importantly, they are more soluble in water than the other congeners, and therefore have a greater tendency to leach from the soil to be incorporated in the water cycle[2] -- whether that is a positive or negative trait is questionable.

To conclude, it is clear that the macroinvertebrates of the Hoosic River do play a significant role in the biomagnification of polychlorinated biphenyls. It is difficult to determine whether this process is at all related to any specific bioaccumulation factors of certain species, or whether the levels detected are at all related to the PCB levels of the surrounding sediment. However, the process speaks for itself, and it underscores the environmental dangers of these substances. PCBs have proven to be a double-edged sword: highly useful in their physical and chemical stability, but extremely dangerous for those same reasons.

Scott,

Lots of very nice work here!
Good job!

[1] "Other substances," such as chlorodibenzofurans (CDFs), which I'm not sure are much better for the environment... (Beeton, pg. 151).

[2] Bush, pg. 101.

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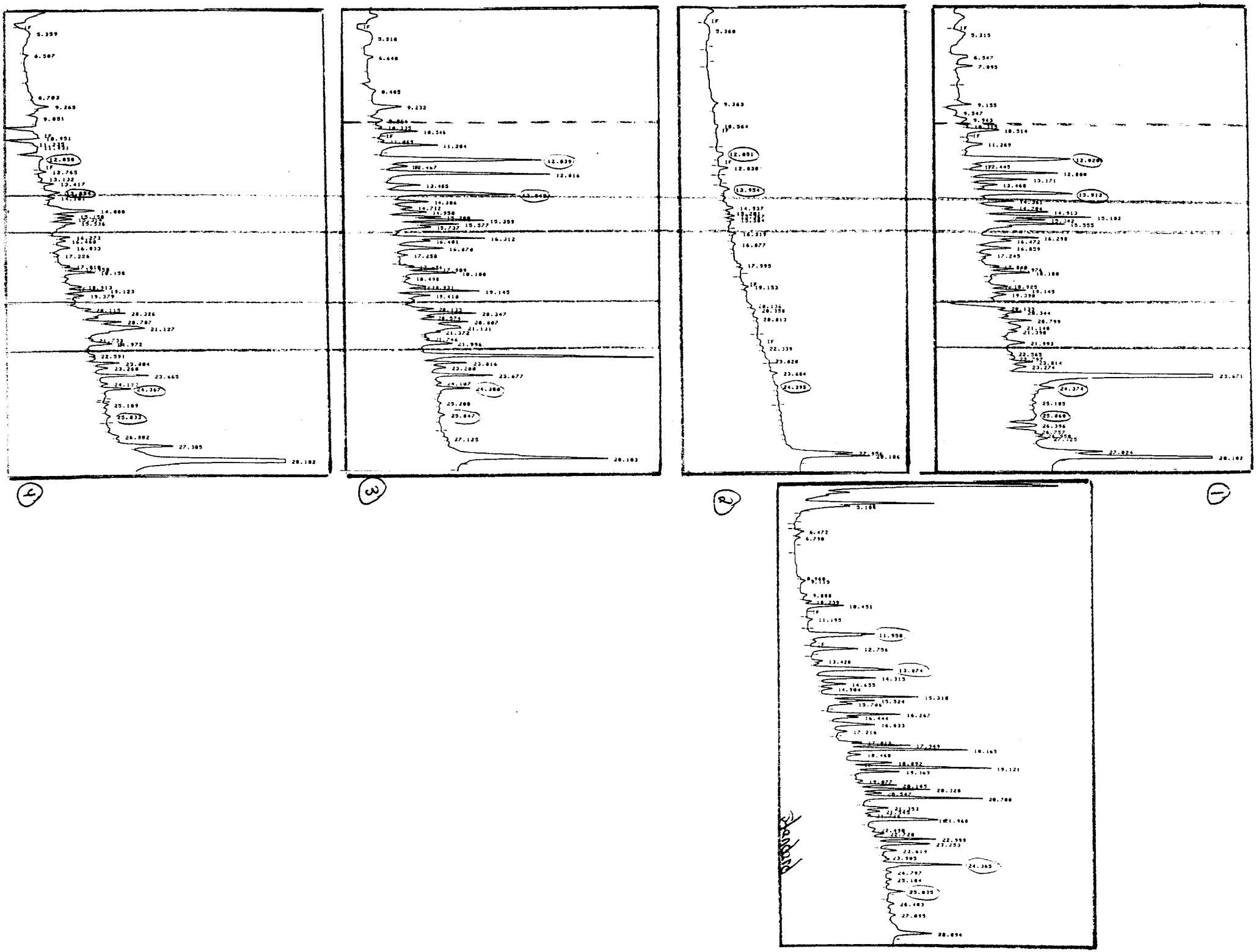
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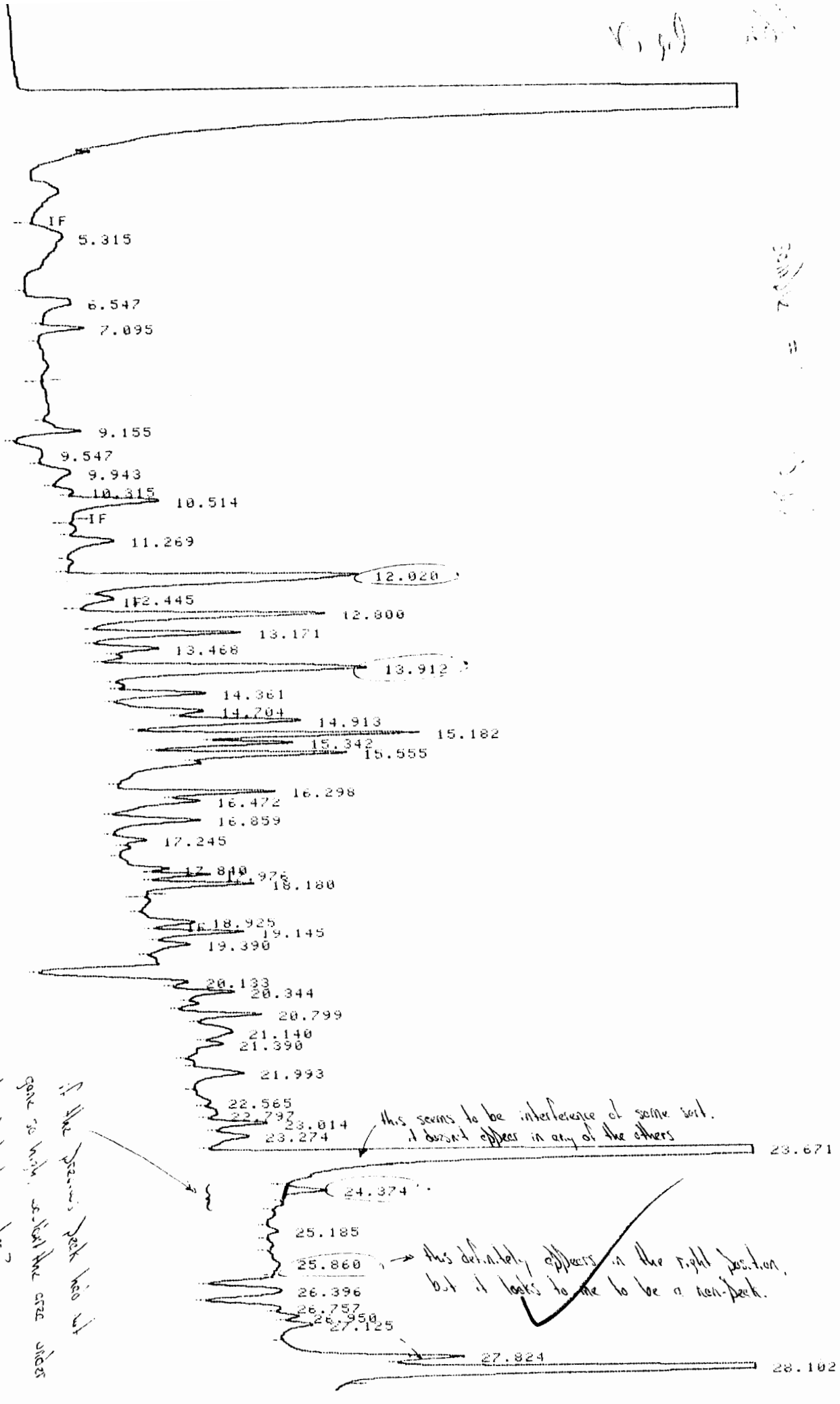
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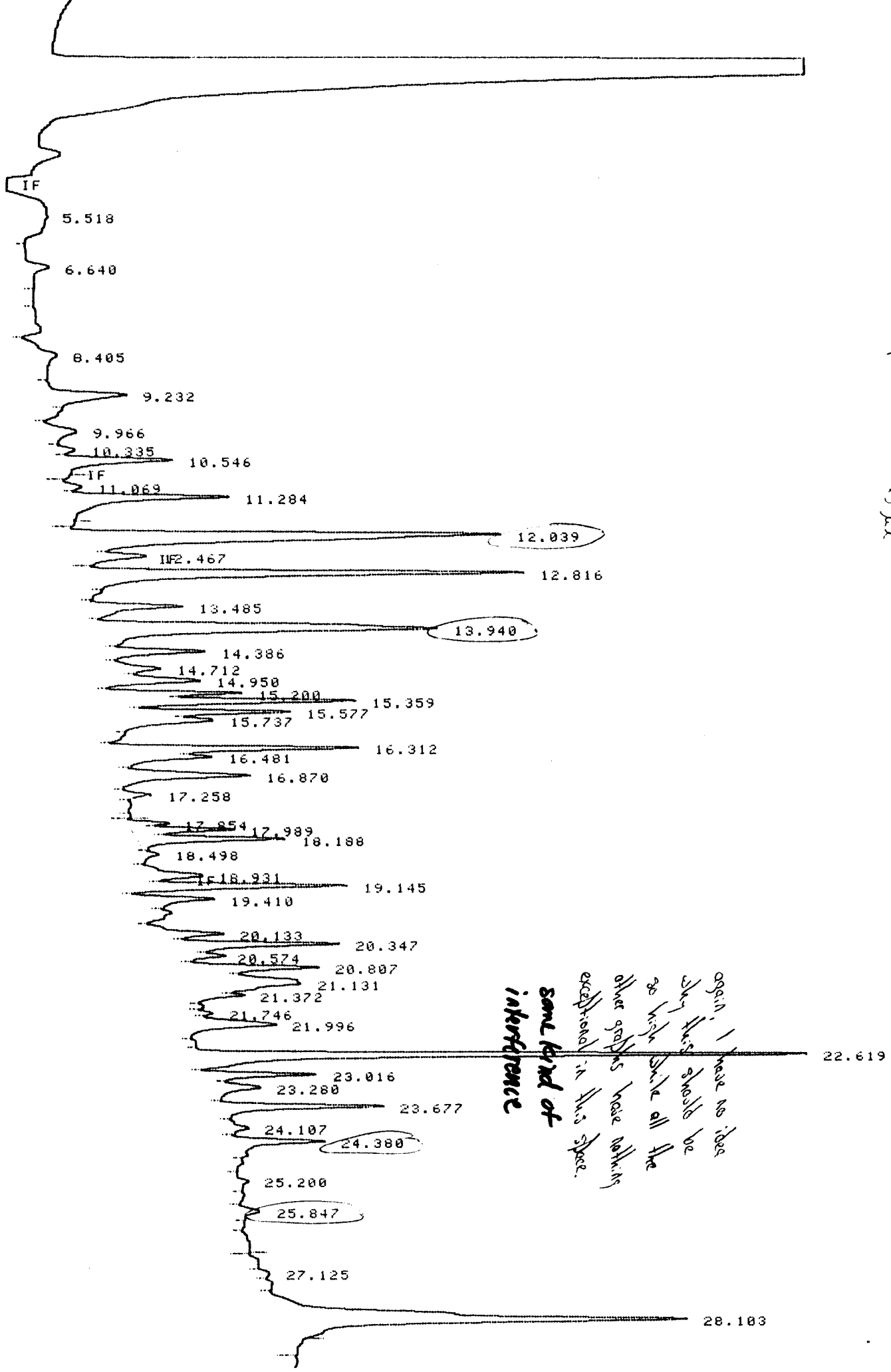
22.797
23.014
23.274
23.671
24.374
25.185
25.860
26.396
26.757
26.950
27.125
27.824
28.102
this seems to be interference of some sort. It doesn't appear in any of the others
this definitely appears in the right position, but it looks to me to be a non-peak.
probably so.

Sample #2 10 µl

These little bumps all occur in pretty much the right positions — there was just not enough bumps to get good readings

It is possible to determine this shoulder area. We might have a reading!

It is possible.



Some kind of interference

again, I have no idea why this should be so high while all the other graphs have nothing exceptional in this space.

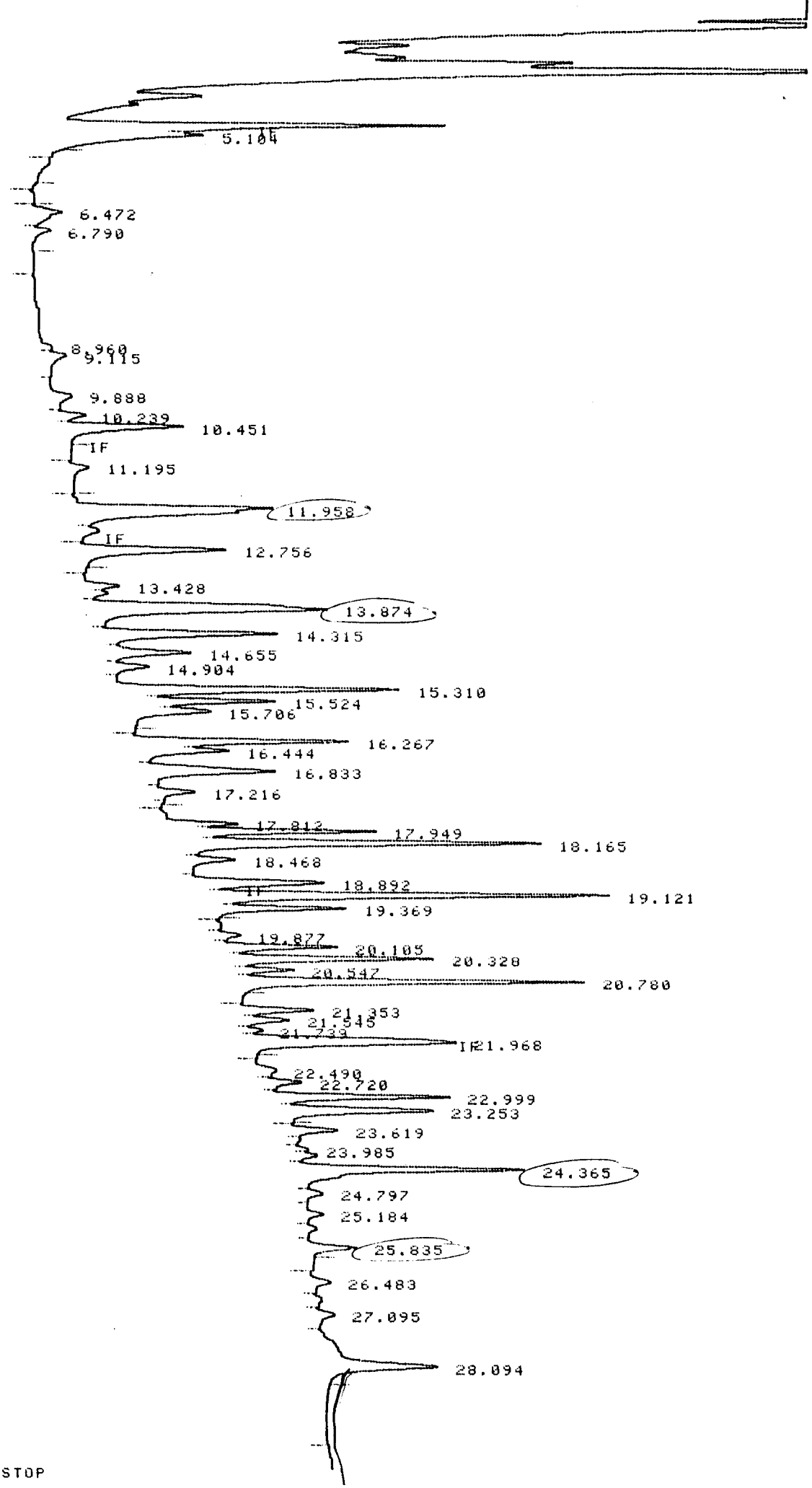
Sample #3 Bill

Sample #4 10 µl

(1)

In this attempt to allow for "human" error - I accepted the wrong peaks!

For some reason, the machine shifted its "expected values" - so that the first value it offers does not correspond to the correct peak. The next one is hard to determine the area between it



Standard (100 ng)